Research Article
Turkish Journal of Maritime and Marine Sciences Volume: 6 Issue: 2 (2020) 239-251

Effect of different time and temperature on fatty acid composition of trout waste hydrolized by Alkali Protease enzyme

Alkali Proteaz enzimi ile hidrolize olan alabalık atığının yağ asidi kompozisyonu üzerine farklı süre ve sıcaklığın etkisi

Türk Denizcilik ve Deniz Bilimleri Dergisi Cilt: 6 Sayı: 2 (2020) 239-251

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ABSTRACT

The raw material consisted of combinations of viscera, backbone and digestive tract and was hydrolysed with Alcalase enzyme at different time and temperature and determined fatty acid compositions of extracted oil from these waste. As a result of study, the major fatty acids in trout waste oil were C16:0 (palmitic acid), stearic acid (C18:0), oleic acid (C18:1n-9c), linoleic acid (C18:2n6), linolenic acid (C18:3n3), palmitoleic acid (C16:1), linoleic acid (C18:2n-6c), eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA). The highest and lowest DHA and EPA concentrations of trout waste oil were obtained by hydrolysis at 40 ºC for 4 hour and 50 ºC 30 min. (4.37 % DHA and 1.62% EPA) and at 50ºC for 30 minute and 50 ºC for 4 hour (5.11% DHA and 1.91% EPA), respectively. The reaction time had significant effects on the fatty acid compositions of extracted oil fraction. It can be recommended that the waste can be an important additive that can meet the fatty acid requirement of human and the cultivated species.

Keywords: Alcalase, hydrolysate, fatty acids, oil, trout waste.

Article Info
Received: 23 October 2020
Revised: 25 November 2020
Accepted: 25 November 2020

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ÖZET

Hammaddeyi iç organlar, iskelet ve sindirim sistemi atıkları oluşturmaktadır. Farklı zaman ve sıcaklıklarda Alcalase enzimi ile hidrolize edilmiş ve bu atıklardan ekstrakte edilen yağın yağ asidi bileşimleri belirlenmiştir. Çalışma sonucunda, alabalık atık yağındaki başlıca yağ asitleri C16: 0 (palmitik asit), stearik asit (C18: 0), oleik asit (C18: 1n-9c), linoleik asit (C18: 2n6), linolenik asit (C18: 3n3), palmitoleik asit (C16: 1), linoleik asit (C18: 2n-6c), eikosapentaenoik asit (C20: 5n-3, EPA) ve dokosaheksaenoik asit (C22: 6n-3, DHA)'tir. Alabalık atığından hidroliz yöntemi ile elde edilen yağın, en yüksek ve en düşük DHA ve EPA oranları 40 °C'de 4 saat ve 50 °C'de 30 dakika hidroliz ile elde edilmiştir (% 4.37 DHA ve % 1.62 EPA) ve 50 °C'de 30 dakika ve 50 °C'de 4 saat (sırasıyla % 5.11 DHA ve % 1.91 EPA). Reaksiyon süresinin, ekstrakte edilen yağ franksiyonunun yağ asiti bileşimleri üzerinde önemli etkileri olmuştur. Atığın insan ve yetiştirilen türlerin yağ asidi ihtiyacını karşılayabilecek önemli bir katkı maddesi olabileceği önerilebilir.

Anahtar sözcükler: Alkalaz, hidrolizat, yağ asitleri, yağ, alabalık atıkları.

1. INTRODUCTION

A large amount of solid wastes consisting of heads, tails, bones, skin, and viscera are discarded by the fish slaughtering and filleting operations (Šližyte *et al*., 2005; Gbogouri *et al*., 2006). These waste is estimated that more than 70% of the total fish captures are processed, which may represent up to 50% of the total fish weight (Shahidi, 2007). For various purposes, optimal utilization of these products is becoming increasingly due to contains valuable food component, one of which is fish oil (Šližyte *et al*., 2005).

In comparison to other animal and vegetable, fish oil has been considered as the most important source of long-chain n-3 polyunsaturated fatty acid, especially eicosapentaenoic acid (EPA, C20:5, n-3) and docosahexaenoic acid (DHA,C22:6, n-3), which cannot be synthesized by human beings (Gbogouri *et al*., 2006; Silva *et al*., 2017). Therefore, Polyunsaturated fatty acids (PUFAs) are important source of essential dietary components for maintaining health and for used in aqua feeds as supply of long chain omega-3s fatty acids for aquaculture (Kery and Murphy, 2007; Chow, 2000; Routray *et al*., 2018).

Researchers have been extensively studied a suitable extraction procedure for the production of high quality fish oil from fish waste resulting from the fish processing plant (Chantachum *et al*., 2000; Gbogouri *et al*., 2006; Wu and Bechtel, 2008; Rubio-Rodriguez *et al*., 2012; Nguyen *et al*., 2013; Liu *et al*., 2020). Recently, enzymatic reaction with proteases hydrolysis is one of the most efficient methods to obtain crude oil from fish waste since it can be simpler and cheaper regarding investment cost and energy expense (Rubio-Rodríguez *et al*., 2010; Nguyen *et al*., 2013). Moreover, those methods do not require organic solvents and high temperatures when compared to main oil extraction method, which are usually used are wet rendering and dry rendering methods (Liaset *et al*., 2003; Rubio-Rodríguez *et al*., 2010; Routray *et al*., 2018).

Many commercial proteases from animal, plant, or microbial sources have been widely used such as Alcalase, Flavourzyme, Neutrase, Protamex, epsine, papain, , Corolase PN-L, Corolase 7089 to enzymatic

hydrolysis (Nguyen *et al*., 2020). As an endopeptidase that can break peptide bonds of nonterminal amino acids, alcalase has been reported efficient for extraction of oil from fish waste by researchers (Šližyte *et al*., 2005; Hathwar *et al*., 2011; Mahmoud *et al*., 2008). Gbogouri *et al*. (2006) demonstrated that Alcalase was the most efficient enzyme regarding oil yield from fresh salmon heads. Batista *et al*. (2009) concluded that Alcalase was efficient for oil extraction from sardine by-products. Similarly, Linder *et al*. (2010) found the highest oil recovery from ground salmon heads at middle temperature (55 °C) using Alcalase. The aim of this work was to evaluate the effect of different time and temperature on fatty acids composition of rainbow trout (*Oncorhynchus mykiss*) waste by enzymatic hydrolysis of using alkali protease.

2. MATERIALS AND METHODS

2.1. MATERIALS

In the study, the waste (skeleton, fin, head, skin and internal organs) of rainbow trout (*Onchoryncus mykiss*), which emerged as the most fish waste in the Black Sea region, was obtained from aquaculture businesses in Ordu province in March.

The wastes were vacuum packed in packages of 1 and 5 kg and placed in foam boxes in ice, brought to Ordu University Fatsa Faculty of Marine Sciences Department of Fisheries Technology Engineering and stored at -40 ° C until protein hydrolyzate was obtained.

2.2.METHODS

2.2.1. Enzymatic Hydrolysis

Trout waste, which was stored in 5 kg packages in frozen state, was minced after thawing at room temperature by using a meat grinder (Empero E.M.P.12.01.P). Then, to ensure the inactivation of endogenous enzymes, the wastes was heated at 90°C for 20 minutes in a water bath (Memmert WNB 22). The minced fish meat, whose enzymes were inactivated, was homogenized (IKA T 25) by adding distilled water at a ratio of 1: 1 after cooling. For the alkaline protease enzyme 1%, three different temperatures were determined as 40°C, 50°C and 60°C for the optimization process. In the hydrolysis stage, alkaline protease, different enzyme times of 30 min, 1, 4 and 8 hours were determined for optimum product efficiency. It was applied to terminate the hydrolysis in samples with a certain time and temperature for at 85°C for 10 minutes. The cooled hydrolysis solution, then it was separated into phases by centrifuge (Sigma 3-30K) at 4100 rpm for 20 min. 4 different phases were formed: the upper phase, the light protein, the second phase, the proteinaceous phase, and the last phase, the insoluble matter phase. The fatty acid composition was analyzed from the oil phase formed in the top phase of the hydrolyzed solution.

2.2.2.Lipid Analyses

Lipid was analyzed according to the method by Bligh and Dyer (1959). Accordingly, a total of 22.11% lipid was determined in the trout waste used (Korkmaz and Tokur, 2019).

2.2.3. Fatty Acid Analysis

Fatty acid methyl esters from extracted lipid were analysed by Ichihara *et al*. (1996) method. 4ml of 2M KOH and 2ml of n-heptane were added on 25 mg of extracted oil sample. Then it was mixed in vortex for 2 minutes at room temperature and centrifuged at 4000 rpm for 10 minutes and the heptane layer was taken for analysis in gas chromatography (GC) device.

The fatty acids composition was carried out using GC (Gas chromatographic) with flame ionization detector (FID) and auto sampling (Perkin Emler, USA) with SGE column at 30m x 0.32mm ID x 0.25μm film thickness. Injector and detector temperatures were set to 220 ° C and then 280°C respectively. Meanwhile, the temperature of the oven was kept at 140°C

for 5 minutes. Afterwards, it was brought up to 200°C by increasing 4°C every minute, and by increasing 1 ° C per minute from 200°C to 220°C. Sample amount is 1 ml and carrier gas control is provided to be at 16 ps. Split application was carried out at a ratio of 1:50. Fatty acids were identified by comparing the FAME mixture consisting of standard 37 components depending on their arrival times. The results of the two GC analysis performed in the same way are expressed in % with ± standard deviation values.

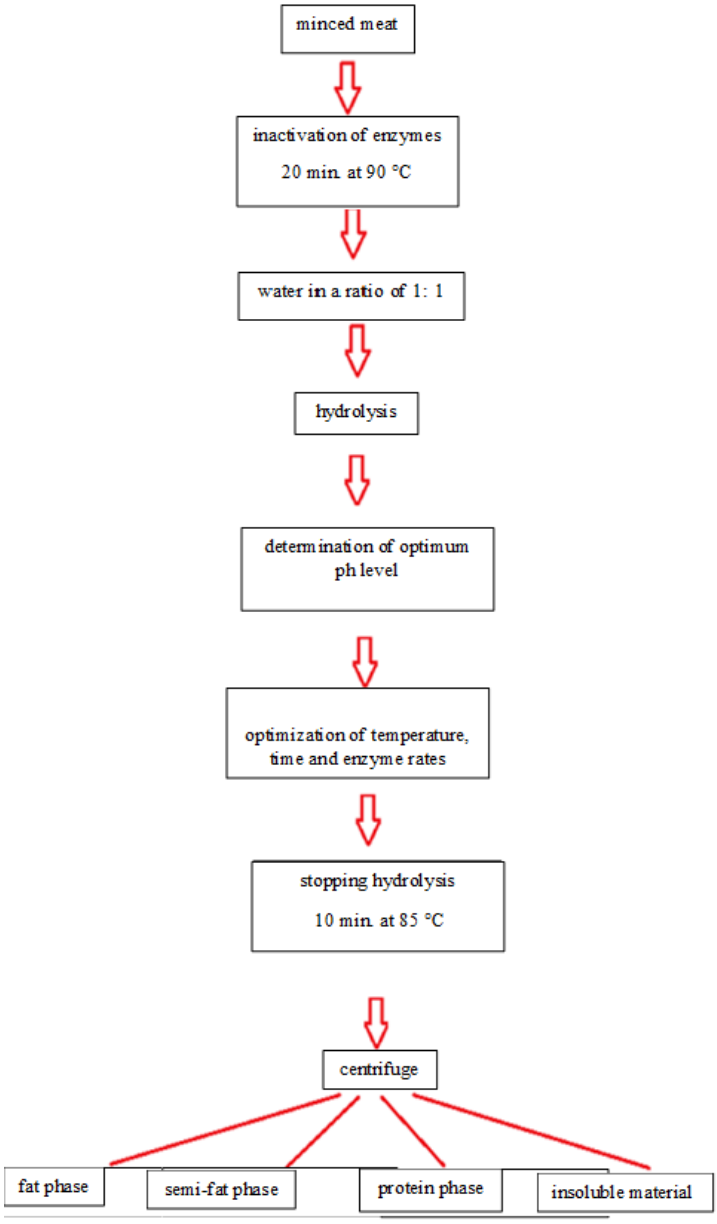

Figure 1. Hydrolyzate production flow chart in optimization of fish waste with alkaline protease enzyme

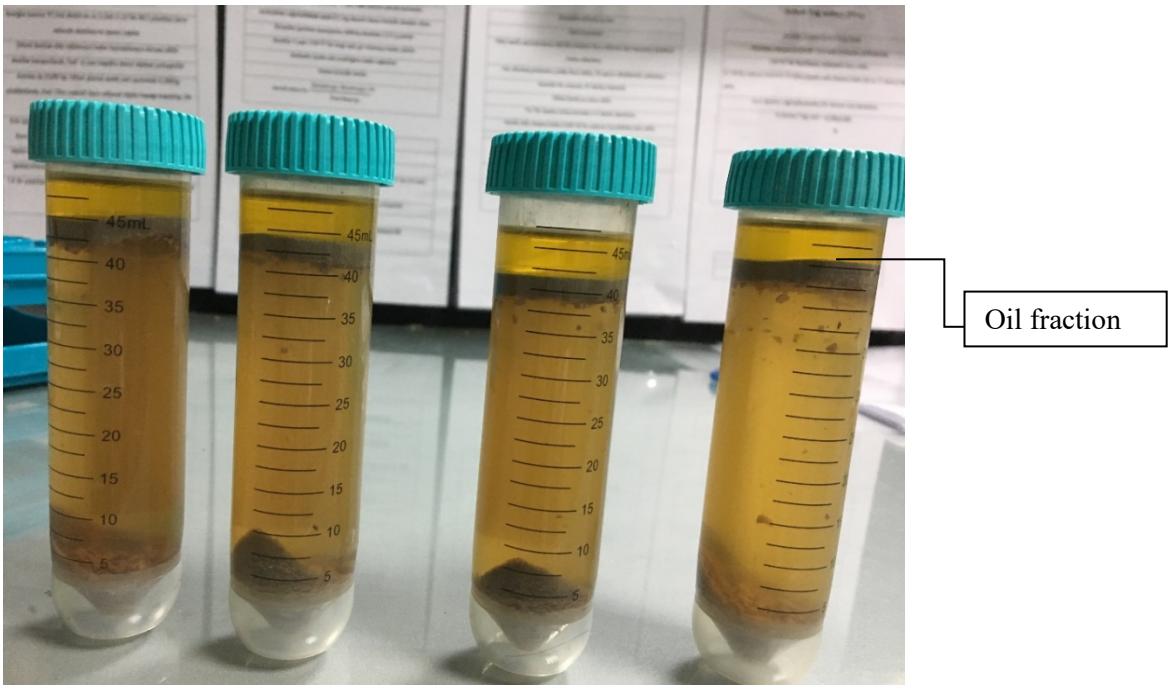

Figure 2. Oil fraction of extracted trout waste by using alcalase anzyme

2.2.4. Statistical Analysis

Duncan multiple comparison test (One-way Anova at $p < 0.05$ significance level) was applied using the SPSS 16.0 package program to determine the effect of time and temperature on the fatty acid determination of the oil phase formed in the top phase of protein hydrolysate solution (Duncan and Beverly, 1955).

3 .RESULTS and DISCUSSION

The effects of different times on fatty acid composition of hydrolyzed fish wastes are shown in Table 1,2, and 3.

Table 1. Effects of Different Times at 40°C, 50°C and 60°C of Hydrolyzed Trout Wastes on Fatty Acid Composition(% total fatty acids)

Fatyacids	**40°C**			
	Time			
	30'	**1h**	**4h**	**8h**
C12:0	0,05±0,00^{a}	0,05±0,00^{a}	0,05±0,00^{a}	0,05±0,00^{a}
C14:0	2,23±0,01^{a}	2,24±0,01^{a}	2,28±0,00^{b}	2,24±0,00^{a}
C15:0	0,23±0,00^{a}	0,23±0,00^{a}	0,27±0,02^{b}	0,23±0,00^{a}
C16:0	14,27±0,06^{a}	14,46±0,02^{b}	15,47±0,01^{c}	14,43±0,00^{b}
C17:0	0,25±0,00^{a}	0,25±0,00^{a}	0,27±0,00^{b}	0,25±0,00^{a}
C18:0	4,44±0,03^{a}	4,51±0,01^{b}	4,88±0,03^{c}	4,51±0,02^{b}
C20:0	0,27±0,00^{a}	0,28±0,00^{a}	0,30±0,00^{b}	0,28±0,00^{a}
C22:0	0,14±0,00^{a}	0,14±0,00^{a}	0,15±0,00^{a}	0,14±0,00^{a}
C24:0	0,06±0,00^{a}	0,07±0,00^{a}	0,07±0,00^{a}	0,06±0,00^{a}
ΣSFA	21,94±0,11^{a}	22,21±0,04^{b}	23,74±0,06^{c}	22,19±0,01^{b}
C16:1	3,89±0,02^{b}	3,87±0,00^{b}	3,82±0,00^{a}	3,87±0,00^{b}
C18 :1n9t	0,08±0,00^{a}	0,08±0,01^{a}	0,09±0,00^{a}	0,08±0,00^{a}
C18:1n9c	39,24±0,12^{b}	39,15±0,03^{b}	37,14±0,10^{a}	39,20±0,02^{b}
C20:1	2,61±0,00^{b}	2,59±0,02^{b}	2,45±0,02^{a}	2,60±0,02^{b}
C22:1n9	0,31±0,05^{b}	0,27±0,00^{ab}	0,24±0,00^{a}	0,27±0,00^{ab}
C:24:1	0,33±0,00^{a}	0,33±0,00^{a}	0,31±0,00^{a}	0,33±0,00^{a}
ΣMUFA	46,46±0,19^{b}	46,28±0,00^{b}	44,05±0,13^{a}	46,34±0,00^{b}
C18:2n6c	18,09±0,01^{b}	17,99±0,01^{a}	19,17±0,06^{c}	17,97±0,00^{a}
C18:3n6	0,28±0,00^{a}	0,28±0,00^{a}	0,24±0,00^{a}	0,28±0,00^{a}
C18:3n6	3,26±0,01^{b}	3,27±0,02^{b}	3,05±0,03^{a}	3,27±0,01^{b}
C20:2	1,49±0,00^{a}	1,48±0,00^{a}	1,53±0,00^{a}	1,48±0,00^{a}
C22:2	0,16±0,00^{a}	0,15±0,00^{a}	0,15±0,00^{a}	0,15±0,00^{a}
ΣPUFA	23,28±0,02^{b}	23,18±0,01^{a}	24,13±0,09^{c}	23,15±0,01^{a}
C20:3n6	0,57±0,00^{a}	0,57±0,00^{a}	0,52±0,00^{a}	0,56±0,00^{a}
C20:3n3	0,40±0,00^{a}	0,40±0,00^{a}	0,37±0,00^{a}	0,40±0,00^{a}
C20:4n6	0,46±0,01^{a}	0,46±0,01^{a}	0,45±0,00^{a}	0,46±0,00^{a}
C20:5n3	1,87±0,01^{b}	1,85±0,01^{b}	1,64±0,03^{b}	1,87±0,01^{a}
C22:6n3	5,01±0,06^{a}	5,03±0,01^{a}	5,11±0,06^{a}	5,02±0,02^{a}
ΣHUFA	8,31±0,08^{b}	8,32±0,02^{b}	8,09±0,09^{a}	8,30±0,01^{b}
Σn3	7,29±0,07^{a}	7,29±0,02^{a}	7,12±0,09^{a}	7,28±0,01^{a}
Σn6	22,66±0,03^{a}	22,57±0,00^{a}	23,42±0,09^{b}	22,54±0,01^{a}
n3/n6	0,32±0,00^{b}	0,32±0,00^{b}	0,30±0,00^{a}	0,32±0,00^{b}
EPA/DHA	0,37±0,01^{b}	0,37±0,00^{b}	0,32±0,00^{a}	0,37±0,00^{b}

* Different letters in the same line indicate the differences at 0.05 significance level.

Table 2. Effects of Different Times at 50°C of Hydrolyzed Trout Wastes on Fatty Acid Composition (% total fatty acids)

Fatyacids	50°C			
	Time			
	30'	1h	4h	8h
C12:0	0,05±0,00^{a}	0,05±0,00^{a}	0,05±0,00^{a}	0,05±0,00^{a}
C14:0	2,03±0,00^{a}	2,18±0,00^{b}	2,21±0,00^{c}	2,22±0,00^{c}
C15:0	0,21±0,00^{a}	0,22±0,00^{b}	0,23±0,00^{b}	0,23±0,00^{b}
C16:0	14,28±0,00^{a}	14,41±0,00^{ab}	14,62±0,00^{c}	14,55±0,00^{bc}
C17:0	0,23±0,00^{a}	0,24±0,00^{a}	0,25±0,00^{a}	0,25±0,00^{a}
C18:0	4,77±0,00^{b}	4,57±0,00^{a}	4,56±0,00^{a}	4,52±0,00^{a}
C20:0	0,27±0,00^{a}	0,28±0,00^{a}	0,28±0,00^{a}	0,28±0,00^{a}
C22:0	0,13±0,00^{a}	0,14±0,00^{a}	0,14±0,00^{a}	0,14±0,00^{a}
C24:0	0,06±0,00^{a}	0,06±0,00^{a}	0,06±0,00^{a}	0,07±0,00^{a}
ΣSFA	22,03±0,03^{a}	22,15±0,15^{ab}	22,41±0,03^{c}	22,30±0,05^{bc}
C16:1	3,67±0,00^{a}	3,82±0,02^{b}	3,86±0,00^{c}	3,87±0,00^{c}
C18 :1n9t	0,08±0,00^{a}	0,08±0,00^{a}	0,08±0,00^{a}	0,07±0,00^{a}
C18:1n9c	39,39±0,00^{bc}	39,47±0,12^{c}	38,85±0,07^{a}	39,02±0,27^{ab}
C20:1	2,54±0,00^{a}	2,38±0,31^{a}	2,50±0,02^{a}	2,55±0,04^{a}
C22:1n9	0,25±0,00^{a}	0,26±0,00^{b}	0,27±0,00^{b}	0,27±0,00^{b}
C:24:1	0,27±0,00^{a}	0,31±0,00^{b}	0,32±0,00^{b}	0,32±0,00^{b}
ΣMUFA	46,18±0,00^{b}	46,31±0,17^{b}	45,89±0,09^{a}	46,11±0,31^{b}
C18:2n6c	19,23±0,03^{c}	18,41±0,09^{b}	18,10±0,02^{a}	18,05±0,06^{a}
C18:3n6	0,26±0,00^{a}	0,27±0,00^{a}	0,28±0,00^{a}	0,28±0,01^{a}
C18:3n6	3,20±0,01^{a}	3,19±0,12^{a}	3,29±0,01^{a}	3,27±0,03^{a}
C20:2	1,56±0,00^{c}	1,50±0,00^{b}	1,47±0,00^{a}	1,47±0,01^{a}
C22:2	0,15±0,00^{a}	0,15±0,00^{a}	0,15±0,00^{a}	0,15±0,00^{a}
ΣPUFA	24,41±0,02^{c}	23,52±0,03^{b}	23,29±0,03^{a}	23,23±0,08^{a}
C20:3n6	0,56±0,00^{a}	0,56±0,00^{a}	0,58±0,00^{a}	0,57±0,01^{a}
C20:3n3	0,38±0,00^{a}	0,40±0,00^{b}	0,40±0,00^{b}	0,40±0,00^{b}
C20:4n6	0,45±0,00^{a}	0,46±0,00^{a}	0,47±0,00^{a}	0,46±0,01^{a}
C20:5n3	1,62±0,01^{a}	1,80±0,01^{b}	1,91±0,01^{c}	1,89±0,06^{bc}
C22:6n3	4,37±0,01^{a}	4,80±0,03^{b}	5,06±0,02^{c}	5,03±0,13^{c}
ΣHUFA	7,38±0,00^{a}	8,01±0,05^{b}	8,41±0,03^{c}	8,35±0,22^{c}
Σn3	6,37±0,00^{a}	6,99±0,05^{b}	7,37±0,03^{c}	7,31±0,19^{c}
Σn6	23,71±0,03^{c}	22,89±0,03^{b}	22,72±0,03^{ab}	22,64±0,12^{a}
n3/n6	0,27±0,00^{a}	0,30±0,00^{b}	0,32±0,00^{c}	0,32±0,01^{c}
EPA/DHA	0,37±0,00^{a}	0,38±0,00^{a}	0,38±0,00^{a}	0,38±0,00^{a}

* Different letters in the same line indicate the differences at 0.05 significance level.

Table 3. Effects of Different Times at 60°C of Hydrolyzed Trout Wastes on Fatty Acid Composition (% total fatty acids)

Fatty acids	60°C			
	Time			
	30'	1h	4h	8h
C12:0	0,05±0,00[a]	0,05±0,00[a]	0,05±0,00[a]	0,05±0,00[a]
C14:0	2,24±0,00[a]	2,22±0,02[a]	2,22±0,00[a]	2,24±0,01[a]
C15:0	0,23±0,00[a]	0,23±0,00[a]	0,23±0,00[a]	0,23±0,00[a]
C16:0	14,37±0,06[a]	14,54±0,04[b]	14,40±0,07[ab]	14,49±0,01[ab]
C17:0	0,24±0,00[a]	0,25±0,00[a]	0,25±0,00[a]	0,26±0,00[a]
C18:0	4,49±0,03[a]	4,57±0,00[b]	4,53±0,03[ab]	4,57±0,00[b]
C20:0	0,28±0,00[a]	0,28±0,00[a]	0,28±0,00[a]	0,28±0,00[a]
C22:0	0,14±0,00[a]	0,14±0,00[a]	0,14±0,00[a]	0,13±0,01[a]
C24:0	0,06±0,00[a]	0,07±0,00[a]	0,07±0,00[a]	0,07±0,00[a]
ΣSFA	22,11±0,10[a]	22,35±0,02[b]	22,18±0,12[ab]	22,32±0,00[ab]
C16:1	3,86±0,01[a]	3,90±0,00[b]	3,88±0,01[ab]	3,89±0,02[ab]
C18 :1n9t	0,08±0,00[a]	0,09±0,00[a]	0,09±0,00[a]	0,09±0,00[a]
C18:1n9c	39,19±0,03[a]	38,88±0,30[a]	39,11±0,06[a]	39,07±0,04[a]
C20:1	2,59±0,00[a]	2,54±0,03[a]	2,58±0,00[a]	2,61±0,05[a]
C22:1n9	0,26±0,00[a]	0,29±0,00[b]	0,28±0,00[ab]	0,29±0,00[b]
C:24:1	0,33±0,00[a]	0,33±0,00[a]	0,33±0,00[a]	0,34±0,00[a]
ΣMUFA	46,31±0,05[a]	46,02±0,34[a]	46,27±0,07[a]	46,28±0,11[a]
C18:2n6c	18,05±0,04[a]	18,02±0,07[a]	18,00±0,02[a]	17,94±0,05[a]
C18:3n6	0,28±0,00[a]	0,29±0,01[a]	0,29±0,00[a]	0,28±0,00[a]
C18:3n6	3,27±0,02[a]	3,25±0,01[a]	3,26±0,02[a]	3,24±0,00[a]
C20:2	1,48±0,00[a]	1,48±0,01[a]	1,49±0,00[a]	1,49±0,01[a]
C22:2	0,15±0,00[a]	0,16±0,00[a]	0,16±0,00[a]	0,16±0,00[a]
ΣPUFA	23,22±0,06[a]	23,20±0,08[a]	23,19±0,05[a]	23,12±0,05[a]
C20:3n6	0,57±0,00[a]	0,58±0,01[a]	0,58±0,00[a]	0,58±0,00[a]
C20:3n3	0,40±0,00[a]	0,40±0,01[a]	0,41±0,00[a]	0,41±0,00[a]
C20:4n6	0,46±0,00[a]	0,47±0,01[a]	0,47±0,00[a]	0,46±0,00[a]
C20:5n3	1,88±0,01[a]	1,90±0,07[a]	1,87±0,00[a]	1,85±0,02[a]
C22:6n3	5,04±0,00[a]	5,07±0,16[a]	5,04±0,02[a]	4,99±0,04[a]
ΣHUFA	8,36±0,01[a]	8,42±0,24[a]	8,36±0,01[a]	8,28±0,05[a]
Σn3	7,33±0,01[a]	7,38±0,22[a]	7,32±0,01[a]	7,24±0,05[a]
Σn6	22,62±0,06[a]	22,61±0,11[a]	22,59±0,05[a]	22,51±0,07[a]
n3/n6	0,32±0,00[a]	0,33±0,01[a]	0,32±0,00[a]	0,32±0,00[a]
EPA/DHA	0,37±0,00[a]	0,37±0,00[a]	0,37±0,00[a]	0,37±0,00[a]

* Different letters in the same line indicate the differences at 0.05 significance level.

The predominant fatty acids in oils extracted from trout waste were C16:0 (palmitic acid), stearic acid (C18:0), oleic acid (C18:1n-9c), linoleic acid (C18:2n6), linolenic acid (C18:3n3), palmitoleic acid (C16:1), linoleic acid (C18:2n-6c), eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA). Yeşilayer and Genç (2013) who compared the fatty acid composition of wild brown trout and farmed rainbow trout were reported similar results for fatty acid composition of farmed rainbow fillets.

DHA (22:6 n-3) was found the highest highly unsaturated fatty acids (HUFA) in oil extracted from trout waste by using alcalase (ranged between 4.37% and 5.11%). The results presented are similar to Yeşilayer and Genç (2013) who reported 5.8% docosahexaenoic acid (DHA) values for farmed rainbow trout fillet. However, Kołakowska *et al*. (2006) and Fiori *et al*.

(2012) found higher amount of DHA content in rainbow trout waste material. Hixson *et al*. (2014) studied the effect of dietary camelina oil on fatty acid composition of farmed rainbow trout muscle. They found that the percentage of DHA in trout muscle decreased from 14.1% to 6.6% by almost half with the addition of camelina oil in trout feed. Most studies have shown that the proportions of EPA and DHA are decreased in fish feed diets containing vegetable oils (Alasalvar *et al*., 2002; Pickova and Mørkøre, 2007; Simopoulos, 2002). These results may explain why trout waste oil contain lower DHA content.

EPA (C20:5n-3) ranged between 1.62% and 1.91% in trout waste oil. Kołakowska *et al*. (2006) reported 1.4 % EPA in oil extracted from the by products of the rainbow trout. Babajafari *et al*. (2017) also found 1.76 % EPA in oil recovered from trout head by using concentrated protease.

The highest and lowest DHA and EPA concentrations were obtained trout waste oil hydrolyzed at 40ºC for 4 hour and 50ºC 30 min. (4.37 % DHA and 1.62% EPA) and at 50ºC for 30 minute and 50 ºC for 4 hour (5.11% DHA and 1.91% EPA), respectively. Although there seems to be differences between different times and temperatures, the values were found to be quite close to each other. Similarly, Głowacz-Różyńska *et al*. (2016) reported the close content of PUFA and EPA+DHA in oils of salmon by products obtained different extraction methods (cold extraction, high temperature extraction and enzymatic hydrolyze).

According to the U.S. Food and Drug Administration, fish oil up to 3 grams daily is "generally recognized as safe" (GRAS) as a food (Liu *et al*., 2019). Although the recommended intake for eicosapentaenoic acid (C20:5 n-3; EPA) and docosahexaenoic acid (C22:6 n-3; DHA) shows a discrepancy between the different scientific panels, only consumption of 1000 mg of trout waste oil would be more than sufficient to meet human nutritional requirements with respect to the amount of DHA and EPA (Zhang *et al*., 2019).

The highest amount of fatty acids detected in the oil fractions extracted from trout waste were total monounsaturated fatty acids (44,05%-46,46%) followed by total polyunsaturated fatty acids (23,12%-24,41%) and total saturated fatty acids (21.94%- 23.74%). Similar result have been found by Routray *et al*. (2018) who studied fatty acid profile of the salmon by products oil extracted by using enzymatic protein hydrolysis. Similarly, Gbogouri *et al*. (2006) showed that the highest fatty acids were monounsaturated fatty acids (39.9-40.8%) followed by polyunsaturated fatty acids (32.3-35.4%). and saturated fatty acids (24.7-27.3%) in fish oil extracted from salmon head by using proteolytic enzyme. Routray *et al*. (2018) stated that the percentage fatty acids composition of the extracted salmon waste were differed as the composition of resultant oil differs with quality of raw material, storage methods, processing techniques and extraction techniques. Moreover, the fatty acid composition of the white muscle from Atlantic salmon very much reflects the salmon's dietary lipid (Torstensen *et al*., 2000). Thus, the fatty acid composition of the salmon oil will depend on the lipid sources used in the feed production (Liaset *et al*., 2003).

Total unsaturated fatty acids were ranged between 76.26%-78.06% of total fatty acids. Consequently, those oils are important source of unsaturated fatty acids. Kołakowska *et al*. (2006) reported similar value (about 77.1% unsaturated fatty acids). Similar results have also been reported by Fiori *et al*. (2012) for oil from trout by products (Głowacz-Różyńska *et al*. (2016) for oil from salmon by products.

The n-3/n-6 ratio in oil from trout waste changed between 0.27 to 0.33. Johansson *et al*. (2000) also found lower n-3/n-6 ratio (0.20) for fatty acid composition of rainbow trout fillet. On the other hand, n-3/n-6 ratio were found higher for rainbow trout waste than our findings by Fiori *et al*. (2012) and

Kołakowska *et al*. (2006). The reason why the n-3 / n-6 ratio was low in this study was thought to be due to the high percentage of linoneic acid in the fatty acid profile of oil. The higher amount of linoleic acid in farmed trout were also found by Yeşilayer and Genç (2013) with respect to aquafeed ingredients, which plant oils used in the commercially produced fish feed contain higher amount of n-6 fatty acid (Hixson *et al*., 2014).

Fish oil as a source of *omega-3* fatty acids has been increasingly applied for its different health beneficial effects. In this study, trout waste are also a useful source of essential fatty acids linoleic and α-linolenic acids. Routray *et al*. (2018) reported that oil from salmon by-products is also a significant source of omega-3 fatty acids and omega-6 fatty acids, as also observed in the current case, which have several beneficial effects including effectiveness against cardio-vascular disorders (Lou *et al*., 2011), auto-immune disorders, psychiatric ailments (Dyall *et al*., 2010), various forms of cancer (Rose *et al*., 1999; Astorg, 2004; Leitzmann *et al*., 2004). The effectiveness of omega-3/6 fatty acids is attributed to lowering of cholesterol (Lopez-Huertas *et al*., 2010), anti-inflammatory properties (Simopoulos, 2002; Figueras *et al*., 2011), romatoid artrit (Wall *et al*., 2010). Monounsaturated fatty acids include omega-9 fatty acids such as oleic acid, which was observed as a major component in the present case. Oleic acid has been observed to decrease cardiovascular risk through reducing blood lipids, mainly cholesterol (Lopez-Huertas *et al*., 2010). It also reduces the tendency of cardiovascular stroke as has been associated with consumption of olive oil, where blood plasma of the participants under study contained higher oleic acid (Samieri *et al*., 2011).

n-3 HUFA requirements for larvae of various marine fish species are reported to range from 0.3 to 39 g kg on a dry basis. According to NRC (1993), the essential fatty acid requirements of fish have been determined 0.8-1% linolenic acid for rainbow trout (Watanabe *et al*. 1982; Castell *et al*., 1972) and 0.5% EPA and DHA in sea bream (Yone *et al*., 1976), 1% EPA and DHA for sea bass (Buranapanidgit *et al*.,1988). It can be thought that the waste can be an important additive that can meet the fatty acid requirement of the cultivated fish species.

As a result of 40ºC hydrolysis of trout waste, the highest total saturated fatty acids (ΣSFA), total polyunsaturated fatty acids (ΣPUFA), total n6 fatty acids (Σn-6) were obtained after 4 hours ($p < 0.05$). The lowest ratio of total monounsaturated fatty acids (ΣMUFA), n-3 and n6 fatty acids (n-3 / n-6) and eicosapentaenoic acid (EPA) and decosahexanoic fatty acids (DHA) were detected at 4 hour hydrolysis ($p < 0.05$).On the other hand, it was found that hydrolysis at other times (30 min., 1 hour and 8 hours) did not make a significant difference ($p > 0.05$).

As a result of 50 ºC hydrolysis of trout waste, the highest ΣSFA, ΣPUFA and ΣHUFA was obtained after 4 hours, 30 minutes and for 4 and 8 hours, respectively ($p < 0.05$). Statistically, the highest n-3 ratio was found in fish hydrolyzed for 4 and 8 hours ($p < 0.05$). Conversely, it was found that hydrolyzing at 50 ºC does not have a statistically significant result with respect to the ΣEPA / DHA ratio in trout wastes ($p > 0.05$).

As a results of 50ºC hydrolysis of trout waste, there was no significant effect of different time on total ΣMUFA, ΣPUFA, ΣHUFA , Σn-3, Σn-6, Σn-3 / n-6 and ΣEPA / DHA ratios ($p > 0.05$). However, the highest total saturated fatty acids content obtained in trout waste oil extracted at 60 ºC for 1 hour by using alcalase ($p < 0.05$).

Ramakrishnan *et al*. (2013) studied that the fish oil was extracted from mackerel (whole fish and different parts of waste such as tail, fin, head, etc.) using different concentrations of alcalase enzyme (0.5, 1 and 2%) at 55° C and different reaction times (1, 2, 3 and 4 h). They found that the enzyme concentration and the reaction time

had significant effects on the oil yield. In this study, fatty acid composition of oil fraction extracted from trout waste can be effected by enzymatic hydrolyzing at the different time and temperature. Similarly, many studies showed that enzyme concentration, the type of enzyme and reaction time play important roles in the quality and recovery of oil from the fish during the enzymatic extraction of oil with proteases (Linder *et al*. 2005; Hathwar *et al*., 2011).

4. CONCLUSION

Due to high consumption of trout fish (Oncorhynchus mykiss) and its usage in fish processing factories, fish waste could be a better proposition in effectively managing. In this study, the fatty acids composition of trout waste oil extracted by enzymatic hydrolyzing (alcalase) were evaluated with different time and temperature. As a results of this study, trout waste oil can be utilized as a source of oils rich in PUFA which have numerous health benefits at all extracted temperature and time. From this point of view, the trout waste oil might be a useful ingredient in functional foods and animal feed.

ACKOWLEDGEMENT

This study was supported by Ordu University Scientific Research Projects AR-1646 and BD-1701.

DISCLOSURE STATEMENT

The authors declare that there is no conflict of interest.

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