J Am Oil Chem Soc (2011) 88:589–593
DOI 10.1007/s11746-010-1689-4

ORIGINAL PAPER

Production of PUFA Concentrates from Poultry and Fish Processing Waste

Dipak Patil · Ahindra Nag

Received: 4 June 2010 / Revised: 29 August 2010 / Accepted: 27 September 2010 / Published online: 15 October 2010

Abstract In fish and poultry processing, viscera are generally considered as a waste product and often discarded. Chicken and hilsa fish (*Hilsa ilisa*) viscera were used for the production of polyunsaturated fatty acids (PUFA) linoleic (18:2n-6), eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). Free fatty acids (FFA) were extracted by alkaline hydrolysis of chicken and fish viscera; yields were 5.2 and 5.9% (w/w) respectively. PUFA concentrates were obtained by a two step process—deduction of saturated fatty acids (FA) by low temperature crystallization in acetone followed by urea inclusion compound-based fractionation. Acetone treatment removed 90 and 96% of saturated FA in chicken and fish viscera respectively with FA to acetone ratio of 1:12 (w/v). Using an urea to FA ratio (w/w) of 4.0, chicken viscera produced a maximum of 84.1% of PUFA concentrates containing 82.1% of linoleic acid with a yield of 10% where as in the case of fish viscera the maximum PUFA concentrates were 91.3% containing 78.2% of EPA-DHA with the yield of 11%. Thus, the utilization of poultry and fish processing waste into the production of PUFA concentrates has been shown.

Keywords Viscera · Urea complex fractionation · Polyunsaturated fatty acid

Electronic supplementary material The online version of this article (doi:10.1007/s11746-010-1689-4) contains supplementary material, which is available to authorized users.

D. Patil · A. Nag (✉)
Department of Chemistry, Indian Institute of Technology, Kharagpur 721302, India
e-mail: ahinnag@chem.iitkgp.ernet.in

Introduction

PUFA especially eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), have been recognized for their biochemical role in health for the prevention and treatment of several human diseases [1]. Similarly, absence of linoleic acid (LA; 18:2n-6) which is an essential fatty acid (FA), in the diet is responsible for the development of abnormalities such as diabetic neuropathy rheumatoid arthritis, reproductive and autoimmune disorders [2, 3].

Different methods are used for the production of PUFA concentrates [4]. This includes supercritical fluid extraction [5] in which the separation depends on the molecular size. In the enzymatic method [6], the enzyme cannot hydrolyze the ester bonds of EPA and DHA due to steric hindrance resulting from five and six double bonds respectively. Urea complexation [7] is a simple and efficient technique in which the extent of linearity in the structure of FA assists in the separation by getting trapped as a guest molecule in the urea complex. Unsaturated FA are non-linear molecules due to the presence of all *cis* bonds whereas saturated and monounsaturated FA have an almost linear structure and hence the latter can be easily trapped by the cavity of the urea complex [8]. The advantage of a urea complexation method is that crystals are extremely stable and filtration does not necessarily have to be carried out at a very low temperature which solvent crystallization of FA would require [9].

The disposal of by-products generated from fish and poultry processing is becoming a major problem for industries causing environmental pollution and loss of valuable nutrients [10]. The waste products of fish processing often end up in landfills and consist of skin, heads, frames and viscera. The average weight of hilsa fish viscera

is about 7% of the total weight. In India chicken viscera are discarded in large quantities (>1,80,000 ton/year) which constitute nearly 30% of the total wastes [11]. A few studies have shown that this processing waste could be used as an animal feed [12–14] and also for the production of oil [15] or biodiesel [16]. Although reports are available on the FA profile of chicken viscera [17] and hilsa fish oil [18], isolation of PUFA from these materials is as yet unexplored.

In the present study, FFA were extracted by alkaline hydrolysis of chicken and fish viscera. The extracted FFA were used for the production of PUFA concentrates by low temperature crystallization using acetone followed by urea fractionation. Ultimately, the objective of this study was to produce PUFA concentrates from the processing waste viscera, by a two step process.

Materials and Methods

Materials

Chicken and hilsa (*Hilsa ilisa*) fish viscera were collected from the local market at Kharagpur, West Bengal, India. Fatty acid methyl ester standards (Supelco TM 37 Component FAME Mix, Catalog No. 47885-U) and BF_3-methanol reagent (14% BF3 in CH3OH, w/v) were purchased from Sigma–Aldrich Chemical Co. Inc (St. Louis, MO). Urea and other solvents were purchased from Sisco Research Laboratory, Mumbai, India.

Extraction of FFA from Viscera

Extraction of FFA from chicken and fish viscera was carried out by alkaline hydrolysis. Viscera (100 g) were added to freshly prepared solutions of sodium hydroxide (0.75 mol) in a mixture of water (80 mL) and ethanol (120 mL). The mixture was refluxed under an inert atmosphere for 2 h at 76–78 °C. The reaction mixture was cooled to room temperature and carefully acidified with HCl (4 N) to acidic pH (2–3) in a cold water bath. The resulting FFA was extracted once with a 1:1 mixture of hexane:diethyl ether. The organic layer was washed with water thoroughly to a neutral pH and dried over anhydrous sodium sulfate. The organic layer was concentrated by rotary evaporation. Samples of three different chicken and fish viscera were analyzed using the above method.

Acetone Treatment

FFA extracted from three different batches of chicken and fish viscera were mixed and treated with acetone to remove saturated FA. The separation was carried out by dissolving the FFA in 12% (w/v) acetone followed by refrigeration at −10 °C for 12 h. The solution was filtered through a Buchner funnel and the FA were recovered from the filtrate by evaporating the solvent with a rotary evaporator. These FA were analyzed by Gas Chromatography (GC) and used for further purification by urea fractionation.

Urea Fractionation

Acetone treated FFA (2 g) was added to different amount of urea in 20 mL of 95% ethanol. The mixture was heated with constant stirring with magnetic stirrer to give the clear solution (74–76 °C). The urea–FA complex was allowed to crystallize at room temperature by occasional shaking to promote the formation of urea complexes. It was then kept in a refrigerator at 5–7 °C for 18 h. Urea–FA crystals were separated with a Buchner funnel and washed with non-complexing solvent (iso-octane) to remove traces of the filtrate. The filtrate was acidified with 4 N HCl followed by extraction with hexane:diethyl ether (1:1). The extracted organic layer was dried using anhydrous Na_2SO_4, concentrated on a rotary evaporator and further analyzed by GC.

FA Analysis

FFA, 50 mg were mixed with 2 mL BF_3-methanol reagent and refluxed for 2 min. After cooling the solution, 1–2 mL of water was added and then the FFA were extracted with hexane. Finally the solvent was evaporated to get the methyl ester. It was diluted with chloroform or hexane and injected into the GC, where the GC oven temperature was heated from 100 to 220 °C at 4 °C/min; Inlet and detector temperatures were 240 and 250 °C, respectively and a DB 5 capillary column was used. The FA ester peaks were identified and quantified by comparing their retention times and peak areas with those of the corresponding standard methyl esters.

Statistical Analysis

Results were expressed as the mean of three sets of experiments. The data expressed in tables was analyzed by one-way ANOVA (Windows Excel program) where the differences were considered to be significant at $P < 0.05$.

Results and Discussion

The FFA obtained from the extraction of chicken and fish viscera were 5.2 and 5.9% (w/w), respectively. The average FA profile of chicken viscera was found to be, C16:0 (16.5%), C18:0 (5.0%), C16:1 (6.1%), C18:1 (38.4%),

Table 1 Fatty acid composition (%) of chicken and fish viscera before and after acetone treatment

Viscera	Saturated	Mono-unsaturated	Total PUFA
Chicken	26.3 ± 2.59	53.8 ± 2.92	19.9 ± 0.81 (18.7 ± 0.79)[b]
Chicken[a]	3.9 ± 0.28	70.1 ± 4.03	26.0 ± 1.27 (25.4 ± 1.29)[b]
Fish	48.8 ± 3.61	38.9 ± 1.96	12.3 ± 1.00 (10.9 ± 0.84)[c]
Fish[a]	4.2 ± 0.39	73.0 ± 3.3	22.8 ± 1.69 (18.3 ± 1.47)[c]

[a] Fatty acid left after acetone treatment

[b] Linoleic acid

[c] EPA-DHA

C18:2 (17.8%) and others (16.2%) whereas of fish viscera it was found to be C16:0 (33.9%), C18:0 (8.9%), C16:1 (5.8%), C18:1 (28.3%), C20:5 (7.5%), C22:6 (3.4%) and others (12.2%). These FFA lipids contain saturated as well as unsaturated FA, in which palmitic and stearic acid are the main saturated FA present in both chicken and fish viscera. Based on previous reports [17, 19], acetone is the most suitable and commonly used solvent for separation of saturated and unsaturated FA. In this study the same solvent was also used to remove saturated FA by adopting the low-temperature crystallization method. Since saturated FA are solid in nature due to the compact packing of its straight chain rather than unsaturated FA which are liquid in nature, the former gets crystallized in acetone at low temperatures. The yield of FA (w/w) obtained after acetone treatment was 68% for chicken viscera and 49% for fish viscera. Table 1 includes the percentage composition of FA of chicken and fish viscera before and after acetone treatment.

Urea complexes were formed by addition of FFA to the solution of urea in alcohol. Exothermic complex formation was initiated by allowing the solution to cool; occasional shaking promoted the formation of hexagonal crystals with FA rather than the tetragonal crystals of pure urea. The amount of urea used for the crystallization removed the maximum amount of monounsaturated FA to leave a greater percentage of PUFA in the filtrate or non-urea complexed fraction (NUCF); however, the percentage recovery of NUCF diminished. In this present case urea was used to remove mainly monounsaturated FA which was left in solution after the acetone treatment. The degree of saturation in the FA molecule results its inclusion in the crystal lattice of urea; the more unsaturated, the less will be the possibility of their inclusion into the urea crystals [20].

Urea complexation with FA was carried out at various urea to FA ratios from 2 to 4 (Tables 2, 3) shows the reduction in the recovered saturated and monounsaturated FA with the increase in the urea level. In chicken and fish viscera, the decrease was up to 61–91 and 85–95% of saturated FA in the NUCF, similarly in the case of monounsaturated FA it was 87–98 and 96–99% respectively. Consequently there is an increase in the percentage of PUFA in NUCF. Using a 4:1 ratio of urea to FA, chicken

Table 2 Effect of urea/fatty acid ratio on fatty acid composition (%) of urea concentrates from chicken viscera

Urea/FFA (w/w)	2.0	2.5	3.0	3.5	4.0
Saturated	4.8 ± 0.07	4.3 ± 1.47	3.8 ± 0.52	3.7 ± 1.29	3.4 ± 0.12
Mono-unsaturated	27.5 ± 4.27	25.5 ± 2.41	24.7 ± 3.25	17.4 ± 3.01	12.5 ± 2.44
PUFA	67.7 ± 5.43	70.2 ± 4.27	71.5 ± 4.53	78.9 ± 2.08	84.1 ± 3.64
Linoleic acid	64.9 ± 0.84	66.7 ± 3.01	70.5 ± 3.87	77.0 ± 2.91	82.1 ± 2.79
Yield of NUCF (%)	**32 ± 3.6**	**22 ± 3.3**	**17 ± 1.5**	**13 ± 1.0**	10 ± 0.9
Recovery of linoleic acid in NUCF (%)	81.8	57.8	47.2	39.4	32.3

Significant differences are shown in bold ($P < 0.05$)

Table 3 Effect of urea/fatty acid ratio on fatty acid composition (%) of urea concentrates from fish viscera

Urea/FFA (w/w)	2.0	2.5	3.0	3.5	4.0
Saturated	3.0 ± 0.61	2.7 ± 1.71	2.6 ± 1.09	2.4 ± 2.71	1.8 ± 2.17
Mono-unsaturated	13.8 ± 2.91	10.9 ± 4.39	7.8 ± 1.93	7.7 ± 1.75	6.9 ± 4.27
PUFA	83.2 ± 4.36	86.4 ± 1.63	89.6 ± 2.56	89.9 ± 2.91	91.3 ± 4.71
EPA	45.9 ± 0.39	46.0 ± 1.39	44.9 ± 2.73	44.8 ± 0.25	43.4 ± 3.01
DHA	23.6 ± 1.51	26.8 ± 2.01	30.0 ± 1.75	31.6 ± 2.91	34.8 ± 3.36
EPA-DHA	69.5 ± 2.08	72.8 ± 4.09	74.9 ± 2.68	76.4 ± 4.81	78.2 ± 4.89
Yield of NUCF (%)	**21 ± 2.7**	**18 ± 2.8**	**15 ± 1.0**	13 ± 0.5	11 ± 0.6
Recovery of EPA-DHA in NUCF (%)	79.7	71.6	61.6	54.3	47.0

Significant differences are shown in bold ($P < 0.05$)

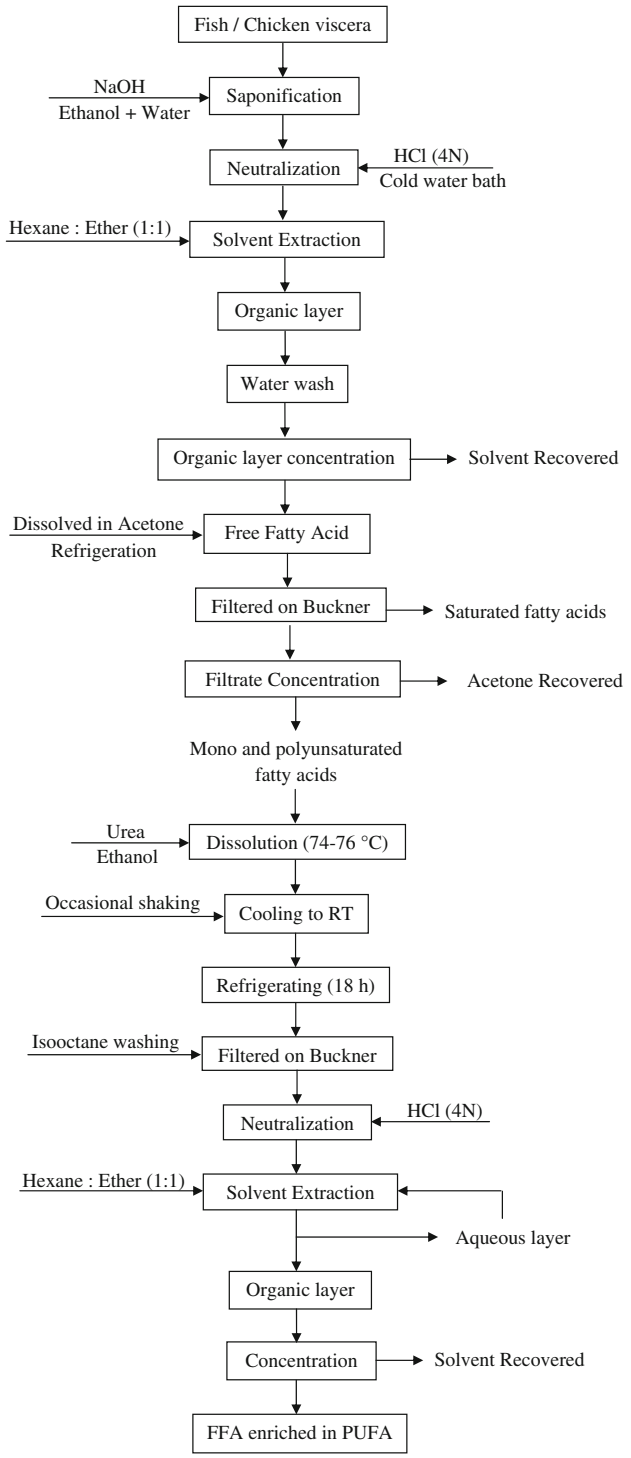

Fig. 1 Flowchart for the preparation of PUFA concentrates from chicken and fish viscera

and fish viscera produced 84.1 and 91.3% of PUFA concentrates in NUCF respectively. Although the total PUFA content in chicken viscera (26.0%) after the acetone treatment was more than that of in fish viscera (22.8%) (Table 1); the PUFA content in NUCF was higher in fish viscera as compared to that of chicken viscera in all urea to FA ratios. This may be due to the greater extent of unsaturation present in fish viscera containing PUFA (EPA-DHA) than that of chicken viscera (linoleic acid).

A yield of 10% linoleic acid was obtained from the FFA of chicken viscera of 82.1% purity along with other PUFA (DHA and linolenic acid). The total yield of EPA-DHA obtained from FFA of fish viscera was 11% of 78.2% along with other PUFA (alpha linolenic acid, n-3; Docosapentaenoic acid, n-3; hexadecadienoic acid, n-4). The overall purity of linoleic acid was higher as compared to EPA-DHA due to the presence of a higher amount of other PUFA in fish viscera as compared to chicken viscera. Zuta et al. [21] reported the production of PUFA from mackerel processing waste in which they got 73.2% PUFA concentrates by applying an urea to FA ratio of 3.5; whereas in the present study 89.9% of PUFA concentrates were obtained from the same urea–FA combination. This could be due to the presence of a higher amount of PUFA in the substrate. Zuta et al. [21] performed urea fractionation directly on the substrate containing 15.2% of PUFA, but in the present study it was initially increased by acetone treatment up to 22.8%.

Production of PUFA by acetone treatment followed by urea fractionation (Fig. 1) as described above involves simple separation technology and requires low energy efficiency. Moreover the procedure involves environment friendly operating conditions and inexpensive renewable materials such as urea and ethanol [22] for separation. These waste products are an excellent source of PUFA and can be purified to a greater extent by optimizing the other parameters in the urea complex fractionation [23].

In conclusion, this report demonstrates the importance of poultry and fish processing waste. The use of this waste material (viscera) can be commercialized for the production of PUFA concentrates via acetone treatment and urea inclusion fractionation which has potential value for large-scale production. The described method can also be applied to other biowaste containing adequate amounts of PUFA to get their concentrates.

Acknowledgments We would like to acknowledge Dr. Santinath Ghosh, Department of Chemical Technology, University College of Science and Technology, University of Calcutta for valuable suggestions.

References

1. Sahena F, Zaidul ISM, Jinap S, Saari N, Jahurul HA, Abbas KA, Norulaini NA (2009) PUFAs in fish: extraction, fractionation, importance in health. CRFSFS 8:59–74
2. Kinsella JE (1986) Food components with potential therapeutic benefits: the n-3 polyunsaturated fatty acids of fish oils. Food Technol 40:89–97

3. Simopoulos AP (1997) Essential fatty acids in health and chronic disease. Food Rev Int 13:623–631
4. Rubio-Rodríguez N, Beltrán S, Jaime I, de Diego SM, Sanz MT, Carballido JR (2010) Production of omega-3 polyunsaturated fatty acid concentrates: a review. Innov Food Sci Emerg Technol 11:1–12
5. Mishra VK, Temelli F, Ooraikul B (1993) Extraction and purification of ω-3 fatty acids with an emphasis on supercritical fluid extraction—a review. Food Res Int 26:217–226
6. Shimada Y, Sugihara A, Tominaga Y (2001) Enzymatic purification of polyunsaturated fatty acids. J Biosci Bioeng 91:529–538
7. Guil-Guerrero JL, Belarbi H (2001) Purification process for cod liver oil polyunsaturated fatty acids. J Am Oil Chem Soc 78:477–484
8. Iverson JL, Weik RW (1967) Correlation of fatty acid structure with preferential order of urea complex formation. J Assoc Off Anal Chem 50:1111–1118
9. Udaya N, Wanasundara X, Shahidi F (1999) Concentration of omega 3-polyunsaturated fatty acids of seal blubber oil by urea complexation: optimization of reaction conditions. Food Chem 65:41–49
10. Giri SS, Sahoo SK, Sahu AK, Mukhopadhyay PK (2000) Nutrient digestibility and intestinal enzyme activity of *Clarias batrachus*(Linn.) juveniles fed on dried fish and chicken viscera incorporated diets. Bioresour Technol 71:97–101
11. Jamdar SN, Harikumar P (2008) Radiation decontamination of poultry viscera. Radiat Phys Chem 77:467–472
12. Arvanitoyannis IS, Kassaveti A (2008) Fish industry waste: treatments, environmental impacts, current and potential uses. Int J Food Sci Technol 43:726–745
13. Rao JR, Mahendrakar NS, Chakrabarty NM, Raghavan SL (1996) Utilisation of fermented fish and poultry offals in feed for common carp (*Cyprinus caprio*). Seaf Export J 27:17–23
14. Strøm T, Eggum BO (1981) Nutritional value of fish viscera silage. J Sci Food Agric 32:115–120
15. Sathivel S, Prinyawiwatkul W, King JM, Grimm CC, Lloyd S (2003) Oil production from catfish viscera. J Am Oil Chem Soc 80:377–382
16. Kondamudi N, Strull J, Misra M, Mohapatra SK (2009) A green process for producing biodiesel from feather meal. J Agric Food Chem 57:6163–6166
17. Patil D, Leonardis AD, Nag A (2010) Synthesis of biosurfactants from natural resources. J Food Biochem. doi:10.1111/j.1745-4514.2010.00414.x
18. Saha S, Bandyopadhyaya I, Dutta J (1990) Lipid of hilsa fish (*Hilsa ilisa*). J Indian Chem Soc 67:414–416
19. Yang TS, Liu TT (2004) Optimization of production of conjugated linoleic acid from soybean oil. J Agric Food Chem 52:5079–5084
20. Spurvey SA, Shahidi F (2000) Concentration of gamma linolenic acid (GLA) from borage oil by urea complexation: optimization of reaction conditions. J Food Lipids 7:163–174
21. Zuta CP, Simpson BK, Chan HM, Phillips L (2003) Concentrating PUFA from mackerel processing waste. J Am Oil Chem Soc 80:933–936
22. Hayes DG (2006) Effect of temperature programming on the performance of urea inclusion compound-based free fatty acid fractionation. J Am Oil Chem Soc 83:253–259
23. Wu M, Ding H, Wang S, Xu S (2008) Optimizing conditions for the purification of linoleic acid from sunflower oil by urea complex fractionation. J Am Oil Chem Soc 85:677–684