JOURNAL OF CHEMICAL ENGINEERING AND INDUSTRIAL BIOTECHNOLOGY
E-ISSN: 0126-8139
VOLUME 10, ISSUE 2, DECEMBER 2024, 32 – 36
DOI: https://doi.org/10.15282/jceib.v10i2.10613

RESEARCH ARTICLE

Two level full factorial design for the extraction of antioxidants from *Decapterus maruadsi* byproduct using wet rendering technique

Muhammad Nurfikri Alif Abdullah, Zatul Iffah Mohd Arshad*, Nurul Aini Azman

Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Lebuh Persiaran Tun Khalil Yaakob, 26300 Kuantan, Pahang, Malaysia.

ABSTRACT - The fish industry is a growing sector in Malaysia. However, the fishing industry produces a significant amount of fish waste that is often discarded directly into the sea, rivers, sewers, and land, causing environmental pollution. Fish waste can be transformed into useful items due to the existence of antioxidants and other essential substances. In this research, a two-level full factorial design was employed to recover antioxidants from *Decapterus maruadsi* waste using wet rendering technique. Design Expert 7.0 software randomly allocated the variables into 16 experiments, with ABTS Radical Scavenging Assay as a response. Optimization for antioxidant activity yielded a 0.08% difference between experimental and projected values. In conclusion, *Decapterus maruadsi* waste can be revalorized as a good source of antioxidants.

ARTICLE HISTORY

Received	:	23rd Apr. 2024
Revised	:	30th Sep. 2024
Accepted	:	25th Oct. 2024
Published	:	30th Dec. 2024

KEYWORDS
Decapterus maruadsi
Wet rendering
Antioxidant
Two-level factorial design
Fish waste

1.0 INTRODUCTION

In 2020, the Malaysian Fisheries Sector, comprising capture fisheries, inland fisheries, and aquaculture, generated 1.85 million metric tonnes valued at RM 14.5 billion [1]. Therefore, the fishing industry in Malaysia is vital for the country's economic development. While there is an increasing demand for Ikan Selayang (*Decapterus maruadsi*) as a primary fish snack, the disposal process of fish waste from the fish snack sector remains ineffective. Hence, fish leftovers can be used to produce top-quality products and serve as a substitute for artificial antioxidants. Many synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary butylhydroquinone (TBHQ), are used as preservatives to prevent food products from deteriorating [2]. However, the overuse of artificial antioxidants could lead to risks like potential carcinogenesis, cytotoxicity, and endocrine disruption [3].

At present, certain fish waste is utilized to produce fertilizers, fish oil, and fishmeal, yielding minimal profits, while the rest is discarded. Hence, the focus on extracting antioxidants from fish waste has increased interest. To date, few methods are used for extracting valuable constituents from fish waste, including extractions assisted by ultrasound and microwave, supercritical fluid extraction using CO_2, and enzymatic hydrolysis [4]. Scaling up the ultrasound-assisted and pressure-assisted extractions are expensive, whereas catalysts are needed to initiate the reaction in the hydrolysis method. Other than these methods, the wet rendering process is selected for its cost-effectiveness because no special equipment is needed to carry out the process. Additionally, the wet rendering method is known for being economical, environmentally friendly, and producing top-quality oils or proteins without the need for harmful chemicals [5]. In the wet rendering process, the efficiency of the extraction is influenced by variables such as temperature, duration, solvent types, and sample-to-solvent ratio [6]. The conventional way to optimize extraction process variables is the one factor at a time (OFAT) method. This method necessitates significant time and financial resources and neglects variable interactions. Hence, the impacts of variables under several experimental settings are assessed using a fractional factorial design [7].

Thus, this research aimed to investigate parameters in the wet rendering procedure, including solvent types (ethanol or methanol), duration of extraction (30 to 150 minutes), concentration of sample-to-solvent ratio (1:10 to 9:10 % w/v), and temperature (0 to 60°C) using a two-level factorial design in Design Expert software, for obtaining antioxidants from *Decapterus maruadsi* waste.

2.0 MATERIALS AND METHODS

2.1 *Preparation of fish byproducts*

Fish wastes were collected and washed using tap water before being stored in a freezer. To maintain the quality of the remaining samples, only one container was transported at a time. The initial process involved boiling the raw material at 100°C for 30 minutes as a pre-treatment step. The fish waste was then cut up, dessicated in an oven at a temperature of 150°C for 50 minutes and was crushed into powder form. After that, the fish waste powder was kept for later use at room temperature in a plastic pouch. The moisture content of the dried fish powder should range from 46% to 68%.

***CORRESPONDING AUTHOR | Z. I. M. Arshad** | ✉ zatul@umpsa.edu.my

2.2 *Wet rendering method*

As shown in Table 1, the study began with varying temperatures and proceeded by adding extraction solvents, either methanol or ethanol. 10 mL of extraction solvents were put into one gram of fish waste powder. Following this, the mixture was put in an incubator set to 60°C and incubated for 30 minutes. The mixture was subsequently strained onto filter paper, and the filtrate was taken for analysis. The variables investigated included solvent type (methanol or ethanol), temperature (ranging from 0 to 60°C), concentration of sample-to-solvent ratio (1:10 to 9:10% w/v), and extraction duration (30 to 150 minutes).

2.3 *Estimation of radical scavenging activity by ABTS assay*

An ABTS•+ solution was prepared using a combination of 7.7 mM ABTS and 2.45 mM potassium persulfate ($K_2S_2O_8$). A 10 mM PBS (pH 7.4) solution was used to dilute the ABTS•+ solution after being stored at room temperature in darkness for 12-16 hours. The solution was then maintained at 30°C in the absence of light until the absorbance reached 0.7 (±0.02) at 734 nm wavelength. Trolox was used as a standard antioxidant, ranging from 0 to 3 µM to calculate the % inhibition value for all samples. 20 µL of Trolox standards were mixed with 180 µL of ABTS+ working solution and vortexed for 30 seconds. The absorbance readings were performed at a wavelength of 734 nm using a UV spectrophotometer (Thermo Scientific™ GENESYS™ 50 UV-Visible, USA). Each run was triplicated and reported as mg Trolox equivalent per liter (mg TE/L) [8].

2.4 *Statistical analysis*

The extraction of antioxidants from *Decapterus maruadsi* wastes was carried out using a fractional factorial design (FFD) (2^{5-1}). The independent factors were duration of extraction (30 to 150 minutes), concentration of sample-to-solvent ratio (1:10 to 9:10% w/v), temperature (0 to 60°C), and solvent types (methanol or ethanol) with ABTS Radical Scavenging Activity as a response. There were 16 experimental runs designed by the statistical software package version 7.0 of Design Expert®, Stat-Ease, Inc., Minneapolis, MN, USA. To evaluate the statistical significance of the model, two-way ANOVA was employed. The statistical significance was attributed to probability values of $p \leq 0.05$.

3.0 EXPERIMENTAL RESULTS

3.1 *Influence of wet rendering parameters on ABTS radical scavenging activity*

Table 1 presents the antioxidant activity data of the wet rendering extract of *Decapterus maruadsi* wastes, with values ranging from 31.99 to 58.28 mg TE/L. The highest antioxidant activity was 58.28 mg TE/L when extracting the *Decapterus maruadsi* wastes for 30 minutes at 60°C using a sample-to-solvent ratio of 9:10 %w/v with methanol as the solvent. Methanol's greater polarity enables it to have better interactions with polar substances, like antioxidants present in fish waste. This characteristic makes methanol a better solvent for extracting these useful compounds in comparison to ethanol. This research aligns with Franco *et al.,* [9] in utilizing methanol as the extraction solvent for obtaining antioxidants from fish waste.

Table 1. Design FFD with the corresponding ABTS Radical Scavenging Activity.

Factors				**Response**
A	**B**	**C**	**D**	**Y**
0	150	Methanol	1.00	43.91 ± 0.033
60	30	Methanol	1.00	53.78 ± 0.004
0	30	Methanol	9.00	51.17 ± 0.005
60	150	Methanol	1.00	47.67 ± 0.056
60	30	Methanol	9.00	58.28 ± 0.004
0	150	Methanol	9.00	53.54 ± 0.021
60	150	Methanol	9.00	55.20 ± 0.002
0	30	Methanol	1.00	31.99 ± 0.104
0	30	Ethanol	1.00	43.51 ± 0.036
60	30	Ethanol	1.00	48.25 ± 0.033
0	150	Ethanol	1.00	40.83 ± 0.047
60	150	Ethanol	1.00	47.07 ± 0.074
0	30	Ethanol	9.00	44.07 ± 0.045
60	30	Ethanol	9.00	56.07 ± 0.003
0	150	Ethanol	9.00	45.96 ± 0.018
60	150	Ethanol	9.00	54.80 ± 0.022

*A– temperature (°C); B – Extraction time (minutes); C – Type of solvent; D – concentration of sample-to-solvent ratio; Y – ABTS Scavenging Activity (mg TE/L).

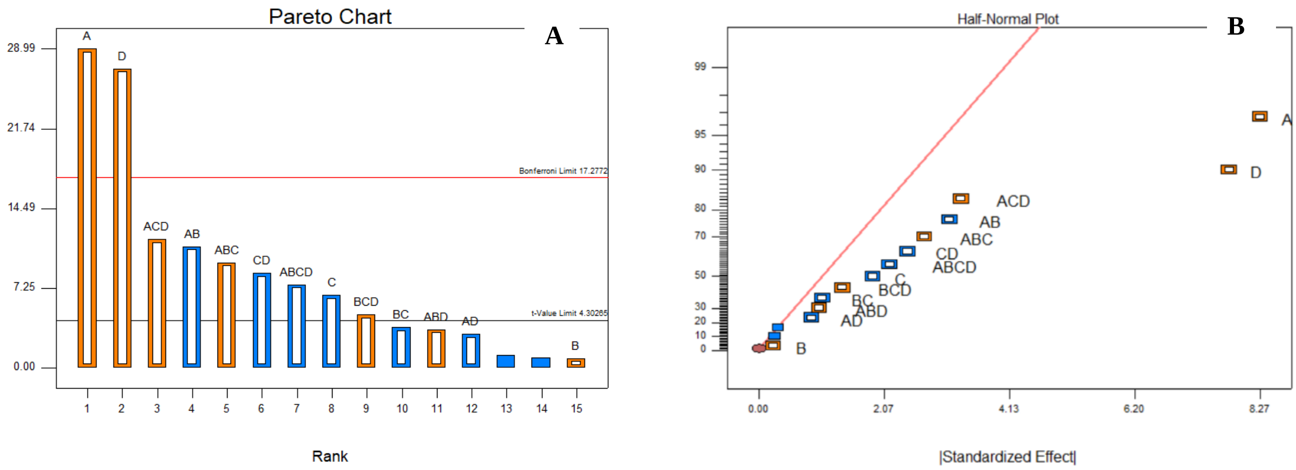

Figure 1. Standardized effects on ABTS radical scavenging activity represented through Pareto chart (A) and Half-Normal Plot (B).

The Pareto chart in Figure 1A illustrates the impact of the variables on the ABTS Scavenging Activity. The chart shows two horizontal lines representing the Bonferroni (17.2772) and t-value (4.30265) thresholds. According to the Pareto chart, a bar exceeding the t-value and Bonferroni limits indicates statistical significance ($p < 0.05$), while a bar below the t-value limit is deemed not significant ($p > 0.05$). Furthermore, variables that have effects exceeding the Bonferroni threshold are considered highly significant, whereas variables with effects falling between the Bonferroni threshold and t-value threshold are still considered significant. In this research, the variables A and D were identified as definitely significant, while ACD, AB, ABC, CD, ABCD, C, and BCD were considered potentially significant (Figure 1A). Based on the half-normal plots (Figure 1B), A and D were identified as outliers from the standardized effects clustered around the linear trend line, making them the most significant variables impacting ABTS Radical Scavenging Activity. A similar trend was also noticed with *p*-value results tabulated in Table 2, in which temperature and concentration ratio of sample-to-solvent were found highly significant ($p < 0.01$), while extraction time and type of solvent remained significant ($p < 0.05$) in obtaining higher antioxidant activity. The model and experimental data agreed well on ABTS activity ($R^2 = 0.9991$). The predicted R^2 value of 0.9409 is close to the adjusted R^2 value of 0.9931. The model's high significance was confirmed by the adjusted R^2 value. The predicted R^2 score indicates that the ABTS model will maintain its accuracy for future data. Additionally, the adequate precision ratio was 48.209, considered favorable as the value was greater than 4.

Table 2. *P*-values of response for every factors.

Source	***p*-value Prob>F $^{a, b}$**
Model	0.0060**
A	0.0012**
B	0.5006
C	0.0224*
D	0.0013**
AB	0.0081**
AD	0.0937
BC	0.0674
CD	0.0133*
ABC	0.0108*
ABD	0.0739
ACD	0.0073**
BCD	0.0404*
ABCD	0.0171*

$R^2 = 0.9991$; R^2-adjusted = 0.9931; R^2-predicted = 0.9409; Adeq Precision = 48.209

^{a} Significant (*P < 0.05) while ^{b} is Highly significant (** P < 0.01).

3.2 Plot of interaction effect against ABTS radical scavenging activity

Figure 2 displays the interaction effects plot between temperature and concentration of sample-to-solvent ratio towards ABTS radical scavenging activity. To achieve maximum extraction yield for interaction effects in Figure 2, the extraction time was held constant at 90 minutes with methanol as the solvent, while other factors were also constant. It was evident from Figure 2 that the red lines performed the best in terms of antioxidant activity at both 0°C and 60°C. The highest antioxidant activity was observed at 60°C with a 9:10 sample-to-solvent ratio. In summary, higher temperature and concentration levels can improve the effectiveness of antioxidants in removing the ABTS•+ radical, as temperature speeds up the reaction rate and higher concentrations result in more reactive substances. Increased levels of antioxidants lead to a greater number of available molecules to interact with and counteract ABTS•+. The ABTS test evaluates the reduction of radical cation absorption, influenced by the level of antioxidants and reaction time [10].

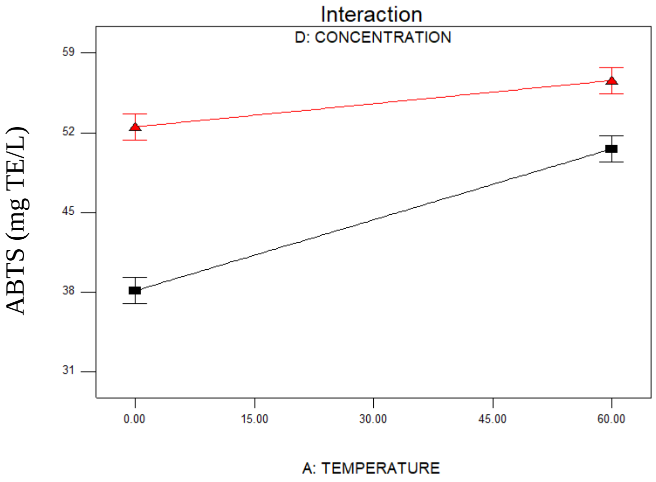

Figure 2. Correlation between factors on the ABTS radical scavenging activity (mg TE/L).

3.3 Optimum results from Design Expert

Design Expert software recommended an extraction time of 30 minutes at a temperature of 60°C, using methanol as the solvent at a concentration of sample-to-solvent ratio 9:10 (w/v%) to achieve an ABTS radical scavenging activity of 57.9962 mg TE/L. To verify the accuracy of the model proposed by Design Expert, an additional validation test was conducted. The experimental result was 58.04 mg TE/L, comparable to the predicted value of 57.9962 mg TE/L, with only a 0.08% error. This shows 99.92% accuracy between the results from the experiment and the predictions of antioxidant activity for *Decapterus maruadsi* wastes. Table 3 compares the results from the literature and the present study on different extraction methods with their ABTS radical scavenging activity. Based on this information, the more advanced the extraction method used, the greater the difference in antioxidant value obtained. Pulsed electric fields and enzymatic hydrolysis are considered more sustainable and eco-friendly extraction methods than wet rendering as they eliminate the need for solvents. Nevertheless, wet rendering can be considered as a choice because it is cost-effective and easy to manage.

Table 3. Type of fish waste, extraction method, parameter and ABTS radical scavenging activity in several fish wastes varieties.

Type of fish waste	**Extraction method**	**Parameter**	**ABTS radical scavenging activity**	**References**
Sea bass (*Dicentrarchus labrax*) wastes	Pulsed Electric Fields	specific energy, field strength, and time of extraction	400 (µmol TE/L)	[11]
Red tilapia scales (*Oreochromis sp.*)	Enzymatic hydrolysis	pH, type of enzyme, enzyme and substrate concentration	209.8 ± 2.96 (µmol TE/g prot)	[12]
Decapterus maruadsi wastes	Wet rendering	temperature, extraction time, type of solvents, and sample-to-solvent concentration ratio	57.9962 (mg TE/L)	This study

4.0 CONCLUSION

The present study employed a two-level factorial design screening to pinpoint key factors in the wet rendering process like temperature, extraction time, solvent type, and concentration of the sample-to-solvent ratio that influence ABTS radical scavenging activity. The antioxidant activity was significantly affected by temperature and the concentration of the sample-to-solvent ratio ($p < 0.01$). The Design Expert produced the best extraction condition resulting in the experimental result of 58.28 mg TE/L for antioxidant activity, achieved with a temperature of 60°C, 30 minutes extraction time, and methanol as the solvent at a 9:10 (w/v%) concentration ratio. To sum up, the wet rendering technique proved to be effective in extracting antioxidants from *Decapterus maruadsi* waste for use in the food and pharmaceutical sectors.

5.0 AUTHORS CONTRIBUTION

Muhammad Nurfikri Alif Abdullah (Conceptualization, Visualization, Writing-original draft)
Zatul Iffah Mohd Arshad (Conceptualization; Formal analysis)
Nurul Aini Azman (Methodology; Writing-review & editing)

6.0 ACKNOWLEDGEMENTS

This work is supported by Universiti Malaysia Pahang Al-Sultan Abdullah under UMP Flagship Research Grant (RDU182204-2).

7.0 REFERENCES

[1] Harun M, Ismail R, Sulaiman N. Status of Fish in Food Security in Malaysia. Journal of Advanced Zoology. 2023 Sep 1;44(3).

[2] Xu X, Liu A, Hu S, Ares I, Martínez-Larrañaga MR, Wang X, Martínez M, Anadón A, Martínez MA. Synthetic phenolic antioxidants: Metabolism, hazards and mechanism of action. Food Chemistry. 2021 Aug 15;353:129488. https://doi.org/10.1016/j.foodchem.2021.129488

[3] Felter SP, Zhang X, Thompson C. Butylated hydroxyanisole: Carcinogenic food additive to be avoided or harmless antioxidant important to protect food supply?. Regulatory Toxicology and Pharmacology. 2021 Apr 1;121:104887. https://doi.org/10.1016/j.yrtph.2021.104887

[4] Ivanovs K, Blumberga D. Extraction of fish oil using green extraction methods: A short review. Energy Procedia. 2017 Sep 1;128:477-83. https://doi.org/10.1016/j.egypro.2017.09.033

[5] Dave J, Ali AM, Kudre T, Nukhthamna P, Kumar N, Kieliszek M, Bavisetty SC. Influence of solvent-free extraction of fish oil from catfish (Clarias magur) heads using a Taguchi orthogonal array design: A qualitative and quantitative approach. Open Life Sciences. 2023 Nov 23;18(1):20220789. https://doi.org/10.1515/biol-2022-0789

[6] Yusoh NA, Man RC, Azman NA, Shaarani SM, Mudalip SK, Sulaiman SZ, Arshad ZI. Recovery of antioxidant from Decapterus Macarellus waste using wet rendering method. Materials Today: Proceedings. 2022 Jan 1;57:1382-8. https://doi.org/10.1016/j.matpr.2022.03.173

[7] Alasalvar H, Yildirim Z. Ultrasound-assisted extraction of antioxidant phenolic compounds from Lavandula angustifolia flowers using natural deep eutectic solvents: An experimental design approach. Sustainable Chemistry and Pharmacy. 2021 Sep 1;22:100492. https://doi.org/10.1016/j.scp.2021.100492

[8] Skowyra M, Calvo MI, Gallego Iradi MG, Azman NA, Almajano Pablos MP. Characterization of phytochemicals in petals of different colours from Viola× wittrockiana Gams and their correlation with antioxidant activity. Journal of Agricultural Science. 2014 Aug 15;6(9):93-105. https://doi.org/10.5539/jas.v6n9p93

[9] Franco D, Munekata PE, Agregán R, Bermúdez R, López-Pedrouso M, Pateiro M, Lorenzo JM. Application of pulsed electric fields for obtaining antioxidant extracts from fish residues. Antioxidants. 2020 Jan 21;9(2):90. https://doi.org/10.3390/antiox9020090

[10] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free radical biology and medicine. 1999 May 1;26(9-10):1231-7. https://doi.org/10.1016/S0891-5849(98)00315-3

[11] Martí-Quijal FJ, Castagnini JM, Ruiz MJ, Barba FJ. Sea bass side streams extracts obtained by pulsed electric fields: Nutritional characterization and effect on SH-SY5Y cells. Foods. 2023 Jul 16;12(14):2717. https://doi.org/10.3390/foods12142717

[12] Sierra-Lopera LM, Zapata-Montoya JE. Optimization of enzymatic hydrolysis of red tilapia scales (Oreochromis sp.) to obtain bioactive peptides. Biotechnology Reports. 2021 Jun 1;30:e00611. https://doi.org/10.1016/j.btre.2021.e00611