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Factors Affecting Vitamin D Content in Sardine, Salmon and Shrimp during Different Cooking Processes

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ABSTRACT

This study intended to examine how food composition and cooking process impacted the post-cooking vitamin D content of farmed salmon, local sardine and shrimp. The edible portions were cooked using three different methods: frying, oven cooking and grilling. Before and after cooking, vitamin D, moisture and oil contents, as well as antioxidant activity, were determined. The findings indicate that local sardine exhibited a significantly higher concentration of vitamin D (49.58 μg/100 g) than farmed salmon (24.86 μg/100 g) on a dry basis. Moreover, oven cooking and grilling were found to be more effective methods of preserving vitamin D content. A significant relationship between the vitamin D content and antioxidant activity of food samples was also observed (r=0.8144, p<0.05). Furthermore, an inverse correlation was noted between vitamin D content and the cooked samples' internal temperature as well as moisture and oil contents (r=-0.2652, r=-0.4158 and r=-0.2334, respectively). These results suggest that internal temperature, moisture content and antioxidant capacity have a significant impact on certain foods' vitamin D content during cooking. Further investigations are warranted to explore the potential of antioxidant addition in stabilising vitamin D during thermal food processing.

KEYWORDS
antioxidants, cooking methods, fish, stability, temperature, treatments

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1. Introduction

Vitamin D deficiency has been correlated to a variety of harmful diseases, including bone disease, diabetes mellitus, hypertension, cardiovascular diseases, autoimmune diseases, infectious diseases and cancer (Iyengar *et al.*, 2022). Globally, vitamin D deficiency is so common that it is regarded as a pandemic and a serious health concern. Between 30% and 93% of people worldwide are vitamin D deficient (Hussein *et al.*, 2022). Fish, especially fatty ones, fish oil and fish products are considered the main dietary sources of vitamin D3 (Kamel *et al.*, 2022; Schmid & Walther 2013). Moreover, cooking can cause significant vitamin loss (Gelaye, 2023). Previous studies have examined vitamin D retention in mushrooms, margarine, fish, bread and eggs (Jakobsen & Knuthsen, 2014; Loˇznjak & Jakobsen, 2018). They concluded that the type of food and cooking method affected vitamin D preservation and stability and could result in significant losses. Due to the scarcity of vitamin D3 sources and the numerous factors that influence endogenous production and often lead to deficiency symptoms, it is therefore important to minimise vitamin D losses during cooking procedures. Ložnjak and Jakobsen (2018) determined the extent of vitamin D2 retention in sunflower oil and mushrooms and vitamin D3 in rainbow trout. The cooking methods considered included blanching for 8–20 minutes at 90°C, using different pH levels, steaming for 6 minutes at 86°C, microwave cooking, frying and oven baking for 10–30 minutes at 110–220°C. Their results revealed that vitamin D retention in mushrooms ranged from 62% to 88%. They also found that while high temperatures reduced vitamin D retention, lemon juice addition had the inverse effect. Moreover, Sobral *et al.* (2018) investigated how cooking fish affected the fatty acid profile and retention of fat-soluble vitamins (A, E and D). They cooked cod and salmon fillets using three different methods: (a) frying with rapeseed oil for 4–7 minutes at 180°C, (b) baking for 20–30 minutes at 200°C and (c) steaming. Their findings revealed a clear impact of frying on fatty acids in cod and salmon. Also, monounsaturated fatty acids increased, while saturated fatty acids and polyunsaturated fatty acids decreased. Vitamins A, E and D remained stable during baking and steaming, but vitamin D3 content in cod and salmon decreased during frying. In particular, this research examines the relationships between vitamin D levels and cooking conditions, as well as the chemical composition of cooked sardine, farmed salmon and shrimp during cooking, to see if it could help consumers get a sufficient amount of dietary vitamin D.

2. Materials and Methods

2.1. Materials:

About three kilos of fresh samples of farmed salmon (each weighing 430±10.33 g), local sardine (each weighing 187±6.24 g) and shrimp (each weighing 11.62±1.81 g) were purchased from a large store in Al Ahsa, Saudi Arabia, and transferred to the experimental lab within 30 minutes while kept in ice boxes. The preselected samples were promptly cleaned, and the edible parts were then prepared using usual kitchen techniques for frying, grilling and oven cooking. Table 1 shows the time and internal temperature used in different cooking methods. No oil was added during grilling and oven cooking, while the sunflower oil used during frying was not vitamin D-fortified.

Table 1: Internal temperature (°C) of samples and time (min) used in the cooking methods.

	Internal temperature and time for each cooking method		
	Frying	Oven cooking	Grilling
Salmon	136°C/10min	71°C/20min	70°C/10min
Sardine	141°C/10min	65°C/30min	56°C/15min
Shrimp	140°C/10min	74°C/30min	70°C/12 min

2.2. Chemical Analysis:

2.2.1. Moisture and Fat Content

The moisture and fat contents were determined according to the AOAC and Latimer (2012). The data were expressed on a dry weight basis (Db).

2.2.2. Antioxidant Capacity (AOC)

Free radical scavenging capacity against DPPH (2,2-diphenyl-2

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picrylhydrazyl) was estimated in selected samples (Zhang & Hamauzu, 2004). One ml of extract was mixed with one ml of DPPH (0.4 mmol). The mixture was kept in the dark for 30 minutes before measuring its absorbance at 516 nm using a UV-VIS spectrophotometer, Apel, Japan.

2.2.3. Cooking Loss After Cooking

The percentage of cooking loss was calculated according to the formula proposed by Küçüközet and Uslu (2018). Cooking Loss % = [(W0 –W1)/W0] x 100

Where: W0 and W1 = weights before and after cooking, respectively.

2.2.4. Determination of Vitamin D3

Huang *et al.*'s (2020) method was used to estimate vitamin D3 as cholecalciferol. The sample weighing 10 g was saponified with 100 mL methanolic KOH (39%) and extracted using 200 mL n-Hexane/L diethyl ether. The extract was evaporated, and the final clean-up step involved semi-preparative HPLC before injection. In the HPLC, 5µL of extract (methanol/water 92:8) was injected (Agilent 1260 infinity series LC system). Vitamin D3 was separated and detected using Agilent ZORBAX C18- HT Eclipse PAH column and diode array detector.

2.3. Statistical Analysis:

The Gen-Stat Release 7.2 (PC/Window XP) program was used to define the significance of sample differences at $p \leq 0.05$ significance levels. Microsoft® Excel 2016 software (Microsoft Office software package, Microsoft Corporation, Redmond, WA, USA) was also used to assess the correlation coefficients.

3. Results and Discussion

3.1. Moisture Content:

Table 2 depicts the moisture content of fresh selected samples and the impact of commonly used cooking methods on the moisture content of sardine, salmon and shrimp. The moisture contents in the fresh salmon, sardine and shrimp samples were 63.17%, 64.61% and 83.11%, respectively. Cooking in different ways resulted in a significant decline in moisture content, especially at higher temperatures such as in frying. Frying significantly decreased moisture content more than oven cooking or grilling. The highest reduction in moisture content was observed in fried shrimp at 27.52%. Fried salmon and sardine had 12.09% and 10.97% moisture content reductions, respectively, when compared to fresh samples.

Table 2: Moisture content of fresh and cooked sardine, salmon and shrimp.

	Moisture content %			
	Fresh	Fried	Oven cooked	Grilled
Salmon	63.17 ± 0.11^c	55.53 ± 0.03^c	61.90 ± 0.04^b	60.38 ± 0.24^c
Sardine	64.61 ± 0.05^b	57.52 ± 0.10^b	58.35 ± 0.12^c	61.11 ± 0.10^b
Shrimp	83.11 ± 0.11^a	60.24 ± 0.09^a	67.67 ± 0.10^a	71.50 ± 0.08^a

Means of triplicate ± standard deviation; Different letter within each row indicates a significant difference ($p<0.05$)

The moisture contents of oven-cooked and grilled samples were found to be significantly lower ($p<0.05$) than that of fresh samples and higher than that of fried samples. Cooking has been observed to reduce moisture content depending on the cooking temperature needed; higher cooking temperatures increased water evaporation, resulting in a decrease in moisture content. Furthermore, the moisture content may vary depending on the fishing season, nutrition type and dealing method. The moisture content of farmed salmon obtained in this study was lower (71.6%) than that of salmon fillets obtained by Choo *et al.* (2018). In line with our findings, earlier studies have shown that frying is more likely to induce moisture loss than other cooking methods (Abraha *et al.*, 2018; Sridonpai *et al.*, 2022). A negative correlation was found between moisture content and cooking method temperature (r=-0.5054) (Table 6).

3.2. Fat Content:

Table 3 shows that fresh salmon has the highest fat content at 42.2% (dry weight [DW]), making it a fatty fish. This percentage decreased significantly as a result of different cooking methods. This may be due to salmon oil loss during various cooking methods, particularly frying. Frying induced the greatest amount of salmon oil loss (9.64%), followed by oven cooking (6.25%) and then grilling (2.59%). Notably, salmon samples lost 33.31% of their weight during frying, as shown in Table 4.

Despite such a loss, sardine's oil content was still lower than that of salmon (22.35% DW). On the one hand, the frying process increased the sardine's oil level by 5.01%. On the other hand, oven cooking and grilling reduced its fat content by 23.53% and 10.11%, respectively. Furthermore, shrimp, which initially had a lower oil content (8.34% DW) than salmon and sardine, showed a significant increase in oil content (11.02%) after frying. This increased oil uptake could be attributed to its higher moisture content. Pravinkumar *et al.* (2015) found that sardine oil content ranged from 7.83% to 16.5% (wet base) in different seasons, which is consistent with this study's fresh sardine oil content (7.91% wet base). They also confirmed that fish frying results in a high percentage of fat, but this varies depending on the type of fish. Gulzar *et al.* (2020) discovered that shrimp's fat content ranged between 2.17% and 6.88%, which is lower than this paper's findings.

Table 3: Fat content of fresh and cooked sardine, salmon and shrimp.

	Fat content % (Db)			
	Fresh	Fried	Oven cooked	Grilled
Salmon	42.22±0.28a	38.15±0.29d	39.58±0.10c	41.13±0.11b
Sardine	22.35±0.44b	23.47±0.24a	17.09±0.19d	20.09±0.24c
Shrimp	8.34±0.17d	11.02±0.48b	12.48±0.35a	10.65±0.04c

Means of triplicate ± standard deviation; Different letter within each row indicates a significant difference ($p<0.05$)

Previous studies (Abraha *et al.*, 2018; Ansorena *et al.*, 2010; Bastías *et al.*, 2017; Karimian-Khosroshahi *et al.*, 2016) underscored that cooking fatty fish may cause loss of their natural fats, which was evident in the salmon used in this study. According to Zotos *et al.* (2013), frying increased sardine's fat content. The oil uptake of fried sardine and shrimp may be due to the 'water–oil replacement' theory, which states that moisture loss during frying forms food gaps that are easily filled with oil, leading to increased fat in fried samples (Castillo *et al.*, 2021; Xu *et al.*, 2020).

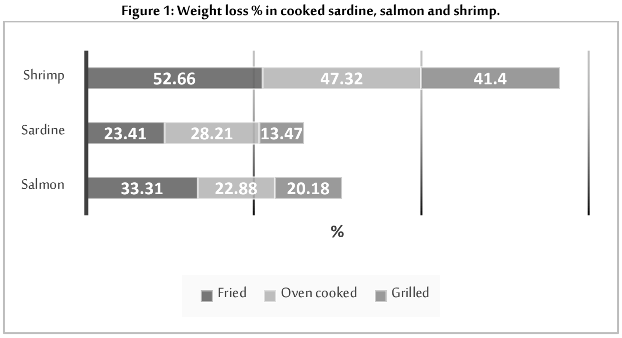
3.3. Cooking Loss Percentage:

Figure 1 shows the cooking loss percentage after different cooking methods were employed. Frying caused the greatest cooking loss percentage in cooked samples, while grilling caused the least. Shrimp had the highest cooking loss percentage, with 56.00%, 47.32% and 41.4% after frying, oven cooking and grilling, respectively. In contrast, grilled sardine had the lowest rate at 13.47%. Shrimp's high moisture content (83.11%) seemed to be the main cause of its high cooking loss percentage. In salmon's case, its cooking loss percentage may be attributed to its fat and moisture contents, as previously mentioned. When compared, sardine lost less weight than salmon because its fat and moisture content loss was lower. Cooking time, temperature, surface contact as well as moisture and fat contents may all play a role in weight loss. Sobral *et al.* (2018) found that in comparison to other methods, frying had the most influence on food weight, with impacts including greater loss of moisture, fat content and quality changes, and a stronger influence on some amino acids.

3.4. Antioxidant Capacity Percentage:

Table 4 shows that fresh sardine's antioxidant activity was significantly higher (66.14%) than that of fresh salmon (64.87%); shrimp had the lowest antioxidant activity (61.32%). After frying the salmon and shrimp, the antioxidant activity increased significantly. Excluding salmon, oven-cooked and grilled sardine and shrimp exhibited higher antioxidant activity compared to their fresh counterparts.

Figure 1: Weight loss % in cooked sardine, salmon and shrimp.



The cooking processes and moisture content of the selected samples in this study may affect their antioxidant activity. Furthermore, because sardine was cooked at a lower temperature than shrimp and salmon, it may have had a higher antioxidant activity (Alamir *et al.*, 2021; Kamel *et al.*, 2022). There are currently few studies on the various antioxidants found in fish. Still, it has recently been reported that vitamin D is one of the various antioxidants that can protect polyunsaturated fats and other components in fish and humans (Alamir *et al.*, 2021; Olubukola Sinbad *et al.*, 2019; Tagliaferri *et al.*, 2019).

Table 4: Antioxidant capacity % of fresh and cooked sardine, salmon and shrimp.

	Antioxidant capacity %			
	Fresh	Fried	Oven cooked	Grilled
Salmon	64.87 ± 0.75^{b}	66.30 ± 1.27^{a}	63.70 ± 0.87^{b}	64.87 ± 1.27^{b}
Sardine	66.14 ± 0.80^{a}	67.14 ± 0.57^{a}	68.57 ± 0.42^{a}	68.57 ± 0.69^{a}
Shrimp	61.32 ± 0.97^{c}	65.56 ± 0.27^{a}	63.23 ± 1.28^{b}	62.65 ± 0.24^{b}

Means of triplicate ± standard deviation; Different letter within each row indicates a significant difference ($p<0.05$)

3.5. Vitamin D Content:

Table 5 presents the effect of different thermal processing (frying, oven cooking, grilling) on the vitamin D content of sardine, salmon and shrimp on a dry base. Sardine and shrimp had high vitamin D content (49.58 and 56.39 µg/100 g, respectively, on a dry base). In comparison, farmed salmon contained 24.86 µg/100 g. The frying process significantly reduced vitamin D content in all samples. Fried shrimp had the greatest reduction (38.92%), while fried salmon had the least (5.31%). Oven cooking and grilling, nonetheless, could preserve vitamin D content in both sardine and salmon. In particular, oven-cooked and grilled sardine had the highest vitamin D content among the cooked samples (50.3 and 51.12 µg/100 g, respectively) and thus seemed to be a good source of the vitamin. Shrimp had the greatest reduction in vitamin D content compared to the other samples; the extensive contact of the shrimp's surface area with high temperature may be responsible for this effect. Thus, the cooking method, the extent of the surface area in contact with oil or the sample's type and internal temperature may all significantly impact the vitamin D content of cooked food. In addition, as vitamin D is a fat-soluble vitamin, the decrease in its content after frying may be due to the following: (a) the exchange of fat with cooking oil, (b) a drop in fat content (as found in salmon), (c) the leaching of the vitamin into the oil during frying or (d) the vitamin's degradation due to high temperature (136–141°C) (Table 3). This study's results regarding salmon's vitamin D content were consistent with the research done by Ostermeyer and Schmidt (2006), which reported a range of 7.8–8.5 µg/100 g for fresh salmon. In comparison, Edouard and Paquotte (2008) revealed that salmon's vitamin D content in their study was 15 µg/100 g. This paper found that the frying process led to a high reduction of vitamin D levels in the selected samples, which may be attributed to either a loss in the natural oil content or to higher temperatures used in frying compared to grilling and oven cooking; exposure to these high temperatures can lead to the degradation (Jakobsen and Knuthsen, 2014; Omotosho *et al.*, 2015) or leaching of the vitamin into the cooking oil during frying (Al-Khusaibi *et al.*, 2023). In addition, Karimian-Khosroshahi *et al.* (2016) found that baking or microwaving rainbow trout did not affect its vitamin D content while frying resulted in the greatest reduction.

Table 5: Vitamin D content of fresh and cooked sardine, salmon and shrimp.

	Vitamin D3 µg/100 g (Db)			
	Fresh	Fried	Oven cooked	Grilled
Salmon	24.86 ± 1.95^{a}	23.54 ± 2.06^{b}	25.62 ± 0.58^{a}	25.53 ± 1.11^{a}
Sardine	49.58 ± 2.49^{a}	32.50 ± 2.25^{b}	50.30 ± 4.86^{a}	51.12 ± 0.36^{a}
Shrimp	56.39 ± 2.50^{a}	34.44 ± 0.61^{c}	36.23 ± 0.96^{b}	37.41 ± 0.73^{b}

Means of triplicate ± standard deviation; Different letter within each row indicates a significant difference ($p<0.05$)

3.6. Correlation Between Certain Parameters and Vitamin D Content of Sardine, Salmon and Shrimp:

Correlation analysis was performed to compare the vitamin D content, moisture, fats and antioxidant capacity of salmon, sardine and shrimp, as well as the cooking time and internal temperature of the cooked samples (Table 6). The strongest correlation was perceived between antioxidant capacity and vitamin D content ($r=0.8144$, $p<0.05$), suggesting that more research is needed to investigate the effect of antioxidant addition to vitamin D-rich foods during cooking on vitamin D stability. According to Kamel *et al.* (2022), there is a relationship between antioxidant capacity and vitamin D content in some processed fish as well as an inverse association between antioxidant capacity and fat content. This study found an inverse correlation between vitamin D content and temperature ($r=-0.26523$, $p<0.05$), which was evident during the frying process; thus, a low cooking temperature is recommended to preserve the vitamin in vitamin D-rich foods. Based on past research, cooking temperature has a negative effect on vitamin D stability (Kamel *et al.*, 2022; Sridonpai *et al.*, 2022). Surprisingly, fat content was also found to have a reverse correlation with vitamin D content ($r=-0.23346$, $p<0.05$). In this study, an increase in antioxidant activity, a decrease in cooking temperature and the level of fat content or uptake seemed to be the most important factors affecting the selected foods' vitamin D content.

Table 6: Correlation between vitamin D content and some characteristics of cooking methods and food composition.

	Vitamin D	Temperature	Time	AOC
Vitamin D*	1			
Temperature**	-0.2652	1		
Time	0.42828	-0.5359	1	
AOC	0.8144	0.12704	0.00502	1
Moisture	-0.4158	-0.5054	0.23	-0.7853
Fats*	-0.2334	-0.0298	-0.3176	0.0904

* On wet base, ** Internal temperature and AOC

4. Conclusion

The results obtained suggest that grilled and oven-cooked sardine is a potential source of vitamin D and should be consumed as a vitamin D-rich food. It was also found that the frying process significantly decreased the vitamin D content in all samples. The greatest reduction was observed in fried shrimp (38.92%), while the least in fried salmon (5.31%). Moreover, cooking temperature, rather than time, was discovered to influence vitamin D concentrations in cooked samples. Notably, excluding salmon, oven-cooked and grilled sardine and shrimp showed an increase in antioxidant activity compared to their fresh counterparts; this indicates that antioxidant activity may be critical in vitamin D retention. Antioxidant capacity, moisture content and cooking temperature were the main factors affecting vitamin D retention after different cooking methods were employed. Further research is needed to confirm that fatty fish are not always the richest source of vitamin D and to examine the effect of antioxidant addition during cooking on vitamin D retention.

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