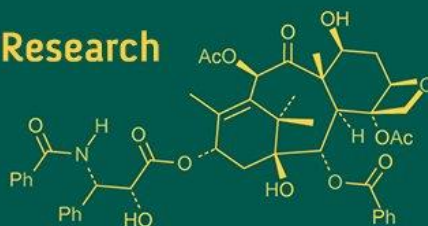


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Extraction of tuna head oil by wet rendering operation and the effect of turmeric rhizome oil on it's quality and storage stability

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Abstract

Euthynnus affinis or kawakawa is known for its high polyunsaturated fatty acids and protein content. The present work evaluated the quality of crude oil obtained from tuna head which is often discarded as waste. The head of mackerel tuna collected were processed for oil extraction using wet rendering process. The crude oil was stored in an amber color bottle and stored at room temperature for evaluating its shelf life. One of the oil samples was incorporated with the turmeric rhizome oil to observe for its quality changes during storage. The physical property like refractive index and the biochemical parameters like PV, FFA, p-anisidine value and TOTOX value were evaluated at periodic intervals during the storage. The fatty acid profile of the crude oil as control and the turmeric rhizome oil as the treatment sample were evaluated once during the storage period. This study focus on using a natural method for extending the shelf life of highly perishable product like fish oil to pave way forward in elucidating the chemical interaction of a natural product in resolving the issue of limited shelf life of Marine lipids in nature.

Keywords: Curcumin, tuna, oil

Introduction

Marine oils refer to fish liver, fish body, marine mammal, crustacean, and cephalopod oils. Fish body oils are the most significant product in this category (Jacobsen *et al.*, 2022) ^[1]. Major sources for the production of fish oils are tuna, anchovies, sea squirt, freshwater crap, sprat, blue whiting, Alaska pollock, pilchard, capelin, menhaden and sardine (Hashim *et al.*, 2021) ^[2]. Fish species have varying oil levels depending on species, age, gender, location, and various environmental factors, including temperature and species origin characteristics such as spawning or migration seasons. Fish by-products are rich in nutrients and bioactive components like proteins and omega-3 polyunsaturated fatty acids. So, fish oil can be extracted from the heads, frames, skins, trimmings, blood, scales, air bladders and other underutilized fish parts (Hashim *et al.*, 2021) ^[2].

Good-quality fresh raw materials and a suitable extraction method are the prerequisites for producing high-quality fish oil (Ahmed *et al.*, 2017) ^[3]. The most common methods for the production of fish oils are the wet rendering method. Furthermore, the fish lipids are extremely vulnerable to oxidation due to the higher

number of double bonds in PUFAs and their location in the fatty acid chain.

Herbs were experimented since traditional days to treat various illness and diseases. Turmeric is an ancient herb mentioned in the Vedic literatures of India, nearly 4000 years ago, where the product was popular for its medicinal value. Belonging to the perennial herbaceous plant of zingiberaceae family, the tuberous rhizome of the plant has been exploited much for its use in culinary, aromatics, food dyes and medicinal industries. The brilliant yellow colour of the spice has given it the title of "Indian saffron" and there are about 3000 publications dealing with turmeric in the modern science since quarter past a century (Benzie and Wachtel, 2011) ^[4]. The significance of this herb in science has led researchers to get into the deeper insights about its structure, function and properties that Unraveled its chemistry and molecular structure.

Curcumin is the active component of the herb turmeric which has versatile properties in treating a wide range of discomfort right from anti inflammation to anti-cancerous properties. Structurally, the compound is 1,7-bis (4- (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, otherwise known as “curcumin I” (Sharifi *et al.*, 2010) [5]. This chemical structure was first elucidated by polish scientists Milobedzka, von Kostanecki and Lampe. For nutritional purposes, curcumin is used at a dose of 5-500 mg/kg based on the type of food and varies widely with the type of foods like dairy products, beverages, cereals, confectionary, meat, eggs and fish (Lakshmi 2014., Solymosi *et al.*, 2015). Present article will focus more on the preservative action of curcumin and turmeric extract on foods with special reference to seafood.

Bioactive Properties of Turmeric Essential Oil

The versatile properties of turmeric as an antioxidant, anti-inflammatory, anti-carcinogenic or other bioactive properties are based upon the composition of the end product. Essential oils are the concentrates of hydrophobic constituents from plants which contain volatile chemical compounds which are basically volatile in nature. Term “Essential oil” is often mis interpreted for its essentiality in food, although the word resembles so. The essential oils are referred to as the “essence of” the specific plant’s fragrance. The common methods of extraction of essential oils involves processes such as distillation, solvent extraction, cold pressing, absolute oil extraction, resin wax embedding and tapping. Turmeric essential oil also carries the essence of the plant and is notable with the properties exhibited by the same. The volatile compound analysis in *C. longa* essential oil by GCMS and NMR reported about 81.2% essential oil components in the same (Avanço *et al.*, 2017) [6]. The main components noted belonged to the terpene class such as sesquiterpenes and the monoterpenes. Terpenes are the most diverse group of organic compounds in nature which are classified based on the number of isoprene unit (functional unit of terpenes) as mono, di, tri, tetra or sesquiterpenes. They form the major component of all essential oils from plants and occur as lipids in nature. Out of the several 100s of compounds isolated from turmeric, the main component of the rhizome contains turmerone and other curcuminoids. These curcuminoids contain curcumin which is known to be a natural antioxidant. The compositional analysis of turmeric has reported a moisture content of > 9%, curcumin (5-6.6%) and volatile oil (<3.5%) (Prasad *et al.*, 2011). The bioactive properties exhibited by the turmeric essential oil are attributed to the various components that are made available in the extraction process. There was a significant antioxidant power exhibited by the turmeric oil using the radical scavenging assay and ferric reducing antioxidant power test (Fernández *et al.*, 2021 and Ivanović *et al.*, 2021). Turmeric oil is rich in bioactive compounds like ar-turmerone, α -turmerone, curone, and others, which contribute to its therapeutic value (Sahoo *et al.*, 2019 and Devkota *et al.*, 2015). Additionally, it plays a role in food preservation, acting as a natural emulsifier and antimicrobial agent in packaging materials. (Tongnuanchan, P. and Benjakul, S., 2014, báñez and Blázquez, 2020).

With this background turmeric was selected as the natural antioxidant to evaluate the quality and stability of marine oil in the present work.

2. Material and method

2.1 Samples and chemicals

Fresh Tuna (*Euthynnus affinis*) were procured from Sassoon dock Landing centre Mumbai, were transported to the laboratory in an ice box with fish-to-ice ratio of 1:1. Fish samples were washed thoroughly with potable tap water and dressed to separate the head, viscera, and skin. All the body parts (Plate 1) were separately sealed in polythene bags, labelled, and kept in a deep freezer (Blue Star, Mumbai, India) at -20 °C until further use. All reagents used were of analytical grade and obtained from Merck, Hi-media and Qualigens. All glasswares used in the experiment were manufactured by Borosil.

2.2 Extraction of oil from turmeric rhizome.

Turmeric rhizome oil was extracted by the hydro-distillation of 200 g of fresh rhizomes using a Clevenger apparatus. The rhizomes were washed thoroughly in running water to remove the mud and dirt adhered to it, followed by drying using a paper towel. The samples were grated finely and placed in a 1000 mL round bottom flask with 500 mL of distilled water. The extraction was done in a Clevenger apparatus for 3-5 h. (Prakash *et al.*, 2012). The extracted oil was stored at 4 °C and protected from light prior to chemical analysis and use. The extracted oil were not profiled before incorporation and hence, it may contain other volatile components.

2.3 Incorporation of the rhizome oil into tuna head oil to evaluate its quality.

The mackerel tuna (*Euthynnus affinis*) head oil prepared by the wet rendering process was subjected for storage study at room temperature to evaluate its quality. Turmeric rhizome oil at 0.3% (w/v) concentration was added as a natural antioxidant to one of the sample as treatment, and the other untreated crude oil served as the control.

2.4 Compositional analysis and quality characteristics of little tuna (e.affinis)

The gross compositional analysis (moisture, crude protein, fat, and ash content) of fresh fish muscle was carried out using the following standard methods (AOAC 2005) and the quality characteristics (TVBN-Malle and Tao (1987), TMA-EU-EC, 2008, TPC -BAM, 2004 of fresh tuna) were evaluated during the study.

2.5 Fat oxidation parameters

The fat oxidation parameters like iodine value, Free fatty acid and Peroxide value and the Total oxidation (TOTOX) were done by various biochemical analysis. (AOAC, 2005).

2.6 Refractive index of oil

Refractive index was measured by Abbe refractometer. The refractometer was calibrated with standard and water before use. The prisms were cleaned with acetone and dried. Few drops of the sample were placed on the prism. Then the prisms were closed and allow standing for 1-2 min. The instrument and lighting was adjusted so as to obtain the most distinct reading possible. After recording the measurement, wipe the prism with tissue to remove the oil and wipe with acetone to clean the prism for the next sample analysis.

2.7 Fatty acid profiling

The prepared FAMES samples were given for outsourcing to determine the fatty acid profile. The samples were

analysed at Envirocare Labs (NABL Certificate No. PC-1041), Thane, Mumbai. AOAC 996.06, 22nd Edition: 2023 was the method followed by the lab for GC-MS analysis of samples.

2.8 Sensory analysis

Sensory analysis was done as per the method by Serfert *et al.* (2010). Vocabulary and attributes of oils, as well as their references, were selected according to the standard practice for sensory evaluation of edible oils and fats. The attributes green, sweet/biscuit-like, metallic, pungent and fishy were selected. The sensory panel consisted of 10 members from our department, including students and staff, who were instructed well about the procedure of sensory evaluation of oils. Oil samples were kept in small glass tubes with cap for evaluating their odour and colour. The panellists were instructed to gently swirl the covered sensory glass and to subsequently uncap the glass and sniff the head-space of the sample with short sniffs and to replace the cover of the glass quickly. The panellists cleared their nasal passage between samples by 1 min resting. Reference samples for each odour were also provided in order to distinguish the odours. They were given sensory score sheets to give scores for odours and colours. The intensities of the odour attributes were evaluated by an intensity scale (0-5) and triangle tests in duplicate. Denotations of the scores of the intensity scale were as follows: 0 = not perceptible, 1 = very slightly perceptible, 2 = slightly perceptible, 3 = considerably perceptible, 4 = strongly perceptible, 5 = very strongly perceptible. For colour analysis also 0-5 scale of yellow colour was given.

2.9 Statistical analysis Results were analysed statistically using Statistical package for social science software (SPSS VERSION 16.0, Chicago IL, USA). Analysis of Variance was carried out and the significant difference among treatments was determined by Duncan's Multiple Range Test (DMRT). The level of significance was set up at $p \leq 0.05$. All the experiments were carried out in triplicates and results were expressed as Mean $\pm$ S.D.

3. Result and Discussion

3.1 Compositional analysis and the quality characteristics of little tuna (*Euthynnus affinis*)

The standard length (in cm) and weight (in Kg) of the selected tuna species for study, the quality characteristics of fresh tuna. The length range of little tuna varied from 14cm to 80 cm along the Indian coast (Rohit *et al.*, 2012). In the present study, the mean, standard length and weight of the little tuna were 38.45 cm and 1.01 Kg, respectively. The size at first maturity for *E. affinis* was estimated at 37.7 cm (Rohit *et al.*, 2012). The fishery along the Maharashtra coast was supported by fishes with a length range of 26-73 cm (Khan, 2004). Tuna is generally processed as raw meat and is mainly marketed as loins or steaks, and the by-products from the species are constituted of skins, heads, bone, viscera and leftover muscle (Gamarro *et al.*, 2013). In the present study, the body composition of *E. affinis* was observed to be constituted by red meat, which has the highest yield (47%), followed by the head (22%). Viscera, dark meat, skin and bone has got 6%, 10%, 6% and 10% yield respectively. Murthy *et al.* (2014) reported that the fillet yield of *Thunnus albacares* is estimated as 45% and the rest (55%) consists of belly flap, bone, skin, tail, gut, and head have great scope as by products. The proximate

composition of different body parts of tuna. The results are in agreement with previous works of Khodammi *et al.* (2012) who observed moisture content as 68.79% in *E. affinis* head. The ash content were reported as 4.77% by the author, in the present study tuna head had higher ash content of 6.2%. Lipid content was 11.68% for the tuna head which is significantly higher in comparison to the edible portion that has 0.52% of fat. Liu *et al.* (2014) reported 0.53% and 1.52% crude lipid from the red and dark meat of *E. affinis* respectively.

3.2 Quality characteristics of tuna head oil and the effect of turmeric rhizome oil on the quality and stability of the crude oil

The yield of the tuna head oil is depicted. Oil or fat could not be extracted from either the dark meat or red meat of tuna. The results indicate that, tuna heads are a major source of oil by wet rendering operation. Nazir *et al.* (2017) also reported that the wet rendering extraction method is the most effective and most potential extraction method to be applied because it produces the highest yield (12.80%) compared to the silage process (6.16%) and solvent extraction method (8.49%). During the storage period of tuna head oil, various biochemical parameters like PV, FFA, p-Anisidine, TOTOX value and iodine value were evaluated at a periodic interval of 3 days each. In addition refractive indices of the two oil samples were also evaluated during the storage period. The samples were stored in closed amber colored bottle and kept at room temperature during the storage period. The PV, FFA, p-anisidine value and TOTOX value significantly increased with storage days for both the control and treatment sample. However, the increase in oxidation parameters was less for turmeric rhizome oil incorporated sample when compared to the control. The iodine value and refractive index for treatment samples were higher than the control, indicating its higher degree of unsaturation. Based on limits for chemical parameters, the control samples were rejected on the 6th day, however the treatment samples was observed to have an extended shelf life until the samples reached the rejection limit for the biochemical and sensory parameters by 12th day of storage.

3.3 Sensory score of fish oil treated with turmeric rhizome oil

Sensory evaluation was carried out during the storage study to compare the odour profiles of crude fish oil and fish oil enriched with turmeric essential oil. The analysis followed the method of Serfert *et al.* (2010), using descriptors based on ASTM E1627-94 (2004). Selected attributes included green, sweet/biscuit-like, metallic, pungent, and fishy odour and the results are represented in the form of a radar diagram. During the storage period, the score for the metallic, fishy and green odour attributes decreased significantly and the score for pungent odour increased significantly for the control sample. Whereas there was no significant decrease in score of spicy and sweet odour attributes of control, which was low from initial days itself. In the case of the treatment group, there was no significant change in the scores for metallic, green and sweet/biscuit-like odour attributes. The scores for green and spicy attributes were higher for treatment compared to control, whereas the scores for metallic, fishy, sweet, and pungent attributes were low for treatment compared to control during storage study. According to the panelists, crude oil's fishy

odour was masked by essential oil's odours. Even though the control and treatment samples were rejected on the 6th and 12th day, respectively, due to exceeding the limits of chemical parameters, the treatment samples were acceptable in terms of sensory attributes even up to the last day of storage. Throughout the storage period, the control oil exhibited a significant decline in green, metallic, and fishy odour attributes, alongside a notable increase in pungency. In contrast, the turmeric oil-treated samples consistently showed higher scores for green and spicy notes, while metallic, fishy, sweet, and pungent attributes were comparatively lower. Although the treated samples were deemed unacceptable by day 12, they demonstrated superior sensory characteristics overall. The incorporation of turmeric essential oil effectively masked undesirable fishy odours, thereby improving the sensory acceptability of the oil.

3.4 Fatty acid profiling of the crude fish oil and the turmeric rhizome oil incorporated fish oil

The saturated fatty acid content was comparable between the control (39.97%) and the treatment (40.60%) samples. Monounsaturated fatty acids (MUFA) were higher in the control (26.25%) than in the treatment (16.80%), whereas the polyunsaturated fatty acid (PUFA) content was notably greater in the treatment (42.60%) compared to the control (33.78%). The treatment sample also exhibited higher levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Among saturated fatty acids, palmitic acid was predominant in both samples. Palmitoleic acid was identified as the principal MUFA, while DHA was the most abundant PUFA, accounting for 19.41% in the control and 22.72% in the treatment, followed by EPA. According to the U.S. Food and Drug Administration (FDA), an optimal dietary omega-6 to omega-3 fatty acid ratio should be approximately 4:1 or lower. In the present study, the omega-3 to omega-6 ratios were 0.73:29.84 for the control and 1.97:33.67 for the treatment, indicating a relatively improved omega-3 profile in the treated oil. Both samples were rich in polyunsaturated fatty acids (PUFAs), highlighting their potential nutritional benefits. Notably, the incorporation of turmeric rhizome oil led to a higher PUFA content in the treated oil compared to the control. This enhancement may be attributed to the antioxidant activity of turmeric oil, which could have mitigated the oxidative degradation of unsaturated fatty acids during storage.

4. Conclusion

The medicinal properties and the protective mechanism of natural herbs have been well exploited since ancient days. However incorporation of the turmeric oil into a compatible form like fish oil which is highly perishable needs to be studied further for its microbial quality and shelf life. Further, insights into the chemical interaction of the curcumin component in the essential oil with the fatty acids in oil would break open the chemistry and resolve the issues of oxidative rancidity in seafood. Hence, a greater knowledge on the use of such indigenous technology for the sustainable use of seafood needs to be given a wider attention of research.

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