INFLUENCE OF EXTRACTION METHODS ON QUALITY OF SHARK LIVER OILS

by

C.V.L. JAYASINGHE*, W.M.K. PERERA ▫ and A. BAMUNUARACHCHI°

• Institute of Post Harvest Technology, National Aquatic Resources Research and Development Agency, Crow Island, Mattakkuliya, Colombo 15, Sri Lanka.

▫ Department of Nutrition and Community Resources Management, Wayamba Campus University of Rajarata, Kuliyapitiya, Sri Lanka.

° Department of Chemistry, Faculty of Applied science, University of Sri Jayawardenapura Nugegoda, Sri Lanka.

ABSTRACT

Shark livers are considered as waste material at most landing sites in Sri Lanka, but they can be utilized as raw material for extraction of fish oil which in turn is useful for medicinal and food purposes. This study evaluated oil extraction methods namely, steam rendering, acid silage, wet rendering, incubation at 30°C and alkali digestion with 2% NaOH at 80°C of silky shark (*Charcarinus falciformis*) livers. Observations were made on the yield, free fatty acid (FFA) content, peroxide value (PV) and thio-barbaturic acid value (TBAV) of the extracts. Results were compared with the oil extracted using the Bligh and Dyer method.

The highest oil yield (44%) was reported by the silage method and this value was not significantly different ($p<0.05$) when compared with that from incubation (43.7%) and steam rendering (41%). The lowest oil yield (25.5%) was obtained from alkali digestion and the value obtained (33.1%) from wet rendering was intermediate. The lowest free fatty acid value (0.04%) was recorded by the alkali digestion and this value was not significantly different ($p<0.05$) when compared with that from wet rendering (0.12%), steam rendering (0.2%) and incubation (0.6%). However, silage gave the highest free fatty acid value (4.2%). Based on the oil yield and the quality, it could be suggested that extraction of oil using steam rendering and silage are suitable for introduction as a small scale industry for coastal communities in Sri Lanka.

INTRODUCTION

In 1997 total marine fish production in Sri Lanka was 248 000mt (Anon., 1998). Sharks contributed 13% to the total. They are a valuable fish as almost every part of the body can be utilized. However, shark livers are presently wasted. Livers contain 30 -75% of oil which is rich in omega -3 poly-unsaturated fatty acids (Jayasinghe *et al.*, 1998). Fish liver oil with its high vitamin A and D levels has been used to prevent night blindness and rickets (Hall, 1992). Liver oil is used in medicinal, food and industrial purposes. Oil composition varies considerably with a number of factors such as species, age, sex and season (Jayasinghe *et al.*, 1998). Different methods are used to extract oils from shark livers (Tanikawa, 1971; Govindan, 1985). The suitability of the extraction methods for a cottage industry has not been assessed. The aim of this study was to compare the available liver oil extraction methods to find out a suitable method for the rural community.

EXPERIMENTAL

Assessment of liver oil extraction techniques

Different oil extraction methods such as acid silage, wet rendering, alkali digestion, steam rendering, incubation and Bligh and Dyer (1959) methods were carried out. Silky shark (*C. falciformis)* liver was minced using a blender (Sumeet, India). Minced liver was divided into 100g portions and the following techniques were applied to extract oil. Yield of oil was measured and quality of oil was assessed by determination of free fatty acid value (AOCS 1992), peroxide value (AOCS, 1992) and thio barbaturic acid value (AOCS, 1990) a few hours after extraction.

Extraction techniques

All methods were tried out in triplicate.

(a) Bligh and Dyer method (control)

The quantity and quality of oil extracted using the Bligh and Dyer method (1959) was used as a control to compare the efficiency of other extraction methods.

(b) Acid silage method (Jayawardena *et al*, 1980).

Added 3.5% (w/w) formic acid to 100 g minced liver and mixed thoroughly for 10 min. Mixtures were kept for 24 hours at room temperature (28±1°C) and the oil was separated by centrifugation (MISTRAL 2000) at 2000 rpm for 10 minutes.

(c) Wet rendering method

Minced liver was mixed with water (20%) and boiled for 30 minutes. The oil was separated by centrifugation.

(d) Alkali digestion method

Added 10% water to liver mince and digested at 40-50°C until liver in liquefied. Then added 2% sodium hydroxide and continued heating at 80°C with pH adjusted to 9. Washed excess alkali with warm water and the oil was separated by centrifugation.

(e) Steam rendering at 95°C for 30 minutes.

Minced liver was placed in a round bottom flask (1000ml) and steam passed through the sample for 2 hrs. The resulting mixture was centrifuged at 2000 rpm for 10 minutes to obtain oil.

(f) Incubation at 30°C for 48 hours

Liver mince was kept in an incubator (Blue, USA.) at 30°C for 48 hours and the oil was separated by centrifugation.

Statistical Analysis (Zar, 1984)

A one-way analysis of variance (ANOVA) with replicates was carried out independently to study the quality of shark liver oil. Significance was accepted at a probability of 5% or less. Bonferroni's Multiple Comparison was used to identify the means, which were significantly different. All means were presented with their standard errors (SEM).

Results:

Table 1. Analysis of crude silky shark liver oil extracted with different techniques.

Parameter	Control (Bligh & Dyer)	Silage	Wet rendering	Alkali digestion	Steam rendering	Incubation
Yield %	63.3 ± 1.3^{1}	$44.3^{a}\pm1.0$	$33.1^{b}\pm0.8$	$25.5^{e}\pm0.7$	$41.7^{a}\pm0.8$	$43.7^{a}\pm0.9$
FFA %	0.6 ± 0.1	$4.2^{a}\pm0.1$	$0.1^{b}\pm0.0$	$0.04^{b}\pm0.0$	$0.2^{b}\pm0.0$	$0.6^{b}\pm0.0$
Peroxide (meq peroxide/g)	1.7 ± 0.0	$6.1^{b}\pm0.1$	$9.1^{a}\pm0.2$	$9.7^{a}\pm0.2$	$1.7^{c}\pm0.0$	$0.02^{d}\pm0.0$
Thio barbaturic acid value	-	$304.6^{bc}\pm5.7$	$245.3^{d}\pm5.8$	$620^{a}\pm10.0$	$274.3^{cd}\pm6.6$	$335.1^{b}\pm8.6$
Refractive index	-	1.48	1.48	1.48	1.48	1.48

[1] *Values in the same row not sharing the same superscript letters differed significantly ($p<0.05$), when analyzed by the Bonferroni's test.*

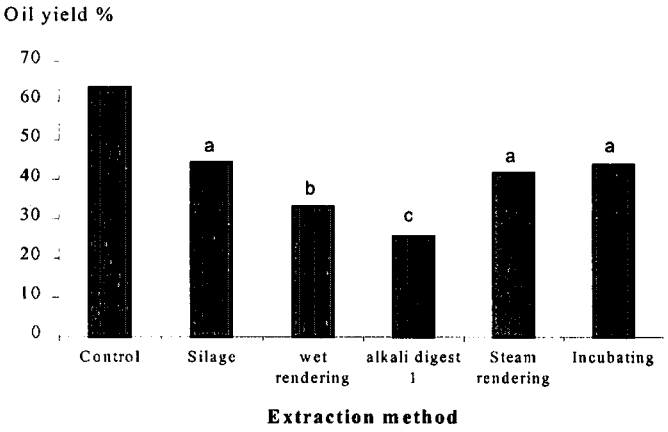

Fig. 1. Oil yields of different extraction methods.

Results of the analysis of oil, using different extraction techniques, are presented in table 1. Control (chloroform -methanol extraction method) recorded the highest oil yield 63.3±1.3%. The value given by acid silage method (44.3±1.0) was not significantly different ($p<0.05$) when compared with incubation (43.7±0.9) and steam rendering methods (41.7±0.8). Lowest oil yield was reported from alkali digestion method (25.5±0.7). Wet rendering method showed an intermediate value (33.1±0.8) (Table 1 and Figure1).

The lowest free fatty acid value was recorded from oil recovered by alkali digestion (0.04%) and this value was not significantly different ($P<0.05$) when compared to wet rendering (0.12%), steam rendering (0.18%) and incubation (0.58%). However, silage recorded the significantly highest (4.2%) free fatty acid value compared to other methods used in the experiment. Extraction methods where heat was applied such as wet rendering, alkali digestion, steam rendering and incubation showed low FFA values compared to non heating methods (Fig.2).

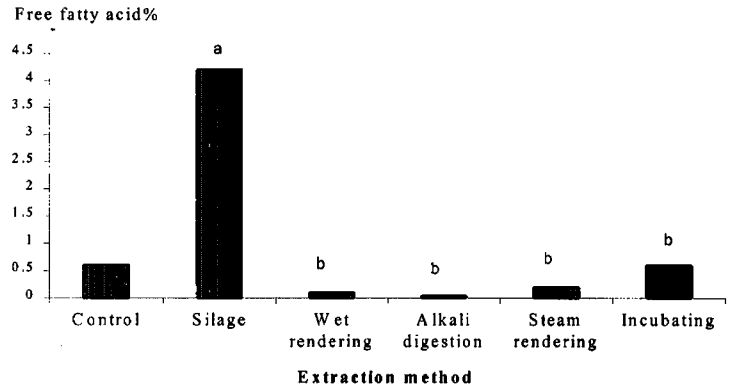

Fig. 2. Free fatty acid values of oils from different extraction methods.

Significantly ($p<0.05$) lower peroxide values were recorded by incubation 0.02 (meq peroxides/g) compared to other methods described in this experiment (Fig. 3). The highest peroxide value 9.7 (meq peroxides/g) was shown by alkali digestion and this value was not significantly ($P<0.05$) different with compared to the value (9.1 meq peroxides/g) given by wet rendering. Steam rendering and silage showed intermediate values 1.7 and 6.1 (meq peroxides/g) respectively.

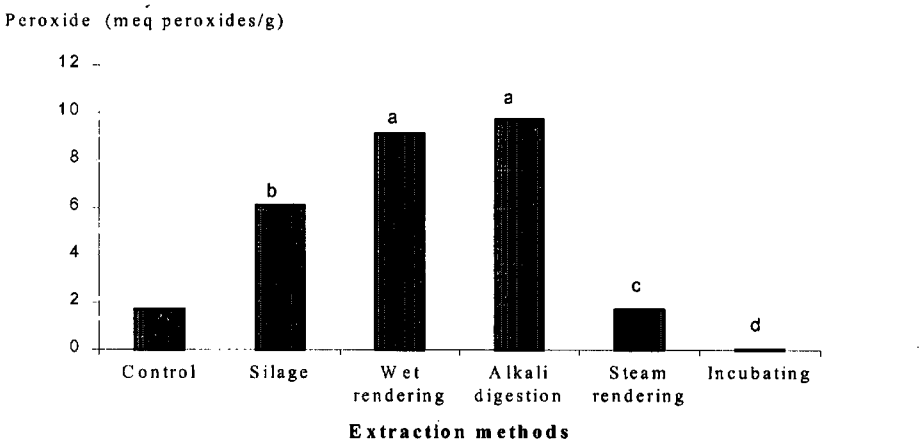

Fig. 3. Peroxide values of oils from different extraction methods.

Denaturing of peroxides was measured by thio barbaturic acid value. The lowest TBA value was shown by oil recovered from wet rendering (245) but this value was not significantly different ($p<0.05$) from oil recovered by steam rendering (274) (Fig.4). Significantly highest ($P<0.05$) TBA values were reported by alkali digestion (620) compared to other methods used in this experiment. However, silage and incubation showed intermediate values (304 and 335).

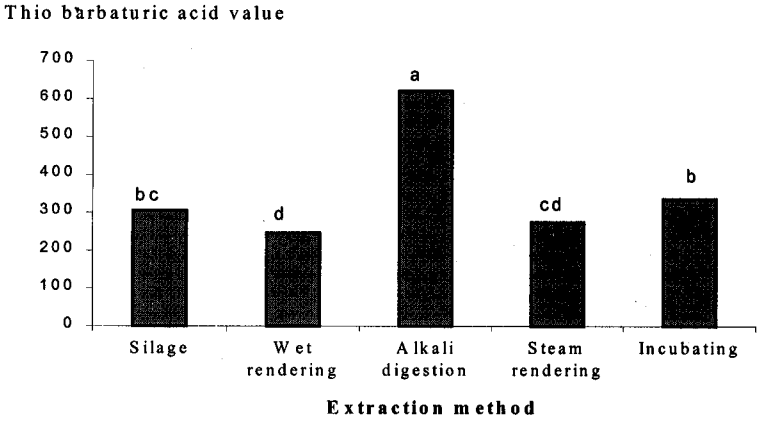

Fig. 4. Thiobarbaturic acid values of oil from different extraction methods.

Refractive index values of recovered oil were identical in all treatments. It showed that the density of the oil does not depend on the procedures, which were used to extract oil.

DISCUSSION

In this study chloroform - methanol extraction recorded the highest oil yield. These results are accordance with the Sunarya *et al.*, (1991), who reported that the oil yields from Bligh and Dyer (chloroform-nethanol extraction) and Soxhlet extraction were similar and higher than from steaming. As the organic chemicals have been used in the extraction process, this method is not recommended to extract oil for food and feed purposes. Acid silage, incubating and steam rendering method recorded the second highest oil yield. It has been explained that steaming results in the thermal rupture of the liver cells and so releases the oil. But, the oil, which is more closely held by the proteinaceous liver tissues, is not released under these conditions (Sunarya *et al.*, 1991; Hall, 1992). If fish oils are obtained from livers by means of wet rendering, a large quantity of vitamin A would remain in the residues left after pressing (Tanikawa, 1971; Govindan 1985). They also described that the amount of oil left in the screening or pressing is 10-30% of the total amount of oil in the raw livers. Therefore, the processing of vitamin rich oil by wet rendering is not recommended. Long processing procedures may also result in low oil yield (Tanikawa, 1971). Oil yield from alkali digestion in this study confirmed the above statement. However, it has been reported that the yield of vitamin A secured by alkali digestion is 70 - 95% of that in raw livers (Tanikawa, 1971).

The highest free fatty acid percentage in oil was recorded from acid ensilage. This may be due to an addition of acid in the processing technique. However, results on the free fatty acid content of the recovered oil suggested that heat treatments might produce low FFA values. A similar observation has been made by Presten (1986) who reported that the oil recovered by heat rendering gives a low free fatty acid and the resulting crude oil is non perishable. Results of the present study confirmed the findings of Summers *et al* (1991). The author demonstrated that the oil produced by heat treatment has a very low percentage of free fatty acids, and no peroxides. The absence of oxidizing peroxides may be advantageous to the quality of the crude oil during long-term storage. Furthermore, Presten (1986) explained that oil obtained by heat rendering could be stored for a long period under tropical conditions without subsequent loss of quality.

Autoxidation and oxidation of oil result in the formation of malonaldehyde, the development of which can be monitored analytically by the determination of thio barbaturic acid value (Summers *et al*, 1992). The highest thio- barbaturic acid value was recorded by alkali digestion and it may be due to the involvement of

alkali in the processing. Steam rendering showed low oil oxidation. However Gopakumar and Thankappan (1986) suggested that the oil should be recovered by steam rendering and protected from atmospheric oxygen by continuous flushing with nitrogen.

According to the cost estimates of the present study it is suggested that steaming and silage methods could be used to extract shark liver oil for food and feed purposes. The present experiment supports the suggestions of Sunarya *et al.*, (1991) that oil extraction by steaming is easier, cheaper, quicker and can probably be introduced to rural communities. It has been reported that solvent extraction methods are not employed for the preparation of vitamin A oils from fish livers, because the equipment is expensive and the recovery of the solvent is not satisfactory (Tanikawa, 1971). Hall (1992) observed that direct steaming at 80-85°C is a simple and economical technique that involves direct steaming. Some procedures require temperature of 70 -75°C. Summers *et al* (1991) reported that the cheapest way of liver oil refining was to ensile the livers with a mixture of formic and phosphoric acids. The silage product is a stable liquid with a malty odour, which has very good storage characteristics. It is a simple process and requires a little capital investment, particularly if non-oily fish are used. The advantage of ensilage for recovering oil is that the neutralized liver slurry can be used as a stock feed or fertilizer (Summers *et al*, 1991).

The centrifuged oil probably contains suspended soap or excess alkali. Therefore, further centrifugation is necessary after washing with warm water (Tanikawa, 1971). The defect of the method is the loss of vitamin A and the decomposing and dissolving of water soluble vitamins which are contained in fish livers besides vitamin A (Tanikawa, 1971). Therefore, it can be assumed that the method of alkali digestion (10% water & 2% NaOH) is not suitable for high quality oil extraction. Kreuzer and Ahmed (1978) have reported contrary results. They suggested that oil extraction by alkali digestion, the addition of 2% (w/v) sodium hydroxide and heating at 80°C for 30 to 45 minutes was very effective.

Silage also can be used in more technologically advanced fisheries. For example, the livers can be ensiled at sea or at the shore at the time of separating livers from the sharks and then subjected to oil extraction (Windsor and Barlow, 1981). Clucas and Sutcliffe (1981) reported that livers with high oil content are usually steamed (85°C) or indirectly heated at 71°C.

Livers with low oil content are treated with alkali, alkali/enzyme digestion and solvent extraction. Excessive heating must be avoided. Since vitamin A is inactivated by light, the oil must be stored in the dark. Hall (1992) recorded that the current level of alkali is important to prevent saponification of fat, and vitamin A absorption in the soap fraction. However, the result of this study suggests that steam rendering and ensilage methods can be used to recover liver oil for food and feed purposes.

Based on the quality and yield of liver oil, it is suggested that steam rendering and silage methods are suitable for extraction of liver oil. This study opens an avenue for a small- scale liver oil production industry among coastal communities in Sri Lanka.

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