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CHANGES IN THE FATTY ACID PROFILE OF THERMAL PROCESSED TILAPIA SANDWICH PASTE WITH PUFA IN RETORTABLE POUCHES

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Abstract: A ready to serve sandwich paste was developed from the meat of tilapia (*Oreochromis mossambicus*) with peeled potato by thermal processing in retortable pouches at a temperature of 121.1°C with different F_0 values of 6,7,8 and 9. The samples processed at 121.1°C at F_0 value 8.08, Cook value 75.02 and total process time 32.23 min were found to be the best. To Study the changes in the fatty acid profile of thermal processed tilapia sandwich spread with PUFA in retortable pouches, cod liver oil were incorporated with 1% and 2% level. The samples were subjected for thermal processing at 121.1°C at F_0 value 8.08, Cook value 75.02 and total process time 32.23 min and for a further storage of 1 yr. at ambient temperature. The fatty acid profile of the tilapia sandwich paste both control and PUFA rich cod liver oil at 1% and 2% level didn't change significantly, whereas, the essential fatty acids (PUFA content) decreased slightly with slight increase in n-3. The ratio between n-3 and n-6 also showed slight increase. In this study the PUFA level was not decreased in the tilapia sandwich paste both in control and in PUFA rich cod liver oil at 1% and 2% level and the reason may be due to the stability and the antioxidant present in the commercial cod liver oil. Thus, the fortification of PUFA in the tilapia sandwich paste in retort pouch did not damage the n-3 and n-6 fatty acids during retorting and storage.

Keywords: Tilapia Sandwich paste, Thermal processing, retort pouch, Fatty acid profile, PUFA.

Introduction

Though marine fish production is stagnant since last 3-5 years, the production of fresh water fish has shown significant progress. Tilapia, one of the fish in the world has been the choice of many. This is due to the efficient conversion of feed, ability to breed easily, resistance to handling stress, and disease and palatability (Akande, 1989). Culture of tilapia in India is now being commercially practiced and is also grown wildly in natural water bodies. Globally bulk of the tilapia is being consumed in fresh, frozen (fillets) and smoked condition (Asiedu et al., 1991*).* Tilapias are less commonly utilized for canning in the Asia-Pacific region, but well

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accepted in the filleted form. The available research literature in India on thermal processing of Tilapia is very limited. Recently, market for processed foods has been growing at a greater rate due to the change in life style and food habits of the people. Since tilapia is an odorless lean variety of fish with white flesh, it could be an ideal choice as raw material for the development of ready to serve fish products like fish curry, sandwich, cutlets, burgers and sticks by using retort pouches for both domestic and international markets (Dhanapal et al., 2010, 2016).

Therefore, the present study was aimed at developing thermally processed sandwich paste from tilapia in retortable pouch and to study the changes in the fatty acid profile of thermal processed tilapia sandwich spread with PUFA in retortable pouches.

Materials and Methods

Tilapia Fish (*Oreochromis mossambicus*) harvested from the natural waters of Muthukur, Nellore District, Andhra Pradesh, India was iced in the ratio of 1:1 and transported to laboratory in chilled condition. The time gap between capture of fish and processing into fish sandwich paste for analysis was less than 30 hours. Fish measuring around 18 to 26 cm (4-5 pieces/kg) were washed with chilled water and dressed to remove bones, skins using meat bone separator (Badder, Germany). The separated tilapia meat is minced in to paste again using meat mincer (Sirman, Italy).

Retort Pouch

Laminated flexible pouch (4-ply), consisting of 12 µ polyester (outer layer), 9 µ aluminium foil and 15 µ nylon layer (middle layer) and 70 µ polypropylene (inner cast) was used for packing the sandwich paste. Pouches (150×200 mm) were used for the present study were purchased from M/s. Pradeep Laminators Pvt. Ltd. Pune. Maharastra, India.

Overpressure Retort

The pilot scale overpressure retorting unit (Alpha Steritech, Bangalore, India) consisting of retort, receiver and the control system was used for thermal processing which ensures close simulation with commercial scale equipment and which produces a high degree of process reproducibility and accuracy.

Ellab CTF 9004(ELLAB Co., Roedovre, Denmark) Precission Thermometer and F_0 value integrator (Version 5.0, Denmark) was used to record core temperature, retort temperature, F_0 value and cook value at a specific time interval of 60 seconds. Temperature range of the instrument is -100^0C to +350°C with resolution of 0.1°C. The F_0 constants were e programmed at T=121.1°C, Z=10°C and Cook value constants at T=100°C.

The vacuum-sealing machine supplied by M/s ACEPACK, Mumbai was used for sealing the pouch with fish sandwich paste. Secondary sealing was done by Foot operated hydraulic heat sealing machine supplied by M/s Alpha Steritech, Bangalore.

Preparation of sandwich paste

The sandwich paste was prepared by the following method. The mince was prepared using meat bone separator (Badder, Germany) from the dressed fish (50.50 %) and boiled for 15 min. Potato was boiled, peeled (13.85%) and was made into paste. Chopped ginger (1.25%), garlic (4.6%) and green chilies (1.85%) were also ground into paste. Chilly powder (1.25%) and turmeric powder (0.5%) together were made into a paste by adding sufficient water (9.25%). Cinnamon, cardamom, anise, pepper, bay leaf and cloves in equal proportion was made into powder and used as spice mix (0.25%). Fish paste was added into the frying pan and fried with half of the oil and kept aside. Half broken mustard (0.25%) and cumin seed (0.75%) were added into the hot oil (11.50%) and fried for 1-2 min. Chopped ginger, garlic and green chilly paste was added and half fried. Chilli powder and turmeric powder paste along with spice mix and salt (1.80%) were also added and fried until characteristic odour of fried spices emerges. The fish paste and potato paste were added and frying continued in a low flame for a while with vigorous stirring till the characteristic smell emerged. Lemon juice extract (2%) and coriander leaves (0.4%) were added and mixed. For fortification of PUFA in sandwich paste, cod liver oil at 1% and 2% level were added and mixed uniformly prior to filling in the pouch for the study.

Proximate composition

The moisture content of fish muscle and sandwich paste of tilapia was determined by using Automatic Moisture Analyzer (Denver Moisture Analyzer, Model IR 120, Bohemia, N.Y., U.S.A.). The sample was heated initially at 100˚C and later at a temperature of 170˚C. Moisture content corresponds to the weight loss of the sample. The crude protein content of the sample was determined by estimating Total Nitrogen by Kjeldahl method (AOAC, 2000). Crude protein content (Total nitrogen × 6.25) was calculated by multiplying the nitrogen content with 6.25. Fat was determined by the method described by the AOAC (2000) using the Soxhlet extraction system. Ash content was determined by ashing in a microwave furnace (Phoenix, Ariz., U.S.A) at 550°C ± 10°C for 5 hours and sodium chloride content was determined by AOAC (2000) method.

pH

The pH was determined by blending 10 grams of tilapia sandwich paste and fish muscle with 90 ml distilled water in a homogenizer (Kinematica AG, Polytron System PT 2100, Lucerne, Switzerland) each for 30 seconds using a digital pH meter (Eutech Instruments, Singapore) standardized at pH 4 and 7 (APHA 1998).

Thiobarbituric acid (TBA) value

TBA value was determined using the method of Tarladgis et al., (1960). The TBA number was expressed as milligram malonoldehyde equivalents per kilogram sample. The absorbance was determined by a spectrophotometer at 532 nm against a blank containing distilled water and TBA solution.

Fatty acid profile

Total lipid content in the fish mince and sandwich paste (about 3g) were extracted using the method described by Folch et al., (1957). The fatty acids in the total lipids were converted into Fatty acid methyl (FAM) esters by transmethylation using methanolic sodium hydroxide, BF_3 methanol and n-heptane (AOAC, 2000). FAM esters were analyzed using Gas chromatography–Mass spectrometry (Shimadzu QP2010quadrupole, Kyoto, Japan) equipped with ionization energy of 70 eV operating in positive electronic impact set to 100 μA, connected to a GC 8060 gas chromatograph (Shimadzu) equipped with a Carbowax (25 m × 0.25 mm; 0.25-μm film thickness) column (Cromlab S.A., J&W Scientific, Folsom, Calif., U.S.A.) and helium 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 min and then to increase at a rate of 10°C per min to a final temperature of 230°C. FAM esters were separated at constant pressure (23.1 kPa). The mass spectrometer was tuned to get the relative abundances of m/z ranging from 40.00 to 550.00. The identification of the methyl esters of fatty acids were done by matching with retention time and fatty acids and reported as percentage of total fatty acids.

Commercial Sterility Test

The thermally processed samples of three types of products processed at different F_0 value and at different temperatures were incubated at 37^0C for 15 days and 55^0C for minimum of 5 days. The incubated pouches were aseptically opened and 1-2 g of the samples was inoculated into the sterilized fluid thioglycolate broth in test tubes. Sterilized liquid paraffin was put on to the top of the broth to create anaerobic condition and incubated at 37^0C for 48 hrs and at 55^0C for 4 days (IS: 2168 1971), respectively.

Statistical analysis

The SPSS 10.00 (SPSS 2000) statistical packaging was used for analysis of the experimental results. The results were expressed as mean ± standard deviation and Duncan test was used to asses statistical significance ($p<0.05$) between the samples stored at ambient temperature during storage study. The correlation coefficients between the parameters were carried out using the same software.

Results and Discussion

The sandwich paste thus standardized at F_0 8 was fortified with 1% and 2% PUFA and a control was kept without fortification for comparative studies. These samples were processed at 121^0C at F_0 8, Cook value 75.02 and total process time 32.23 min. (Dhanapal et al., 2016) and studied for a further storage of 1 yr. at ambient temperature. The aim of this was to see the effect of retorting on the tilapia sandwich paste fortified with different level of PUFA and control sandwich paste during storage.

Changes in chemical composition of sandwich paste

The pH, moisture, protein, fat and ash content of the sandwich paste (control & treated sample) are presented in Table 1&2. During the period of storage, no significant change was observed in the content of moisture, protein, fat and ash. pH of the products showed a slight decrease during storage. Heating of muscle or isolated myofibrils usually results in an increase of pH (Hamm, 1966; Roberts and Lawrie, 1974; Fogg and Harrison, 1975) whereas, the moisture content showed no significant change throughout the storage period (Table 2). Thankamma *et al.* (1998) observed similar changes in the moisture content of the fish paste stored in the retort pouch. The initial decrease in the moisture content of all the samples may be due to the heat induced coagulation of protein and cross linking of peptide bonds. The high content of fat in the paste is due to the fat added to make the paste smooth and more spreadable.

Table 1: Biochemical changes of tilapia sandwich paste in retort pouch during storage

Period (month)	pH			Moisture (%)		
	Control	T1	T2	Control	T1	T2
0	5.53±0.01	5.59±0.01	5.55±0.01	67.18±0.01	65.93±0.04	66.34±0.05
3	4.85±0.03	5.03±0.03	4.88±0.02	64.87±0.20	64.63±0.25	64.83±0.09
6	5.13±0.03	5.10±0.00	4.93±0.01	66.79±0.09	66.37±0.20	65.80±0.23

9	5.10±0.00	5.00±0.00	5.00±0.00	64.94±0.12	65.98±0.09	65.61±0.09
12	5.00±0.00	4.91±0.01	4.83±0.02	66.08±0.11	67.48±0.19	65.29±0.07

Note: T1 - 1% cod liver oil, T2 - 2% cod liver oil & C - Control (without cod liver oil); n=3

Table 2: Chemical changes of tilapia sandwich paste in retort pouch during storage

Period (month)	**Protein**			**Fat**			**Ash**		
	C	**T1**	**T2**	**C**	**T1**	**T2**	**C**	**T1**	**T2**
0	10.16± 0.74	10.44± 0.10	10.01± 0.26	13.35± 0.04	13.35± 0.05	13.48± 0.04	2.95±0 .02	2.99±0 .01	2.94±0. 03
3	10.03± 0.01	10.45± 0.04	10.08± 0.21	13.59± 0.15	13.53± 0.17	13.47± 0.14	3.07±0 .08	3.17±0 .17	3.01±0. 08
6	10.37± 0.07	10.49± 0.04	10.30± 0.03	13.42± 0.01	13.59± 0.02	13.71± 0.13	3.02±0 .02	3.01±0 .01	3.06±0. 01
9	10.01± 0.04	10.36± 0.13	10.26± 0.02	13.71± 0.10	13.78± 0.03	13.73± 0.01	3.11±0 .02	3.05±0 .01	3.02±0. 01
12	9.97±0. 07	10.19± 0.06	10.26± 0.08	13.67± 0.07	13.73± 0.03	13.79± 0.04	3.03±0 .03	3.08±0 .01	3.00±0. 03

Note: T1 - 1%cod liver oil, T2 - 2% cod liver oil & C- Control (without cod liver oil); n=3

Biochemical and fatty acids changes of sandwich paste in retort pouch during storage

The changes in the TBA of tilapia sandwich paste for both control and treated product is given in the Fig 1. The TBA value of control and treated tilapia sandwich paste showed increasing trend unto sixth month and later decreased till the end of the study period. In addition, brine (NaCl) has been reported to enhance the lipid oxidation and that of the highly unsaturated lipids (Harris and Tall 1994). Several studies have confirmed that pressure increases the rate of lipid oxidation in muscle systems, and have attributed this effect mainly to the water content and/or metal ions released from hemoprotein complexes during pressure treatment (Tanaka *et al.* 1991; Cheah and Ledward, 1995; Cheah and Ledward, 1997). Bindu *et al.* (2004, 2007) and Siriamornpun *et al.* (2008) reported that the TBA value showed an increasing trend during storage period in retort pouch and can respectively. A decreasing trend in TBA value has been reported in canned fish by Mallick *et al*. (2006) and Tanaka *et al*. (1985). Aubourg *et al.* (1997) has suggested that the decrease in TBA value of canned fish meat might be due to dilution of secondary oxidation product by the fill oils, or their

extraction from the meat to the fill oils, or loss to the aqueous exudates from the meat. However in the case of Tilapia sandwich paste, the TBA content gradually increased in the initial period of storage. The increase in the TBA value is probably due to the fact that the processed tilapia sandwich paste produced primary oxidative compounds during heat processing changed to secondary compounds and decrease towards the end of storage may be caused by the involvement of TBA in protein-lipid interactive reactions.

The fatty acid profile results of tilapia sandwich paste are presented in Fig 2, 3, & 4. The fatty acid profile of the tilapia sandwich paste both control and PUFA rich cod liver oil at 1% and 2% level didn't change significantly, whereas, the essential fatty acids (PUFA content) decreased slightly with slight increase in n-3. The ratio between n-3 and n-6 also showed slight increase. In this study the PUFA level was not decreased in the tilapia sandwich paste both in control and in PUFA rich cod liver oil at 1% and 2% level and the reason may be due to the stability and the antioxidant present in the commercial cod liver oil. Thus, the fortification of PUFA in the tilapia sandwich paste in retort pouch did not damage the n-3 and n-6 fatty acids during retorting and storage. A comparison of different time/ temperature sterilizing conditions (F_0 value =7 min) showed that treatments with shorter times but higher temperatures lead to a higher hydrolysis development (Aubourg *et al*., 1997). Hale and Brown (1983); Aubourg *et al.* (1990) reported that no significant change in PUFA concentrations could be noted on heat processing of various sea foods like sardine, mackerel, tuna and crab in sealed containers. So it is concluded that either the fortified samples with cod liver oil (PUFA) or that present in the control was stable at F_0 value 8.

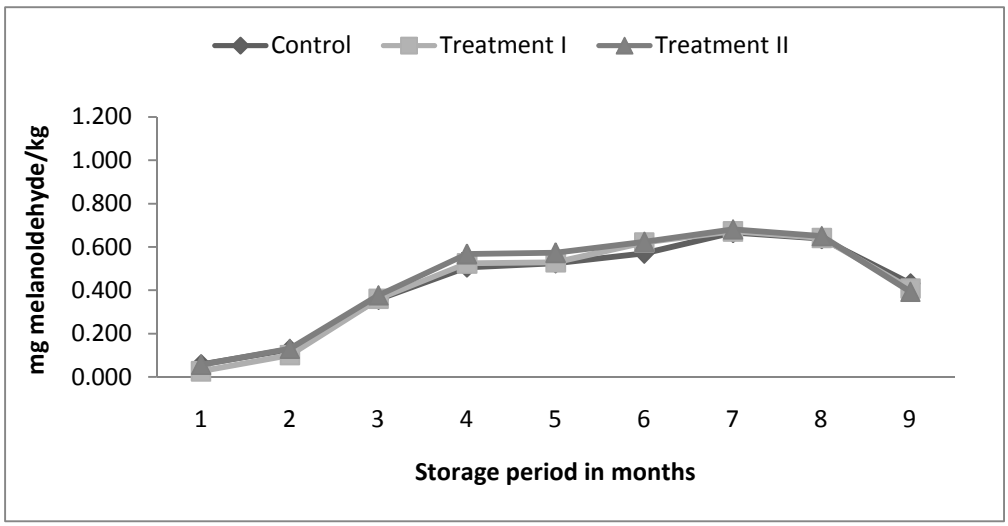

Fig 1: Changes in TBA of sandwich paste during storage

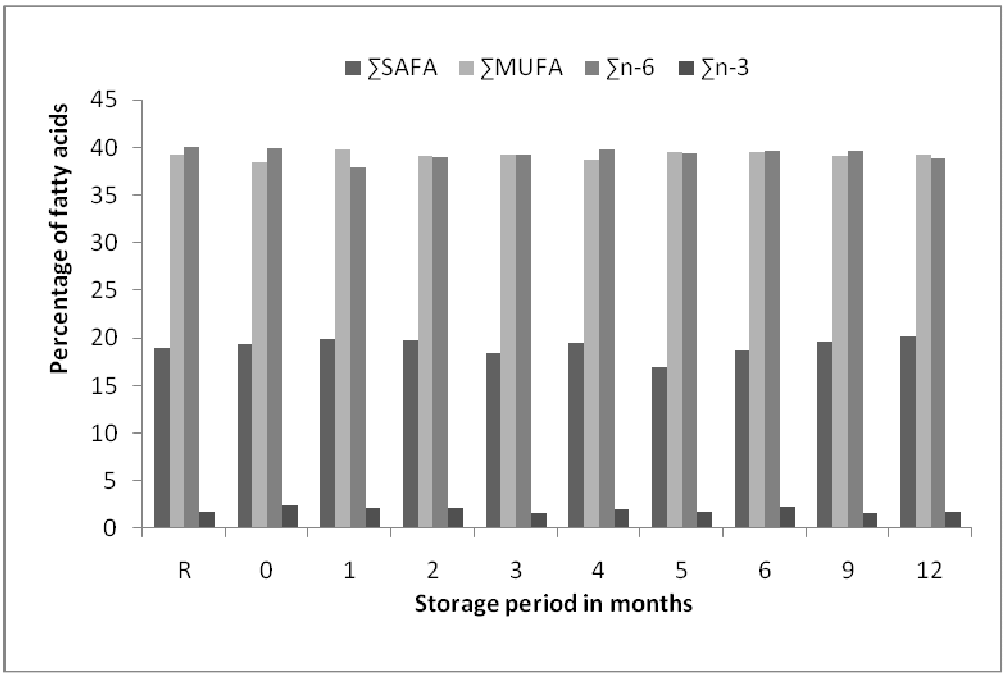

Fig. 2: Changes in fatty acid profile of sandwich paste of control during storage

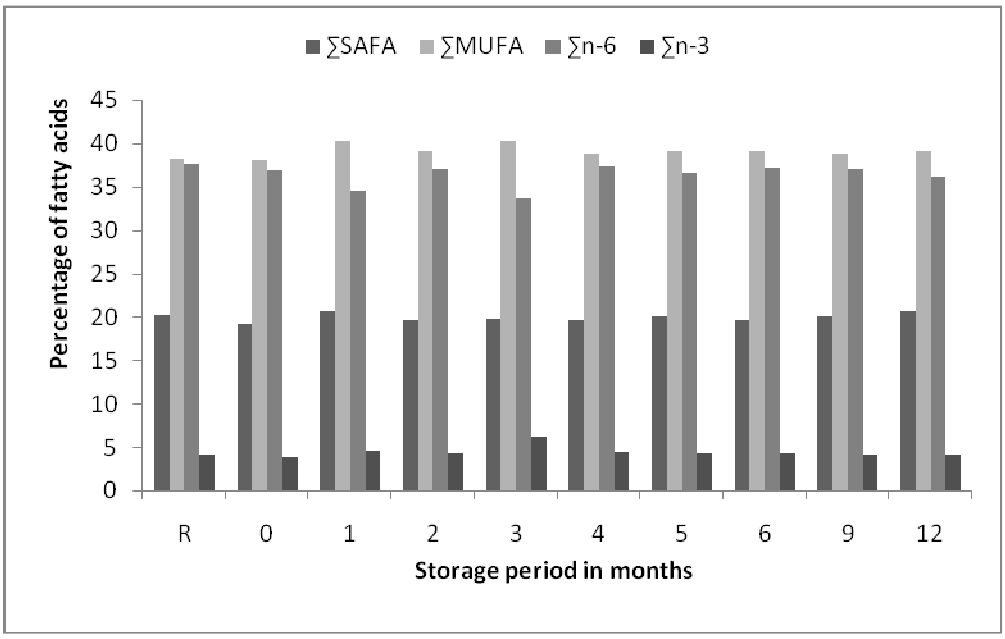

Fig. 3: Changes in fatty acid profile of sandwich paste of 1% PUFA treated during storage

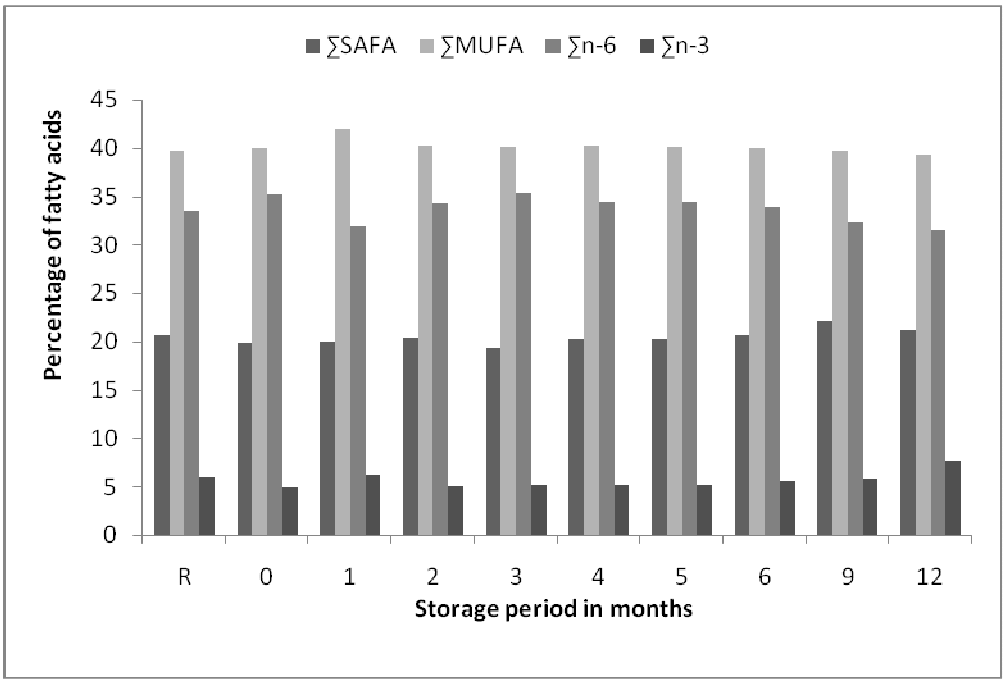

Fig. 4: Changes in fatty acid profile of sandwich paste of 2% PUFA treated during storage

The overall mean score in both control and treated tilapia sandwich paste were found to be good even at the end of 360 days of storage and there was no significant difference in the taste & overall acceptability. Fortification of fish sandwich paste with cod liver oil had no impact on its quality. Similar observations were reported by Evancho *et al.* (1973) and Mallick *et al.* (2006).

Commercial Sterility

The Sandwich paste processed at either of the temperatures, whether control or treated showed no growth after processing and during the storage at ambient temperature, which indicates that the process given to the products, was sufficient to attain sterility.

Conclusion

The study demonstrated that the temperature of 121.1^{o}C and F_0 value of 8 and cook value of 75.02 min were optimum for thermal processing of tilapia sandwich paste. A convenient ready to serve thermal processed tilapia sandwich paste fortified with PUFA can be prepared in tilapia mince using retort pouches that remain in good quality after a period of 1 year at ambient temperature. Thus, the fortification of PUFA in the tilapia sandwich paste in retort pouch did not damage the n-3 and n-6 fatty acids during retorting and storage.

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