



Fatty acid composition and tocopherol content of processed marine fish and contribution of omega-3 fatty acids

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ABSTRACT

The present study analysed fatty acid composition and tocopherol content of raw, curried and fried fish. Of the saturated fatty acids, C12:0, C14:0 and C16:0 had significant increase in fried and curried fish than raw fish. Of monounsaturated fatty acids, C18:1c significantly increased in fried and cooked form whereas polyunsaturated fatty acids, C20:5n3 (EPA) and C22:6n3 (DHA) showed significant decrease in fried and curried fish. In cooked and fried fish there was significant reduction of tocopherol content. There was increase in hypocholesterolaemic and hypercholesterolaemic fatty acid (HH) ratio in fried and curried fish. Fish fried in coconut oil and fish curry in coconut cream were not found to be healthy processing methods and both processing methods lead to significant reduction in potential health benefits of omega-3 fatty acids in the fishes.

Keywords: Coconut, Docosahexaenoic acid (DHA), Eicosapentaenoic acid (EPA), Fish curry, Fried fish, Health

Introduction

Fish in human diet provides beneficial health effects due to presence of omega-3 polyunsaturated fatty acids (omega-3PUFAs) such as eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) (Kocatepe *et al.*, 2011). These fatty acids have beneficial effects in the prevention of atherosclerosis, heart attack, coronary heart diseases (Harris and Shacky, 2004), cancer (Gerber *et al.*, 2005) and inflammatory diseases (Billuzzi, 2001). Fish is consumed as fish curry made with coconut cream or fried in coconut oil in Sri Lanka and South Asia. Fish is usually cooked in different ways for consumption in the world except the far east countries where raw fish is eaten. Cooking (boiling, baking, roasting, grilling and frying) improves hygienic quality of the food by inactivation of pathogenic microorganisms and enhances digestibility and bio-availability of nutrients in the digestive tract (Kocatepe *et al.*, 2011). During cooking, chemical and physical reactions take place which either improves or impairs the nutritional value of food, while the content of thermolabile compounds and fat-soluble vitamins or polyunsaturated fatty acids are often reduced (Tokur, 2007). The effect of processing on the stability of omega-3 PUFA fatty acids in fish has been reported (Garcia-Arias *et al.*, 2003; Aro, *et al.*, 2005). The culinary practices

during cooking, makes them very prone to lipid oxidation and loss of long chain omega-3 PUFAs, which reduces the nutritive value of fish (Aubourg, *et al.*, 2010). Fat content and fatty acids (FAs) of fish lipids are extremely variable, even within species, depending upon different abiotic and biotic factors such as the species, capture area, temperature, fishing season, pH, salinity and reproductive cycle (Kaushik, *et al.*, 2006; Lei, *et al.*, 2013). Furthermore, saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) change with cooking and frying and its amount differs and depends primarily on the total lipid content that can be affected by numerous biological factors (Dundstan, *et al.*, 1996). SFAs are dominated by palmitic acid (C16:0), followed by myristic acid (C14:0) and stearic acid (C18:0), which are more abundant in warm water fish than in cold water fish and also present pentadecylic acid (C15:0), margaric acid (C17:0) and arachidic acid (C20:0) occurring at about 1% or less and generally considered to be of limited nutritive value (Ackman, 1974). The MUFAs are dominated by oleic acid C18:1n9, followed by three times less abundance of C16:1n7. In carnivorous species, DHA are most abundant than EPA and marine fish have more PUFAs than freshwater fish (Dundstan, *et al.*, 1996). The changes in lipid content and fatty acid composition in the frying process are higher than in other cooking

processes (Larsen *et al.*, 2010). The tocopherol contents of seafood has been reported by Gotoh *et al.* (2009). Many studies have reported that suboptimal levels of alpha-tocopherol in plasma are associated with several health problems such as coronary heart disease, arterogenesis, stroke, cancer and other degenerative and neurological diseases (Ribarova *et al.*, 2003).

Materials and methods

Marine fish (eight species *viz.*, *Photopectoralis* (= *Leiognathus*) *bindus*, *Mugil cephalus*, *Rasterliger kanagurata*, *Chirocentrus dorab*, *Selar crumenophthalmus*, *Katsuwonus pelamis*, *Sphyrænea jello* and *Dussumieria acuta*) were purchased from the local market and were used to prepare curry in coconut cream using traditional Sri Lankan recipe and also fried in coconut oil (Turkey brand, Sri Lanka). The ingredients used for making fish curry were: fish (2 kg), tomatoes (chopped, 100 g), onion (sliced, 100 g), garlic (10 cloves sliced), curry leaves (2 springs), green chillies (3), chilli powder (25 g), turmeric powder (5 g), cumin powder (10 g), coriander powder (25 g), coconut milk (2 l), salt (50 g), tamarind pulp (25 ml), water (3 l), coconut oil (25 ml), fenugreek seeds (10 g), cinnamon (1 stick), mustard seeds (10 g) and fennel seeds (10 g). Fish curry and fried fish were made three times consistently using the ingredients and triplicate samples (100 g) were taken for lipid analysis. The raw fish (100 g), the processed or cooked fish (100 g) and fried fish (100 g) were taken for further lipid and tocopherol analysis. Muscle tissue from each fish was homogenised using a grinder. Approximately 3 g samples of homogenised muscle from each fish were weighed in triplicate using an analytical balance (AG204, Mettler, Toledo) and placed in conical flasks. Muscle tissue samples were hydrolysed by adding 8 ml of distilled water and 10 ml of concentrated HCl and incubated at 95°C in a boiling bath for 45 min. The samples were cooled and transferred to Mojonnier flasks. Fat was serially extracted three times with 25 ml volumes of petroleum ether: diethyl ether (1:1 v/v) according to modified method of AOAC (2000). The upper phase containing the lipids was evaporated to dryness and weighed for further analysis. The FAs in the total lipid were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and trans-esterified with 14% BF₃ (v/v) in methanol.

The fatty acid methyl esters (FAMES) were analysed on a Shimadzu-14A model gas chromatograph (GC) (Shimadzu, Japan), equipped with a flame ionisation detector (FID) and fitted with a capillary column (Superlcowax-10 polythene glycol, length -100 m, ID = 0.25 µm) (Sigma-Aldrich Co LLC, St. Louis, MO). Injector and detector temperatures were 200 and 220°C. The oven program was initially held at 60°C for 10 min,

then increased at a rate of 1°C min⁻¹ to 200°C over 10 min and then held at 200°C for 55 min. Total run time was 55 min. The flow rate of the N₂ carrier gas was 1°C min⁻¹. GC analysis of FAMES was repeated three times for each sample. FAMES were identified by comparison of peak retention times to those of standards (NU prep check-SD 461, USA). Samples were run in split mode (50:1). Results were expressed as FID response area as relative percentages of peak area obtained from GC-FID chromatogram. The results are presented as mean±SD (Table 1).

Ratio between hypocholesterolemic and hypercholesterolemic fatty acids (Santos-Silva, *et al.*, 2002) was calculated using the equation:

$$HH = (C18:1n-9+C18:2n-6+C20:4n-6+C18:3n-3+C20:5n-3 + C22:5n-3+C22:6n-6)/(C14:0+C16:0)$$

The basic extraction procedure and fast HPLC method were used as described by Nirungsan and Thongnoppua (2006) to determine the level of tocopherol in freshwater and marine fish.

Means were compared using Tukey's multiple comparison. Data were analysed statistically using one-way analysis of variance (ANOVA), $p < 0.05$ or Student's *t*-test. SAS 9.1.3. was used for statistical analyses.

Results and discussion

All eight varieties of processed fish *viz.*, *P.* (= *L.*) *bindus*, *M. cephalus*, *R. kanagurata*, *C. dorab*, *S. crumenophthalmus*, *K. pelamis*, *S. jello* and *D. acuta* are commonly consumed fish species in Sri Lanka. All fish cooked as curry or fried showed a significantly high level of SFA owing to the presence of lauric acid (C12:0) and palmitic acid (C16:0) in coconut milk and coconut oil, respectively (Table 1). The fatty acid composition of raw (fresh) fish was compared to that of fish curry made with coconut cream and fish fried in coconut oil. The fatty acid composition during the cooking and frying processes are given in Table 1.

Fish fried with coconut oil showed a significant rise in MUFA content in all samples, whereas the omega-3 PUFA (EPA and DHA) content decreased in both fried and curried fish in most samples (Fig.1). Saturated SFA content also showed an increase in both fried and cooked fish in all eight samples and these results are in agreement with those described by Memon *et al.* (2014). The change in omega-3 fatty acid during processing showed significant difference (Table 1) and this may be due to oxidation of omega-3 fatty acid in fried fish than in curried fish. The fatty acid composition of cooked fish showed a decrease in SFA, MUFA and PUFA contents. However, the omega-3 fatty acid content did not

increase in curried samples but decreased in fried samples (Memon *et al.*, 2014). The results show that the major fatty acids among SFAs and MUFAs in each cooked fish sample were palmitic (C16:0) and oleic (C18:1) acids. In addition, linoleic acid (C18:2) and DHA (C22:6) were the predominant PUFAs in both cooked and fried fish (Table 2). The lauric acid (C12:0) content was markedly high in the curry because it was made with coconut cream, which contains a high amount of this fatty acid (45.98 g 100 g⁻¹). In contrast, fried fish did not show a high level of lauric acid (Table 2) because it was oxidised at the frying

stage. Table 3 shows the fatty acid composition of coconut cream and oil, where the lauric acid (C12:0) and myristic acid (C14:0) were found significantly high. These fatty acids were significantly high in fried and curried fish (Table 2).

The omega-3:omega-6 fatty acids ratio has been suggested as a useful indicator of the nutritional value of fish oils. As stated by Kalyoncu *et al.* (2009), this ratio is a key factor for synthesis of hormones known as eicosanoids which is essential for the normal growth and development

Table 1. Polyunsaturated fatty acids (Omega-3 FA) in raw, fried and curried fish

Species	Omega-3 FA (mg 100 g ⁻¹)		
	Raw fish	Fried fish	Curried fish
<i>P. (=L.) bindus</i> (Orangefin pony fish)	23.65±0.73	2.44±0.36	11.27±0.59
<i>M. cephalus</i> (Grey mullet)	15.53±0.42	5.38±0.77	13.20±1.01
<i>R. kanagurata</i> (Indian mackerel)	23.90±0.08	5.40±1.01	24.29±1.49
<i>C. dorab</i> (Wolf herring)	25.04±0.70	6.96±0.92	15.50±0.74
<i>S. crumenophthalamus</i> (Bigeye scad)	23.68±3.12	4.09±1.26	4.35±0.34
<i>K. pelamis</i> (Skipjack tuna)	33.91±2.25	7.61±0.63	15.65±1.16
<i>S. jello</i> (Pickhandle barracuda)	22.82±0.16	5.21±1.13	22.13±1.00
<i>D. acuta</i> (Rainbow sardine)	26.10±0.26	3.97±0.65	8.03±2.60

Data presented as Mean±SD

Table 2. Lipid composition and lipid indices in raw, curried and fried fish

Species	Type of fish	SFA	MUFA	PUFA	Omega-3 -PUFA	Omega-6 -PUFA	PUFA/SFA	Omega-3: Omega6 PUFA	Omega-6: Omega-3-PUFA	SFA : PUFA	AI	TI
<i>P. (=L.) bindus</i>	R	40.99	16.88	31.22	23.65	6.77	0.76	3.49	0.29	1.31	1.02	0.93
	F	42.92	41.27	12.55	2.44	9.81	0.29	0.25	4.02	3.42	0.8	0.89
	C	56.49	21.07	19.11	11.27	7.03	0.34	1.6	0.62	2.96	1.86	0.96
<i>M. cephalus</i>	R	34.91	20.94	15.03	7.29	5.28	0.43	1.38	0.72	2.32	0.91	1.29
	F	43.13	38.37	15.46	5.38	9.38	0.36	0.57	1.74	2.79	0.88	0.91
	C	50.38	16.3	29.15	13.2	15.68	0.58	0.84	1.19	1.73	1.54	0.58
<i>R. kanagurata</i>	R	24.4	18.3	51.2	25.2	26.1	2.1	0.96	1.04	0.48	0.14	0.26
	F	47.48	35.42	13.54	5.4	7.85	0.29	0.69	1.45	3.51	1.13	0.91
	C	32.43	18.96	42.29	23.48	18.31	1.3	1.28	0.78	0.77	0.61	0.56
<i>C. dorab</i>	R	30.18	15.17	25.35	10	15.34	0.84	0.4	2.49	1.04	0.54	1.09
	F	40.76	38.77	19.15	5.21	13.21	0.47	0.76	1.32	2.47	0.75	0.86
	C	37.1	20.52	36.42	22.13	13.63	0.98	0.77	1.3	1.02	0.9	0.65
<i>S. crumenophthalamus</i>	R	42.82	21.78	31.47	23.68	7.28	0.73	3.25	0.31	1.36	0.87	0.9
	F	37.36	37.79	16.82	4.09	11.95	0.45	0.34	2.93	2.22	0.69	0.79
	C	42.43	40.17	14.5	4.35	9.83	0.34	0.44	2.26	2.93	0.75	0.88
<i>K. pelamis</i>	R	28.22	18.25	46.05	33.91	11.22	1.63	3.23	0.31	2.41	0.38	0.62
	F	39.14	38.47	17.54	7.61	9.61	0.45	4.29	0.23	0.31	0.67	0.86
	C	37.35	22.16	33.24	15.65	17.26	0.89	0.23	4.26	1.08	0.91	0.57
<i>S. jello</i>	R	37.87	24.63	25.35	10	15.34	0.67	3.02	0.33	0.61	1.11	1.03
	F	38.72	39.31	19.15	5.21	13.21	0.49	0.79	1.26	2.23	0.67	0.78
	C	41.5	17.18	36.42	22.13	13.63	0.88	0.91	1.1	1.12	1.03	0.65
<i>D. acuta</i>	R	37.87	11.49	32.48	17.24	6.13	0.86	2.81	0.36	1.17	1.06	1.1
	F	38.7	40.96	17.92	3.97	13.5	0.46	0.29	3.4	2.16	0.72	0.85
	C	73.6	8.58	14.21	8.03	5.55	0.19	1.45	0.69	5.18	3.53	0.6

Data presented as mean (mg 100 g⁻¹) and ratio; SFA - Saturated fatty acid, MUFA - Monounsaturated fatty acid, PUFA - Polyunsaturated fatty acid, AI - Atherogenic index, TI -Thrombogenic index, R- Raw fish, F- Fried fish, C - Curried fish

Table 3. Fatty acid profile of coconut cream and coconut oil. Values are Mean±SD (g 100 g⁻¹)

No. of carbons	Fatty acid	Coconut cream	Coconut oil
C6:0	Caproic acid	0.64±0.03	0.33±0.12
C8:0	Caprylic acid	7.97±0.39	5.53±1.21
C10:0	Capric acid	5.36±0.20	4.42±0.37
C12:0	Lauric acid	45.98±0.88	40.86±1.68
C14:0	Myristic acid	20.91±0.17	19.55±0.78
C16:0	Palmitic acid	8.89±0.47	11.91±0.71
C18:0	Stearic acid	4.11±0.49	3.63±0.27
C18:1n9	Oleic acid	5.04±0.45	10.79±0.79
C18:2n6	Linoleic acid	0.95±0.15	2.77±0.20
C18:3n3	Linolenic acid	0.13±0.03	0.13±0.01
C22:0	Behenic acid	ND	0.03±0.01
C24:0	Lignoceric acid	ND	0.06±0.01

ND.: Not determined; C22:6 and C20:5 were not detected in coconut oil and milk

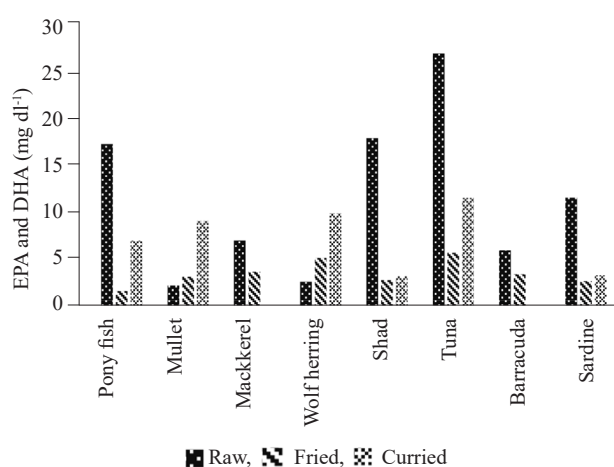


Fig. 1. EPA and DHA content in raw, fried and curried fish.

of the human body. According to current World Health Organisation (WHO) recommendations, the total daily omega-3:omega-6 ratio in the human diet should not be higher than 1.5. Results of the present study reveal that pony fish had the highest omega-3:omega-6 ratio. Of the eight fish species tested, the omega-3:omega-6 ratio, in the descending order (from 4.0 to 0.40), are as follows: Orange fin pony fish > Bigeye scad > Skipjack tuna > Pickhandle barracuda > Rainbow sardine > Grey mullet > Indian mackerel > Wolf herring (Table 2). The omega-3:omega-6 ratio in tissues of marine fishes varies from 5 to 10 and in freshwater fishes from 1 to 4 as stated by Gladyshev *et al.* (2006). The traditional method of Sri Lankan cooking (oil fry and curry) showed omega-3:omega-6 ratio of less than 1 compared to raw fish. The decrease in PUFA:SFA ratio from raw to fried and curried fish and the omega-3:omega-6 ratio in raw fish to fried and curried fish indicated an increase in C20:4n6 and C22:6n6 content (Table 2). The minimum PUFA:SFA ratio is 0.45 as stated by Domingo (2007).

The PUFA:SFA ratio is less than 1 (Table 2) in most fried and cooked fish samples. The Sri Lankan style of fish preparation led to a decrease in overall EPA and DHA content (Fig. 1) which could be interpreted due to two mechanisms that may occur during frying: absorption of culinary fat by the fish and leaching of soluble fat molecules out of the fish. Therefore, it is noted that when an exchange of fat between the fish and culinary fat takes place, loss of specific fatty acids such as EPA and DHA takes place as observed by Sioen *et al.* (2006) that, during frying, a decrease in total fatty acid content in oily fish changed the fatty acid profile of culinary fat. Recommended intake of EPA plus DHA for humans by the WHO is 1 g day⁻¹ (Gladyshev *et al.*, 2006).

The total EPA and DHA to C16:0 ratios is meant as a good index to assess lipid oxidation as stated by Osman *et al.* (2001). Our data showed that processed fish made into curry (particularly mackerel and barracuda) had higher lipid oxidation activities than fried samples. Overall, fish processed into curry had higher hypocholesterolaemic:hypercholesterolaemic (HH) ratios (Table 4). The HH ratio is useful in relating PUFA, considered hypocholesterolaemia. The two saturated fatty acids, namely, myristic acid (C14:0) and palmitic acid (C16:0), are considered hypercholesterolaemic (Testi *et al.*, 2006). Asghari *et al.* (2012) showed that HH value in fried samples increased while decreased significantly in cooked samples. The HH index was higher in all processed fish (fried and curried) compared to raw fish in these findings. Testi *et al.* (2006) found higher values of HH in fish, varying from 2.03 to 2.46. The present study showed that the HH ratio in fried and curried fish ranged from 1.12 to 1.84 (Table 4) and revealed a decrease in cholesterol levels. The content of health beneficial FAs (EPA and DHA) decreased significantly by the traditional cooking methods. By using coconut oil in frying and coconut cream in curry, the nutritional quality decreased

Table 4. Hypocholesterolaemic:hypercholesterolaemic fatty acid (HH) ratio in raw, fried, and curried fish

Fish	Raw (HH)	Fried (HH)	Curried (HH)
Orangefin pony fish	0.68	1.30	0.70
Grey mullet	0.51	1.22	1.41
Indian mackerel	4.17	1.12	2.05
Wolf herring	0.55	1.25	1.72
Bigeye scad	0.86	1.55	1.31
Skipjack tuna	1.32	1.29	1.84
Pickhandle barracuda	0.96	1.56	1.33
Rainbow sardine	0.24	1.41	0.5
R value	-0.5	-0.45	0.64
p value	0.21	0.26	0.09

compared to raw fish, and the content of health beneficial fatty acids and tocopherol was significantly lowered in processed fish (Fig. 2).

The high tocopherol content of raw fish reduced upon cooking in coconut milk, but the reduction was lower when the fish was fried in coconut oil (Fig. 2). This may be due to the presence of plant tocopherol in coconut oil. Tocopherol is a vital nutrient, but it is unstable and can be lost during processing, heating and cooking. An interrelated series of reactions, including hydrolysis, oil oxidation and polymerisation of fat molecules can occur (Saguy and Dana, 2003). Our results show that tocopherol content was markedly high in raw grey mullet (2322 $\mu\text{g } 100 \text{ g}^{-1}$), wolf herring (1468 $\mu\text{g } 100 \text{ g}^{-1}$), skipjack tuna (1084 $\mu\text{g } 100 \text{ g}^{-1}$), Indian mackerel (470 $\mu\text{g } 100 \text{ g}^{-1}$) and rainbow sardine (980 $\mu\text{g } 100 \text{ g}^{-1}$). Fried mackerel (1711 $\mu\text{g } 100 \text{ g}^{-1}$) and rainbow sardine (1410.87 $\mu\text{g } 100 \text{ g}^{-1}$) showed markedly high level of tocopherol. Tocopherol content in fried grey mullet was reduced by half compared to raw fish (923.45 $\mu\text{g } 100 \text{ g}^{-1}$). Curry prepared from all fish samples showed very low levels of tocopherol ranging

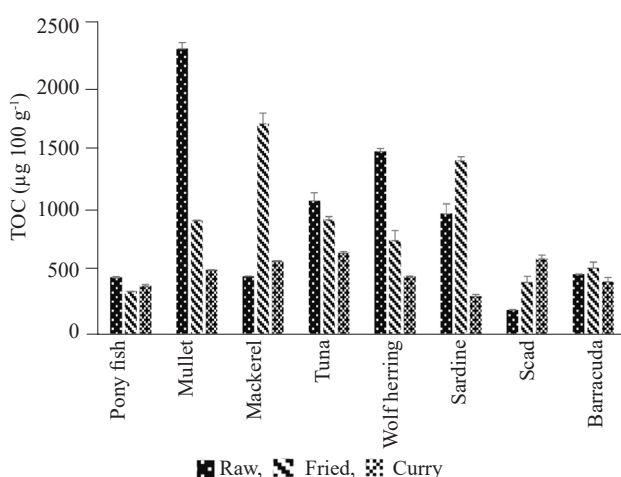


Fig. 2. Tocopherol content in raw, fried and curried fish. TOC Tocopherol

from 468.88 to 661.29 $\mu\text{g } 100 \text{ g}^{-1}$ (Fig. 2). In contrast, the tocopherol content of fried Indian mackerel and rainbow sardine increased significantly, which could be because of the coconut oil and other ingredients added for marination particularly turmeric powder. Our results agreed with those obtained by Kulas *et al.* (2002), which showed that the total tocopherol content varied in raw and cooked fish. In our results, tocopherol content was low in all curried and fried fish except Indian mackerel and rainbow sardine (Fig. 2).

It has been reported that α -tocopherol is lost due to hot oven-cooking followed by refrigeration for 7 days in trout fed high levels of dietary vitamin E. But, hot smoking showed no effect on the α -tocopherol content of smoked fillets compared to that of raw fillets. Similarly, heating processes such as boiling, grilling and frying significantly decreased α -tocopherol content of fish meat (Gotoh, *et al.*, 2011). Loss of tocopherol (vitamin E) content is positively related to lipid degradation, which is affected by cooking temperature, cooking time and oxidative conditions (Wyatt, *et al.*, 1999). Raw horse mackerel has higher vitamin E (tocopherol) content than fried and grilled fish, whereas cooked seabass and hake contains considerably higher vitamin E levels than raw fish (Dias, *et al.*, 2003).

It is concluded that the lipids in fish fillets play an important role in providing taste, flavour, smell and texture to the fish. Our results show that fatty acid compositions changed significantly during fish preparation by either cooking in coconut cream or frying in coconut oil and resulted in lower nutritive values than raw fish. Particularly, the healthy lipids EPA and DHA were lost during fish preparation. Likewise, tocopherol being heat sensitive, showed significant loss after both cooking methods. The H/H ratio significantly increased in fried fish, adding to the evidence that consuming fried fish is less beneficial to human health than eating fish cooked in coconut cream.

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