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Effect of Garlic Paste Addition on the Fatty Acid Profile and Oxidative Stability of Shrimp Analogue Fortified with PUFA-rich Fish Oil During Frozen Storage at -20°C

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Abstract

The current study aimed to develop a shrimp analogue product fortified with PUFA-rich fish oil and improve its oxidative stability during five months of frozen storage at -20°C. The shrimp analogue, prepared from pink perch (*Nemipterus japonicus*) surimi, was added with 2.5% cod liver oil and 4% starch (1:1 ratio of native and modified potato starch). The product was prepared with and without antioxidants. Garlic (*Allium sativum* L.) paste (2 g/100 g surimi) as the natural antioxidant, and butylated hydroxyanisole (BHA) (0.02% of the weight of the cod liver oil) as the synthetic antioxidant. The lipid oxidation indices such as PV and TBARS were monitored monthly. Garlic paste significantly ($p \leq 0.05$) reduced lipid oxidation compared to the control, while BHA was the most effective antioxidant. The fatty acid profiling by GC-MS revealed that the predominant fatty acid in all three samples was oleic acid (18:1, n-9), the monounsaturated fatty acid (MUFA). The dominant saturated fatty acids (SFAs) were myristic acid (14:0), palmitic acid (16:0), and stearic acid (18:0) while important n-3 PUFAs were EPA (20:5, n-3), DHA (22:5, n-3) and α-linolenic acid (18:3, n-3). After 150 days of frozen storage, the ΣPUFA/ΣSFA ratio was highest in BHA (1.03) and garlic (0.90) added samples, compared to control (0.85). The addition of garlic positively influenced the fatty acid profile and reduced lipid oxidation leading to superior nutritional quality for the shrimp analogue.

Keywords: Surimi, antioxidant, lipid oxidation, imitation shrimp

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Introduction

Shrimp is a major item in the seafood diet around the world, which is preferred for its unique taste, ease of preparation, and consistent quality (Haridevamuthu et al., 2024). The increasing demand for shrimp, coupled with its limited availability, has led to the development of shrimp analogues or shrimp-like products containing a percentage of shrimp. Surimi is the main ingredient in seafood analogue products like shrimp analogues and imitation crab claws, which is concentrated myofibrillar protein obtained after washing and stabilizing deboned fish mince with cryoprotectants (Zaghbib, Felix, Romero, Arafa, & Hassouna, 2016). Surimi-based shrimp analogue products are designed to mimic the chewiness, cohesiveness, flavour, taste, and nutritional content of real shrimp, while also replicating its shape, colour, and appearance (Sun, Sun, Thavaraj, Yang, & Guo, 2017). According to the Marine Products Export Development Authority (MPEDA) report, the export of surimi and surimi analogues from India ranked as the fifth most important item, with a quantity of 135,327 MT. This marked a 4.12% increase in quantity and generated ₹ 2,414.43 crores (US$294.43 million) in the financial year 2023-24.

One limitation of surimi-based products is their low content of nutrients such as desirable n-3 polyunsaturated fatty acids (n-3 PUFAs), which have significant health benefits such as anticholesterolemic and antithrombotic effects (Yagi et al., 2017). To address this, shrimp analogues prepared from surimi can be fortified with n-3 PUFA-rich oils. Capsules of fish oil supplements invariably add to the overall daily energy intake and for many people, this is not desirable. So, an alternative way to ensure an optimal n-3 PUFA intake is needed. Fortification of foods with fish oil is one option for increasing dietary intake of n-3 PUFAs. The incorporation of

oils is highly desirable to improve the texture, nutritional quality and flavour of surimi-based products (Shen et al., 2024). Fish oil is a rich source of n-3 PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (He et al., 2022), which are known to have cardioprotective effects and growth-promoting efficiency in children.

Commercial surimi and surimi-based products are generally stored in frozen condition before they reach the market (Jia et al., 2019). However, the inclusion of n-3 PUFAs presents challenges as they are highly unstable and are prone to oxidation (Kunyaboon, Thumanu, Park, Khongla, & Yongsawatdigul, 2021). This can lead to off-flavour development and quality deterioration of surimi during long-term frozen storage. Also, a high consumption of saturated fatty acids (SFA) is linked with elevated serum cholesterol levels and is strongly correlated with coronary death rates. Increasing the dietary ratio of PUFA to SFA has been recommended for the prevention of cardiovascular diseases (CVDs) (Kang, Shin, Park, & Lee, 2005). Hence, preventing fat oxidation and attaining an appropriate shelf-life for n-3 PUFA-enriched products requires meticulous attention to various factors such as the quality of the oil added, the processing conditions employed, the inclusion of antioxidants, as well as the packaging and storage conditions. The addition of synthetic antioxidants is a common approach in the seafood industry to maintain the lipid stability and shelf-life of surimi and surimi products. Recently, there has been a trend towards the use of more natural antioxidants, such as those found in herbs and spices, instead of synthetic antioxidants (Wu et al., 2022; Jannat-Alipour, Rezaei, Shabanpour, Tabarsa, & Rafipour, 2019). Consumers don't prefer many of the more effective synthetic antioxidants because of concerns over their potential health risks and toxicity (Pourmollaei, Nouri, Jafarpour, & Mokhtarpour, 2021). The commonly used commercial synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxy anisole (BHA) have been associated with potential carcinogenic effects on the urinary bladder at high doses (Sun et al., 2017). This underscores the need to explore natural alternatives to synthetic antioxidants. Considering all these, the present study aimed to develop a shrimp analogue product fortified with PUFA-rich cod liver oil and assess its oxidative stability during frozen storage, when added with natural and synthetic antioxidants. Garlic contains several organosulfur compounds and is known for its significant antioxidant and antibacterial properties and thus has been used for medicinal purposes by many populations around the globe (Majumdar et al., 2015). In this study, garlic (*Allium sativum* L.) paste was used as a natural antioxidant and the synthetic antioxidant, butylated hydroxy anisole (BHA), was used for comparison.

Materials and Methods

Frozen surimi prepared from Pink Perch (*Nemipterus japonicus*) was obtained from a reputed processing facility in Taloja, Maharashtra, India. It contained 4% commercial cryoprotectant mix (sucrose/sorbitol, 1:1 ratio). After reaching the laboratory, 1 kg blocks of surimi were vacuum-sealed and stored at −20°C until use. Both native and hydroxypropylated potato starches were sourced from the Jaisheel Brothers (Vile Parle, Maharashtra, India). Butylated hydroxyl anisole (BHA) was purchased from Merck (India). Other food-grade ingredients included paprika colourant (E160c), shrimp flavour (Ms Bio Co., Ltd., Busan, Korea), glycine (E640), and sodium tri-polyphosphate (E451). Cod liver oil was acquired from a chemist in Mumbai, India, while other ingredients like salt, sugar, vinegar, and shrimp were purchased from local retail shops.

The surimi blocks of 1 kg weight, which were partially thawed at 0°C for 10h, were chopped in a food processor (Philips, India) at low speed and mixed for 1 minute. The mixture was then combined with 2.5% salt and 0.2% sodium tri-polyphosphate and mixed for another minute. Following this, 1% sugar, 0.5% acetic acid, 0.4% glycine, and 2.5% cod liver oil were added. To ensure the oxidative stability of PUFA-fortified shrimp analogue, natural and synthetic antioxidants were added. The antioxidants used were garlic paste (GAR) at 2g/100g surimi, and butylated hydroxy anisole (BHA) at 0.02% of the weight of the cod liver oil. The surimi base material without any antioxidants was named CON (control). The base material was mixed with 4% starch (native and modified potato starch in a 50:50 ratio). This mixture was further blended for 2 minutes, stuffed into plastic casings (2.5cm diameter, 15 cm length), and refrigerated at < 5°C for 10h to cold set. The stuffed surimi mixture was then heat-set in a 50°C water bath for 20 minutes, followed by 3 minutes in a 90°C water bath. After cooling, it was sliced into filaments and mixed with 25% unheated surimi paste, 25% cooked shrimp paste (shrimp boiled for 10 minutes and ground),

and 5% shrimp flavour for 1 minute. The paste was moulded into shrimp shapes using plaster of Paris mould (10 cm in length and 2.5 cm in width) and 1% paprika colour was applied manually. The shrimp analogue was heat-set at 100°C for 5 minutes in a water bath, cooled, and frozen in an air blast freezer (Tecnomac, Italy) for 60 minutes to reach an internal temperature of –18°C. The samples were then stored at –20°C and drawn randomly at regular intervals for quality evaluation.

Extraction of lipids from the sample was done by the Folch method (Folch, Lees, & Stanley, 1957) with slight modification. The extracted lipid underwent Fatty Acid Methyl Ester (FAME) preparation and the fatty acid profile was analyzed using GC-MS. The AOAC (AOAC, 2009) method was followed to esterify the lipid extract. Fatty acids were separated using a Shimadzu Qp2010 quadrupole Gas Chromatography-Mass Spectrometer (GC-MS) instrument equipped with a carbowax (30m × 0.25mm ID; 0.25μm film thickness) capillary column (Cromlab S.A.). Helium was used as the carrier gas. Injector and detector temperatures were set at 250°C. Injection was performed in split mode (1:15). The column temperature was programmed initially at 50°C for 2 minutes and then increased at a rate of 10°C per min to a final temperature of 230°C. The FAM esters were separated at constant pressure (23.1 kPa) and peaks were identified by comparing the mass spectra with the mass spectral database. The fatty acid (FA) composition of the samples was expressed as a percentage of the total FAs. The sum of total saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), and ΣPUFA/ΣSFA ratio were calculated with the results of fatty acids.

The modified International Dairy Federation method (IDF, 1991) was used in this study to determine the peroxide value of the samples. The IDF method for peroxide determination is a spectrophotometric method that is based on the ability of the peroxides to oxidise ferrous ions to ferric ions. The results are expressed as meq of O_2 per kg fat.

The Thiobarbituric acid reactive substances (TBARS) value was measured by the method described by Tarladgis, Watts, Younathan, and Dugan (1960), which is a spectrophotometric evaluation of malondialdehyde (MDA). The results are expressed in mg malonaldehyde/kg sample.

The sensory quality of the shrimp analogue samples was evaluated monthly by 10 trained panellists by the method of Meilgaard, Carr and Civille (1999). The overall acceptability of the samples was determined by assessing the appearance, colour, odour, taste, texture, and flavour and a score was given based on a 9-point hedonic scale (1 for dislike extremely to 9 for like extremely). A sensory score of 6 and above was considered good, while a score below 5 was considered poor. Before serving, samples were heated in a microwave oven (700 W) for 5 minutes, and panellists were provided with a glass of water to cleanse their palate.

The data were analyzed using the Statistical Package for Social Sciences (SPSS) software, version 29, and the results are expressed as mean ± standard error. A one-way analysis of variance (ANOVA) was performed to analyze the data. The level of significance was set at $p \leq 0.05$. Duncan's Multiple Range Test (DMRT) was used to determine the significant differences between the treatments and storage periods.

Results and Discussion

Regardless of the obvious benefits of n-3 PUFAs, consumption of foods containing adequate levels of n-3 PUFAs tends to be very low. Fortification of food products with n-3 PUFA by fish oil addition may slightly improve the fatty acid (FA) profile and level of PUFA in the diet and tissues of the human body. The FA profile of shrimp analogues with and without antioxidants during frozen storage analysed by GC-MS is shown in Tables 1, 2, and 3. The predominant fatty acid in all three samples was the monounsaturated fatty acid (MUFA), oleic acid (18:1, n-9), which ranged from 23.22 to 23.68% in different shrimp analogue samples at the beginning of frozen storage. The dominant saturated fatty acids (SFAs) were myristic acid (CH_3 $(CH_2)_{12}COOH$, 14:0), palmitic acid (CH_3 $(CH_2)_{14}COOH$, 16:0), and stearic acid (CH_3 $(CH_2)_{16}COOH$, 18:0) while the most significant PUFA was linoleic acid (18:2, n-6).

Most important n-3 PUFAs were EPA (20:5, n-3), DHA (22:5, n-3) and α-linolenic acid (18:3, n-3). There was a decrease in the EPA and DHA content of all the samples during frozen storage. However, the highest initial and final values of EPA and DHA were shown by shrimp added with BHA, the synthetic antioxidant and lowest by the control sample. The EPA and DHA levels in the garlic paste-added samples were intermediate compared to the levels in the other two groups, both at the beginning and end of the study. The levels of EPA in the control

sample at the start and end of the frozen storage trial were 5.75±.001% and 4.01±.007%, respectively while it was 5.80±.003% and 4.40±.003% in GAR samples. At the end of 150 days of frozen storage, the DHA content was 5.25±.000% and 5.45±.001% in CON and GAR samples, respectively. The EPA and DHA levels in BHA shrimp analogues at the end of the frozen storage study were 5.00±.003 and 6.60±.003%. The changes in the fatty acid profiles give a clear idea about the effectiveness of the antioxidants against oxidation. The higher content of EPA and DHA in GAR and BHA samples can be credited to the protective action of the antioxidants. Garlic naturally contains many antioxidant molecules, including flavonoids and thiosulfinates, which are chemically reactive and can easily lead to the formation of organosulfur compounds like disulphides and trisulphides (Mancini et al., 2020). Therefore, the

Table 1. Changes in the fatty acid profile of shrimp analogue without antioxidant (CON) during frozen storage at –20°C

Fatty acid	% of total fatty acids					
	0^{th} day	30^{th} day	60^{th} day	90^{th} day	120^{th} day	150^{th} day
SFA						
12:0	0.12±0.001^{a}	0.16±0.000^{b}	0.18±0.000^{d}	0.17±0.001^{c}	0.17±0.000^{c}	0.17±0.000^{c}
14:0	5.60±0.001^{a}	7.33±0.003^{d}	6.68±0.000^{c}	6.59±0.003^{b}	6.59±0.007^{b}	6.59±0.007^{b}
15:0	0.57±0.003^{a}	0.79±0.003^{e}	0.72±0.001^{d}	0.68±0.003^{b}	0.70±0.000^{c}	0.70±0.000^{c}
16:0	14.01±0.009^{a}	16.24±0.003^{b}	17.15±0.010^{c}	17.65±0.003^{e}	17.45±0.000^{d}	17.45±0.002^{d}
17:0	0.57±0.000^{b}	0.65±0.003^{e}	0.41±0.001^{a}	0.59±0.000^{c}	0.62±0.001^{d}	0.62±0.001^{d}
18:0	4.68±0.006^{a}	4.75±0.007^{b}	5.02±0.003^{e}	4.84±0.023^{c}	4.91±0.003^{d}	5.11±0.003^{f}
19:0	0.46±0.001^{d}	0.23±0.000^{c}	0.22±0.010^{bc}	0.21±0.003^{ab}	0.20±0.003^{a}	0.21±0.003^{ab}
20:0	0.29±0.006^{e}	0.24±0.003^{b}	0.27±0.001^{c}	0.21±0.003^{a}	0.21±0.000^{a}	0.28±0.001^{d}
MUFA						
16:1, n-9	6.44±0.003^{a}	7.62±0.003^{d}	7.28±0.001^{b}	7.36±0.003^{c}	7.88±0.002^{e}	7.89±0.000^{e}
16:1, n-7	0.23±0.001^{b}	0.26±0.003^{d}	0.19±0.000^{a}	0.23±0.003^{b}	0.25±0.000^{c}	0.25±0.000^{c}
18:1, n-9	23.68±0.003^{a}	23.94±0.003^{b}	25.83±0.007^{c}	26.24±0.030^{d}	26.27±0.030^{d}	26.40±0.003^{e}
18:1, n-5	0.45±0.001^{c}	0.17±0.000^{a}	0.21±0.013^{b}	0.16±0.001^{a}	0.16±0.003^{a}	0.16±0.000^{a}
20:1, n-9	5.56±0.003^{e}	4.58±0.003^{c}	4.73±0.003^{d}	4.48±0.001^{b}	4.47±0.000^{a}	4.48±0.001^{b}
22:1, n-9	4.48±0.003^{d}	2.90±0.003^{b}	3.04±0.020^{c}	2.70±0.003^{a}	2.70±0.003^{a}	2.93±0.030^{b}
22:1, n-7	0.33±0.001^{c}	0.35±0.001^{d}	0.23±0.007^{b}	0.21±0.003^{a}	0.21±0.003^{a}	0.21±0.003^{a}
PUFA						
18:2, n-6	8.43±0.005^{c}	8.51±0.000^{d}	8.22±0.017^{a}	8.31±0.007^{b}	8.31±0.007^{b}	8.29±0.010^{b}
18:3, n-3	3.02±0.006^{b}	3.17±0.000^{c}	2.90±0.027^{a}	2.92±0.003^{a}	2.90±0.003^{a}	2.92±0.003^{a}
18:4, n-3	1.01±0.006^{ab}	1.16±0.007^{c}	1.01±0.010^{ab}	0.97±0.033^{a}	1.00±0.003^{ab}	1.02±0.010^{b}
20:2, n-7	0.72±0.003^{d}	0.63±0.001^{c}	0.61±0.000^{b}	0.60±0.007^{a}	0.59±0.001^{a}	0.59±0.000^{a}
20:3, n-7	0.48±0.003^{c}	0.16±0.002^{b}	0.09±0.007^{a}	0.08±0.003^{a}	0.09±0.003^{a}	0.09±0.000^{a}
20:4, n-6	1.49±0.003^{c}	1.37±0.003^{a}	1.37±0.001^{a}	1.42±0.003^{b}	1.42±0.003^{b}	1.42±0.000^{b}
20:3, n-3	0.27±0.000^{d}	0.27±0.001^{cd}	0.21±0.001^{a}	0.26±0.000^{b}	0.26±0.003^{b}	0.27±0.000^{bc}
20:4, n-3	0.94±0.000^{d}	0.85±0.001^{c}	0.76±0.003^{b}	0.73±0.000^{a}	0.73±0.001^{a}	0.73±0.000^{a}
20:5, n-3	5.75±0.001^{f}	5.29±0.007^{e}	4.86±0.003^{d}	4.64±0.001^{c}	4.42±0.003^{b}	4.01±0.007^{a}
22:4, n-3	0.42±0.001^{c}	0.37±0.001^{a}	0.39±0.003^{b}	0.43±0.001^{d}	0.43±0.000^{d}	0.45±0.000^{e}
22:5, n-3	2.21±0.006^{d}	1.73±0.003^{c}	1.58±0.017^{b}	1.54±0.007^{a}	1.53±0.002^{a}	1.53±0.003^{a}
22:6, n-3	7.82±0.001^{f}	6.26±0.007^{e}	5.82±0.000^{d}	5.78±0.003^{c}	5.50±0.003^{b}	5.25±0.000^{a}

*Means in the row with different superscripts are significantly different ($p \leq 0.05$)

garlic paste addition positively influenced the FA profile of shrimp analogue samples. Similarly, ginger powder addition (1% and 2%) was found to be useful in modifying the FA profile of rabbit burgers stored at 4°C for 7 days (Mancini et al., 2017). Conversely, another previous study reported that garlic powder incorporated at 0.25% didn't greatly modify the FA profile of raw and cooked rabbit meat burgers stored for a week at 4°C possibly due to the low concentration of garlic used (Mancini et al., 2020).

The quality of fat is typically determined by its relative content of SFAs, MUFAs, and PUFAs. It is understood that an increase in the unsaturation of fatty acids makes them more vulnerable to oxidation and it is wise to add antioxidants to protect them against oxidative rancidity (Mhaskar & Varma,

Table 2. Changes in the fatty acid profile of shrimp analogue added with garlic paste (GAR) during frozen storage at –20ºC

Fatty acid	% of total fatty acids					
	0th day	30th day	60th day	90th day	120th day	150th day
SFA						
12:0	0.12±0.000^{a}	0.14±0.00^{c}	0.13±0.000^{b}	0.13±0.000^{b}	0.13±0.001^{b}	0.13±0.001^{b}
14:0	5.63±0.004^{a}	7.07±0.003^{d}	5.84±0.003^{b}	6.51±0.000^{c}	6.51±0.003^{c}	6.51±0.003^{c}
15:0	0.62±0.000^{a}	0.68±0.000^{c}	0.67±0.002^{b}	0.77±0.000^{d}	0.77±0.000^{d}	0.77±0.003^{d}
16:0	14.2±0.003^{a}	16.1±0.023^{c}	15.19±0.003^{b}	17.11±0.000^{d}	17.11±0.000^{d}	17.11±0.000^{d}
17:0	0.55±0.001^{a}	0.62±0.003^{c}	0.58±0.001^{b}	0.69±0.007^{d}	0.70±0.003^{d}	0.70±0.003^{d}
18:0	4.61±0.003^{a}	5.00±0.003^{c}	4.95±0.003^{b}	5.00±0.003^{c}	5.00±0.003^{c}	5.00±0.003^{c}
19:0	0.45±0.000^{d}	0.23±0.000^{b}	0.33±0.003^{c}	0.18±0.000^{a}	0.18±0.000^{a}	0.18±0.001^{a}
20:0	0.31±0.003^{d}	0.25±0.001^{a}	0.30±0.003^{c}	0.27±0.003^{b}	0.27±0.001^{b}	0.27±0.001^{b}
MUFA						
16:1, n-9	6.50±0.003^{a}	7.61±0.007^{d}	6.78±0.003^{b}	7.53±0.001^{c}	7.53±0.000^{c}	7.53±0.003^{c}
16:1, n-7	0.23±0.000^{b}	0.25±0.001^{d}	0.19±0.001^{a}	0.24±0.001^{c}	0.24±0.001^{c}	0.24±0.001^{c}
18:1, n-9	23.22±0.004^{c}	23.21±0.007^{b}	22.87±0.003^{a}	25.65±0.007^{d}	25.66±0.003^{d}	25.71±0.003^{e}
18:1, n-5	0.44±0.000^{d}	0.18±0.001^{a}	0.20±0.003^{b}	0.27±0.001^{c}	0.27±0.000^{c}	0.27±0.001^{c}
20:1, n-9	5.55±0.003^{d}	4.50±0.000^{a}	5.45±0.001^{c}	4.63±0.000^{b}	4.63±0.001^{b}	4.63±0.003^{b}
22:1, n-9	4.48±0.005^{d}	2.90±0.003^{a}	4.11±0.003^{c}	3.03±0.010^{b}	3.02±0.003^{b}	3.02±0.003^{b}
22:1, n-7	0.31±0.003^{d}	0.29±0.001^{c}	0.25±0.003^{b}	0.24±0.001^{ab}	0.24±0.003^{ab}	0.23±0.010^{a}
PUFA						
18:2, n-6	8.40±0.003^{e}	8.09±0.010^{b}	7.91±0.003^{a}	8.28±0.001^{c}	8.28±0.003^{c}	8.38±0.000^{d}
18:3, n-3	3.02±0.000^{a}	3.01±0.010^{a}	4.15±0.040^{b}	3.02±0.003^{a}	3.02±0.000^{a}	3.02±0.003^{a}
18:4, n-3	1.00±0.003^{a}	1.19±0.010^{d}	1.15±0.003^{c}	1.03±0.003^{b}	1.03±0.000^{b}	1.03±0.001^{b}
20:2, n-7	0.69±0.000^{c}	0.59±0.007^{a}	0.71±0.000^{d}	0.66±0.000^{b}	0.66±0.004^{b}	0.65±0.006^{b}
20:3, n-7	0.45±0.000^{d}	0.14±0.001^{c}	0.12±0.001^{b}	0.12±0.007^{ab}	0.11±0.001^{ab}	0.11±0.003^{a}
20:4, n-6	1.48±0.003^{c}	1.34±0.003^{a}	1.57±0.001^{e}	1.42±0.007^{b}	1.42±0.007^{b}	1.40±0.006^{b}
20:3, n-3	0.27±0.000^{b}	0.35±0.003^{d}	0.36±0.000^{e}	0.26±0.007^{a}	0.30±0.003^{c}	0.30±0.000^{c}
20:4, n-3	1.00±0.003^{c}	0.96±0.007^{b}	0.97±0.001^{b}	0.78±0.013^{a}	0.78±0.010^{a}	0.79±0.000^{a}
20:5, n-3	5.80±0.003^{d}	5.85±0.003^{e}	5.81±0.007^{d}	4.67±0.003^{c}	4.50±0.000^{b}	4.40±0.003^{a}
22:4, n-3	0.50±0.003^{c}	0.41±0.001^{b}	0.58±0.003^{e}	0.35±0.003^{a}	0.45±0.002^{c}	0.45±0.001^{c}
22:5, n-3	2.41±0.003^{e}	1.79±0.010^{c}	2.20±0.003^{d}	1.48±0.007^{a}	1.69±0.003^{b}	1.69±0.001^{b}
22:6, n-3	7.77±0.000^{f}	7.20±0.003^{e}	6.60±0.000^{d}	5.68±0.003^{c}	5.50±0.003^{b}	5.45±0.001^{a}

*Means in the row with different superscripts are significantly different ($p \leq 0.05$)

2015). Changes in the ΣPUFA and ΣSFA levels of shrimp analogues with and without antioxidants during frozen storage are shown in Table 4. The ΣPUFA levels decreased and ΣSFA levels increased significantly ($p \leq 0.05$) during storage, which led to a decreased ΣPUFA/ΣSFA ratio in all the samples during storage. Because of more oxidation, the decrease in the ΣPUFA level was highest in the sample without antioxidants. The ideal ΣSFA: ΣMUFA: ΣPUFA ratio in a healthy diet recommended by the American Heart Association (AHA) is 1:1:1 (Hayes, 2002). The ΣPUFA/ΣSFA ratio at the end of the storage trial was significantly ($p \leq 0.05$) higher in the shrimp analogues added with BHA (1.03±0.000), followed by GAR (0.90±0.001) indicating that the antioxidants had delayed fat oxidation to a certain extent. Similarly, an earlier report mentioned that PUFA content was more in γ-

Table 3. Changes in the fatty acid profile of shrimp analogue added with synthetic antioxidant (BHA) during frozen storage at –20ºC

Fatty acid	% of total fatty acids					
	0th day	30th day	60th day	90th day	120th day	150th day
SFA						
12:0	0.13±0.000^{b}	0.12±0.000^{a}	0.17±0.000^{c}	0.28±0.000^{d}	0.28±0.000^{d}	0.28±0.000^{d}
14:0	5.54±0.017^{a}	6.27±0.001^{d}	6.20±0.003^{b}	5.71±0.003^{b}	5.71±0.000^{b}	5.71±0.003^{b}
15:0	0.58±0.001^{a}	0.68±0.001^{b}	0.70±0.000^{d}	0.69±0.000^{c}	0.69±0.004^{c}	0.69±0.000^{c}
16:0	14.07±0.070^{a}	16.63±0.003^{c}	16.81±0.007^{d}	15.37±0.000^{b}	15.37±0.000^{b}	15.37±0.001^{b}
17:0	0.57±0.003^{b}	0.62±0.002^{c}	0.48±0.000^{a}	0.73±0.002^{c}	0.73±0.001^{c}	0.73±0.000^{c}
18:0	4.68±0.003^{a}	5.08±0.003^{c}	5.01±0.003^{b}	5.46±0.003^{e}	5.46±0.000^{e}	5.44±0.001^{d}
19:0	0.46±0.003^{d}	0.28±0.001^{c}	0.22±0.003^{b}	0.18±0.001^{a}	0.18±0.003^{a}	0.18±0.001^{a}
20:0	0.28±0.003^{b}	0.27±0.001^{a}	0.29±0.001^{b}	0.32±0.001^{c}	0.31±0.007^{c}	0.32±0.001^{c}
MUFA						
16:1, n-9	6.45±0.000^{a}	7.67±0.003^{d}	6.49±0.007^{b}	6.86±0.001^{c}	6.86±0.000^{c}	6.86±0.001^{c}
16:1, n-7	0.25±0.003^{d}	0.21±0.010^{b}	0.19±0.000^{a}	0.23±0.001^{c}	0.23±0.002^{c}	0.23±0.000^{c}
18:1, n-9	23.58±0.023^{b}	23.46±0.007^{a}	24.01±0.010^{c}	24.49±0.003^{d}	25.19±0.003^{e}	26±0.003^{f}
18:1, n-5	0.51±0.010^{c}	0.19±0.000^{b}	0.19±0.002^{b}	0.16±0.001^{a}	0.16±0.002^{a}	0.16±0.000^{a}
20:1, n-9	5.50±0.003^{e}	4.55±0.002^{a}	4.73±0.007^{b}	4.88±0.000^{d}	4.78±0.003^{c}	4.78±0.003^{c}
22:1, n-9	4.46±0.020^{e}	3.08±0.023^{a}	3.31±0.013^{c}	3.46±0.007^{d}	3.27±0.003^{b}	3.27±0.004^{bc}
22:1, n-7	0.33±0.001^{d}	0.21±0.007^{a}	0.24±0.003^{b}	0.27±0.001^{c}	0.27±0.000^{c}	0.27±0.000^{c}
PUFA						
18:2, n-6	8.44±0.003^{d}	8.11±0.000^{b}	8.27±0.027^{c}	7.95±0.003^{a}	7.94±0.010^{a}	7.95±0.000^{a}
18:3, n-3	2.96±0.040^{b}	2.89±0.000^{a}	3.29±0.010^{d}	3.14±0.000^{c}	3.14±0.002^{c}	3.14±0.000^{c}
18:4, n-3	1.03±0.033^{a}	1.03±0.000^{a}	1.01±0.010^{a}	1.11±0.003^{b}	1.11±0.003^{b}	1.11±0.003^{b}
20:2, n-7	0.71±0.003^{c}	0.61±0.001^{ab}	0.62±0.002^{b}	0.70±0.003^{c}	0.61±0.010^{ab}	0.60±0.000^{a}
20:3, n-7	0.47±0.000^{d}	0.12±0.001^{b}	0.11±0.007^{a}	0.13±0.000^{c}	0.13±0.002^{c}	0.13±0.000^{c}
20:4, n-6	1.48±0.003^{b}	1.35±0.002^{a}	1.36±0.033^{a}	1.77±0.003^{d}	1.77±0.003^{d}	1.61±0.007^{c}
20:3, n-3	0.26±0.002^{a}	0.26±0.000^{ab}	0.27±0.003^{b}	0.29±0.005^{c}	0.29±0.003^{c}	0.29±0.000^{c}
20:4, n-3	0.96±0.003^{d}	0.74±0.000^{a}	0.82±0.017^{b}	0.88±0.001^{c}	0.88±0.003^{c}	0.88±0.002^{c}
20:5, n-3	5.81±0.007^{f}	5.67±0.001^{e}	5.60±0.003^{d}	5.51±0.003^{c}	5.30±0.001^{b}	5.00±0.003^{a}
22:4, n-3	0.45±0.000^{b}	0.49±0.000^{c}	0.39±0.003^{a}	0.53±0.000^{d}	0.55±0.002^{e}	0.53±0.001^{d}
22:5, n-3	2.21±0.030^{e}	1.65±0.000^{a}	1.80±0.000^{b}	1.88±0.004^{c}	1.88±0.003^{c}	1.88±0.001^{c}
22:6, n-3	7.82±0.001^{f}	7.75±0.001^{e}	7.41±0.010^{d}	7.01±0.003^{c}	6.91±0.007^{b}	6.60±0.003^{a}

*Means in the row with different superscripts are significantly different ($p \leq 0.05$)

Table 4. Changes in the ΣPUFA/ΣSFA ratio of shrimp analogues during frozen storage at –20°C

Sample	ΣPUFA/ΣSFA					
	0th day	30th day	60th day	90th day	120th day	150th day
CON	1.24±0.000^{Af}	0.98±0.000^{Ae}	0.91±0.001^{Ad}	0.89±0.000^{Ac}	0.88±0.001^{Ab}	0.85±0.000^{Aa}
GAR	1.24±0.001^{Ad}	1.03±0.000^{Cb}	1.15±0.002^{Cc}	0.91±0.000^{Ba}	0.91±0.000^{Ba}	0.90±0.001^{Ba}
BHA	1.24±0.004^{Ae}	1.02±0.000^{Ba}	1.04±0.001^{Bb}	1.07±0.000^{Cd}	1.06±0.000^{Cc}	1.03±0.000^{Cb}

*Means in the row with different superscripts$^{(a-f)}$ are significantly different ($p \leq 0.05$)
*Means in the same column with different superscripts$^{(A-C)}$ are significantly different ($p \leq 0.05$)
CON=Control, GAR=Garlic, BHA=Butylated hydroxy anisole

irradiated meat emulsions added with microencapsulated rosemary and mint oil as antioxidants during refrigerated storage (Akhter, Masoodi, Wani, & Rather, 2022). Also, the PUFA and total n-3 PUFA levels of chilled stored rabbit burgers added with turmeric powder (*Curcuma longa* L.) were reportedly higher than the control formulation (Mancini et al., 2015).

Lipid oxidation is a critical issue in fish and fishery products during long-term storage as it leads to the development of undesirable flavour, odour, taste and nutrient loss (Cullere et al., 2019). Peroxide value (PV) is a measure of the first stage of oxidative rancidity, which determines peroxides and hydroperoxides formed in fat. Changes in the PV of shrimp analogue samples added with and without antioxidants are presented in Fig. 1. The PV increased significantly ($p \leq 0.05$) with storage time till 60 days of frozen storage and further there was a significant ($p \leq 0.05$) decrease in PV in all the samples. In the case of the control sample, the PV increased from an initial value of 1.85±0.12 meq of O_2 per kg to 5.67±0.40 meq of O_2 per kg on 60th day and then decreased to 4.60±0.42 meq of O_2 per kg on 150th day of frozen storage. The decrease in PV value can be attributed to the conversion of hydroperoxides and the formation of secondary oxidation products such as aldehydes and ketones. The lowest final value of PV (2.90±0.26) was seen in the BHA-added samples. Lajolinna, Laine, and Linko. (1983) reported a range of 10-30 mill equivalents of O_2/Kg of fat as the maximum permissible limit of PV. In the present study, PV stayed within the permissible quality limit in all the samples at the end of the frozen storage study (<10 meq of O_2 per kg). Antioxidants prevent lipid oxidation. So, PV was lower in the samples containing antioxidants than in the control sample. Both BHA and garlic exhibited antioxidant action. Synthetic antioxidant, BHA was more effective in preventing lipid oxidation than the natural antioxidant, garlic. Similarly, it was reported that the addition of 0.02% BHA/BHT showed significantly higher antioxidant action than rosemary extract and suppressed lipid oxidation in cooked ground beef (Ahn, Grün, & Fernando, 2002). Garlic prevented lipid oxidation as indicated by the lower value of PV (3.70±0.16 meq of O_2 per kg) in garlic-added samples as compared to the control at the end of 5 months of storage. The antioxidant efficiency of garlic is due to the presence of several phytochemical components, which are accountable for relieving oxidative stress (Akullo, Kiage-Mokua, Nakimbugwe, Ng'ang'a, & Kinyuru, 2023). Garlic contains several potent bioactive compounds and allicin is one such major sulphur-containing compound (Mancini et al., 2020). An earlier study reported that the addition of marjoram and zataria essential oils to common carp surimi resulted in a decreased PV of 3.91 meq/kg and 4.06 meq/kg, respectively, compared to the control (4.69 meq/kg) during frozen storage at -18°C (Pourmollaei et al., 2021).

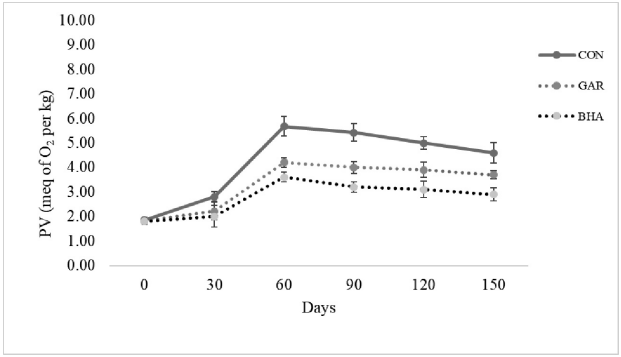

Fig. 1. Peroxide value (PV) of shrimp analogues during frozen storage at –20°C

Table 5. Changes in the sensory score of shrimp analogues during frozen storage at –20°C

Sample	Sensory score for overall acceptability					
	0th day	30th day	60th day	90th day	120th day	150th day
CON	9.0±0.2^{Ae}	8.5±0.2^{Ad}	8.2±0.3^{Ac}	8.0±0.2^{Abc}	7.6±0.1^{Ab}	6.9±0.1^{Aa}
GAR	9.0±0.2^{Aef}	8.8±0.2^{Bde}	8.6±0.2^{Bd}	8.2±0.1^{Bc}	8.0±0.1^{Bb}	7.6±0.2^{Ba}
BHA	9.0±0.2^{Aef}	8.9±0.1^{Be}	8.7±0.1^{Bcd}	8.6±0.2^{Cbc}	8.4±0.2^{Cb}	7.9±0.1^{Ca}

*Means in the row with different superscripts$^{(a-f)}$ are significantly different ($p \leq 0.05$)
*Means in the same column with different superscripts$^{(A-C)}$ are significantly different ($p \leq 0.05$)
CON=Control, GAR=Garlic, BHA=Butylated hydroxy anisole

TBA value is a measure of the second stage of oxidative rancidity of lipids. It has been widely used to indicate lipid oxidation in fish and fishery products. There was a significant increase ($p \leq 0.05$) in the TBA value of all samples during frozen storage due to malonaldehyde formation. The results are illustrated in Fig. 2. The increase in the TBA value was highest in the control samples followed by garlic-added samples and the increase was lowest in BHA-added samples. TBA value of 3-4 mg malonaldehyde/kg sample would lead to souring and quality loss in fish meat (Huss, 1988). In the present study, the TBA value of all samples increased as the storage time increased but it was within the acceptable limit. However, it is also reported that TBARS values between 1.5 and 3.0 mg of malonaldehyde per kg are associated with a rancid flavour in seafood (Zaghbib et al., 2016). The TBA value of CON was 1.86±0.05 mg malonaldehyde/kg sample after 5 months of frozen storage, which was reduced by 29% and 46% in GAR and BHA samples, respectively leading to the prevention of rancid flavour and taste. Lipid oxidation was prevented by garlic paste effectively and it was reported that fresh garlic or garlic powder have more physiological action in comparison to garlic oil, aged garlic and steam-distilled garlic because they do not contain significant amounts of alliin or allicin. Whole garlic exhibits direct antioxidant effects by effectively scavenging exogenously produced hydroxyl radicals (Majumdar et al., 2015). Similarly, Jannet-Alipour et al. (2019) reported that the addition of edible green seaweed, *Ulva intestinalis* powder (2.77 g kg^{-1}), and its sulphated polysaccharide (0.5 g kg^{-1}) with antioxidant action resulted in lower values of TBARS in fish-surimi restructured products throughout frozen storage. Another work recorded a significant reduction in TBARS value in grass cap surimi added with young apple polyphenols at 0.10% and stored at 4°C (Sun et al., 2017). Sensory evaluation of the frozen stored shrimp analogue samples was done each month by 10 regular members. The average of the sensory score given by the members every month for overall acceptability is presented in Table 5. Results indicated that the incorporation of antioxidants resulted in better retention of the sensory quality of shrimp analogues during frozen storage. The initial sensory score for all the samples was 9.0 and the final score was >7 for GAR and BHA samples suggesting the good quality of product at the end of 150 days of storage. There was significant ($p \leq 0.05$) variation in the sensory score of all three samples and the overall sensory acceptability of shrimp analogue was rated by the panellists as follows: BHA>GAR>CON. The BHA-added shrimp analogues had a sensory score of 7.9 while it was only 6.9 for the control sample at the end of the frozen storage study. The sensory score was 7.6 for GAR shrimp analogues at the end of the storage trial. The garlic paste as well as cod liver oil addition didn't negatively affect the sensory properties of shrimp analogues. Garlic possesses a pungent flavour but it was reported that the incorporation of garlic's aqueous extract (GAE) at 1% didn't impart

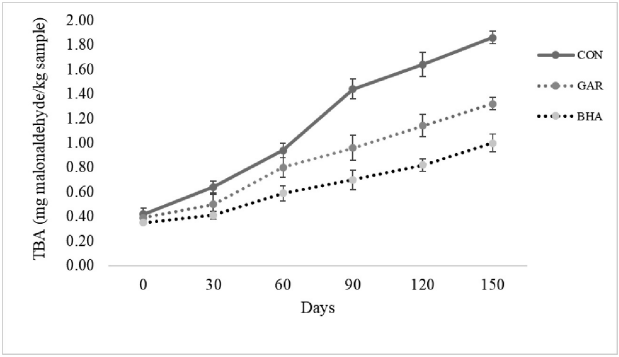

Fig. 2. TBA value of shrimp analogues during frozen storage at –20°C

any negative garlic flavour to the restructured products from Thai pangas surimi during refrigerated storage (Majumdar et al., 2015).

In food fortification, the addition of fish oil is limited by its fishy flavour and the high susceptibility of n-3 PUFAs to oxidation. The oxidation will lead to an undesirable taste and odour, ultimately reducing the shelf-life of products. Consequently, palatability represents a critical factor limiting the extent of fortification with n-3 PUFA (Pérez-Mateos, Lanier, & Boyd, 2006) As per this study, there was no fishy taste recorded by the panel in GAR and BHA samples throughout the storage. The addition of shrimp flavour also might have contributed to masking the other flavours.

Surimi is mainly obtained from underutilized/low-cost fish with white meat, which can be used for developing high-value products such as shrimp analogues/imitation shrimps. Surimi is a high-protein and low-fat food, which is deficient in n-3 PUFAs. The surimi-based products such as shrimp analogue can be fortified with n-3 PUFA-rich fish oil to improve its fatty acid profile. The n-3 PUFAs have beneficial health effects for humans but are also highly susceptible to oxidation during long-term frozen storage, which necessitates antioxidant addition. The results of the present study suggest that garlic and BHA delayed lipid oxidation and undesirable quality changes in shrimp analogue as indicated by lower PV and TBARS values as well as better sensory score and fatty acid profile. It can be concluded that garlic is an efficient natural antioxidant for surimi products incorporated with n-3 PUFAs with promising potential for preventing lipid oxidation as a substitute for synthetic antioxidants.

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