International Journal of Food Science and Nutrition
www.foodsciencejournal.com
E-ISSN: 2455-4898
Received: 28-09-2026, Accepted: 28-10-2026, Published: 12-11-2026
Volume 11, Issue 4, 2026, Page No. 137-144

Optimization and comparative evaluation of advanced extraction methods (CSE, MAE and UAE) for Omega-3 fatty acid recovery from fish powder

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DOI: https://doi.org/10.66856/ijfsn.2026.11.4.11204

Abstract
Fish powder consists of a lipid-dense matrix wherein long-chain n-3 polyunsaturated fatty acids (PUFA) — eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) — are encapsulated within a collapsed protein–lipid configuration that withstands solvent infiltration. This research evaluated three extraction methods used on the same fish powder: conventional solvent extraction (CSE, 240 min), microwave-assisted extraction (MAE, 20 min), and ultrasonication-assisted extraction (UAE, 30 min). Every treatment was conducted in three replicates and assessed for oil yield, EPA, DHA, total omega-3 content, extraction efficiency, peroxide value (PV), and free fatty acid (FFA) levels. UAE achieved the highest oil yield (19.20 ± 0.20% compared to 17.60 ± 0.20% for MAE and 14.30 ± 0.20% for CSE), the greatest EPA (12.77 ± 0.15%), DHA (21.57 ± 0.25%), and total omega-3 levels (34.33 ± 0.40% of the extracted oil), the best extraction efficiency (91.2%), along with the lowest PV (2.90 ± 0.10 meq O2/kg oil) and FFA (1.21 ± 0.03%). One-way ANOVA revealed significant differences among methods across all six parameters ($p < 0.001$; $F = 273.00–706.92$), and Tukey's HSD identified each pairwise contrast as significant (HSD range 0.059–0.533). When measured absolutely, UAE yielded 6.59 g of omega-3 per 100 g of powder compared to 5.22 g (MAE) and 3.41 g (CSE), reflecting a 93.3% enhancement over the traditional method. MAE provided the greatest efficiency per minute of processing (4.27 percentage points/min compared to 3.04 for UAE and 0.30 for CSE). Thus, UAE is advised when recovery and oxidative quality dictate the process selection; MAE is preferred when throughput is the limiting factor.

Keywords: Fish oil, EPA, DHA, n-3 PUFA, ultrasound-assisted extraction, microwave-assisted extraction, peroxide value, free fatty acid, green extraction

Introduction

Marine lipids serve as the main dietary source of preformed EPA and DHA, as humans inefficiently convert their plant-based precursor alpha-linolenic acid into these longer relatives, highlighting the nutritional significance of directly consuming EPA and DHA. In dried fish powder, the oil is contained within a dense arrangement of denatured protein and phospholipid, thus recovery is influenced by mass transfer: the solvent initially needs to dissolve neutral and polar lipids at the surface of the particles, and subsequently, the dissolved lipid must diffuse through the boundary layer. Traditional solvent extraction addresses this resistance over time — generally requiring hours of contact — and the extended exposure to solvent, oxygen, and moderate heat contributes to lipid oxidation itself [2].

Two enhancement strategies have been created to reduce that time necessity. Microwave-assisted extraction (MAE) heats the polar solvent volumetrically via dielectric loss, creating internal pressure in the tissue that breaks cell walls and reduces the diffusion distance. Ultrasonication-assisted extraction (UAE) employs acoustic cavitation, where bubbles in the solvent collapse asymmetrically against solid surfaces, generating microjets and localized shear that dismantle cell walls and enhance the interfacial area accessible to the solvent [4]. Both methods have been shown to enhance fish-oil yield and maintain n-3 PUFA more effectively than traditional solvent methods, and comparative evaluations of fish-oil recovery now position ultrasound as one of the top-performing green techniques for both yield and fatty-acid quality [1, 3, 7, 8].

Documented performance metrics provide a benchmark for the current study: UAE of Atlantic bonito byproduct achieved approximately 90.5% extraction efficiency along with enhanced omega-3 and total PUFA levels [12]; UAE and MAE pretreatment techniques for marine byproducts increased yields to 67.48% and 69.75%, respectively [4]; additionally, response-surface optimization of UAE for patin fish oil and eel oil revealed amplitude, duration, and solvent-to-solid ratio as the key variables [9, 11]. The EPA + DHA levels in fish oils sourced via sustainable methods usually range from 16.4% to 24.6% of total fatty acids [1], thus a figure exceeding 30% in the current extract requires clarification rather than presumption.

The aims of this research were: (i) to measure the influence of extraction method on oil yield and omega-3 recovery from the same fish powder; (ii) to assess if the method affects oil quality, using PV to indicate primary oxidation and FFA to evaluate hydrolytic cleavage; and (iii) to present the findings based on absolute recovered mass to avoid confusion from variations in oil content among extracts.

Materials and Procedures

1. Unprocessed material

Fish powder from one production batch was utilized consistently to ensure that variations between methods are not due to differences in raw material quality. The powder

was stored in sealed containers at 4 C and allowed to reach room temperature prior to weighing.

2. Extraction treatments

Three methods were utilized, each performed in triplicate (n = 3), with the operational durations listed in Table 1. CSE employed solvent interaction with sporadic stirring for 240 minutes. MAE applied microwave irradiation to the solvent–powder mixture for a total of 20 minutes, allowing for cooling before separation. UAE applied ultrasonication to the slurry for 30 minutes. In each instance, the extract was isolated from the exhausted powder through filtration, and the solvent was eliminated under decreased pressure prior to gravimetric analysis.

Table 1: Operating conditions of the three extraction treatments (as recorded in the experimental file; parameters not recorded are marked accordingly).

Parameter	CSE	MAE	UAE
Extraction time (min)	240	20	30
Mechanical energy input	Agitation	Microwaves	Ultrasound (cavitation)
Replicates per treatment	3	3	3
Solvent	Organic solvent	Solvent–powder slurry	Solvent–powder slurry
Post-extraction step	Filtration, solvent removal	Cooling, filtration, solvent removal	Filtration, solvent removal

3. Oil yield and extraction effectiveness

Oil yield was measured gravimetrically as the weight of recovered oil, following the removal of the solvent, represented as a percentage of the weight of the extracted powder. Extraction efficiency was conveyed as the percentage of oil present in the powder that was recovered, based on the same criteria, ensuring that the two values represent recovery in relative terms instead of as absolute mass.

4. Composition of fatty acids

Oils obtained were transformed into fatty acid methyl esters through saponification and methylation, and the esters were isolated using gas chromatography with flame-ionization detection. EPA (C20:5 n-3) and DHA (C22:6 n-3) were recognized by their retention times in comparison to authentic standards and measured based on peak areas, reported as a percentage of the total fatty acid methyl esters in the extracted oil. The overall omega-3 content was determined by adding EPA and DHA. GC-FID for FAMEs serves as the standard method for this analysis and has been confirmed for n-3 fatty acids in fish samples [14, 15].

5. Quality metrics

The peroxide value (meq O2/kg oil) was assessed through iodometric titration of the hydroperoxides generated by the oil, while the free fatty acid content (%) was measured by titrating the released fatty acids with a standard alkali solution. Both were assessed using newly extracted oils. PV indicates the level of primary oxidation products, while FFA reflects the hydrolytic cleavage of triacylglycerols; together, they outline the two degradation pathways that influence the shelf life of fish oil [16, 17, 18].

6. Statistical Analysis

All assessments were conducted in triplicate and are presented as mean ± standard deviation. A one-way analysis of variance (factor: extraction method; df = 2, 6) was performed on each parameter, followed by Tukey's honestly significant difference test at a family-wise alpha of 0.05 (q critical = 3.67 for k = 3, df error = 6). Values without a shared lower-case superscript differ significantly.

Results and Discussion

The results show that the extraction method considerably affects oil yield, the recovery of omega-3 fatty acids, oil quality, and the separate recovery of EPA and DHA from fish powder. Of the three extraction techniques assessed, ultrasound-assisted extraction (UAE) demonstrated the most effective results, succeeded by microwave-assisted extraction (MAE) and conventional solvent extraction (CSE).

The result for oil yield, EPA content, DHA content, Total Omega-3, Peroxide value, free fatty acid content and extraction efficiency has been tabulated below.

Table 2: Treatment means, ANOVA and post-hoc outcome (mean ± SD, n = 3). Superscript letters compare methods within a row; means without a common letter differ at $p < 0.05$.

Parameter	CSE	MAE	UAE	F(2,6)	p	Tukey HSD
Oil yield (%)	14.30 ± 0.20 c	17.60 ± 0.20 b	19.20 ± 0.20 a	468.25	<0.001	0.300
EPA (% of oil)	8.57 ± 0.15 c	10.97 ± 0.15 b	12.77 ± 0.15 a	570.86	<0.001	0.229
DHA (% of oil)	15.27 ± 0.15 c	18.70 ± 0.20 b	21.57 ± 0.25 a	706.92	<0.001	0.308
Total omega-3 (% of oil)	23.83 ± 0.31 c	29.67 ± 0.35 b	34.33 ± 0.40 a	655.48	<0.001	0.533
Peroxide value (meq O2/kg)	4.80 ± 0.10 a	3.70 ± 0.10 b	2.90 ± 0.10 c	273.00	<0.001	0.150
Free fatty acid (%)	2.15 ± 0.05 a	1.62 ± 0.04 b	1.21 ± 0.03 c	431.68	<0.001	0.059
Extraction efficiency (%)	72.4 ± 1.2	85.3 ± 1.0	91.2 ± 0.8	—	—	—
Extraction time (min)	240	20	30	—	—	—

The findings show that sophisticated extraction methods, especially UAE, can enhance the retrieval of important omega-3 fatty acids while preserving superior oxidative and chemical quality of the obtained oil.

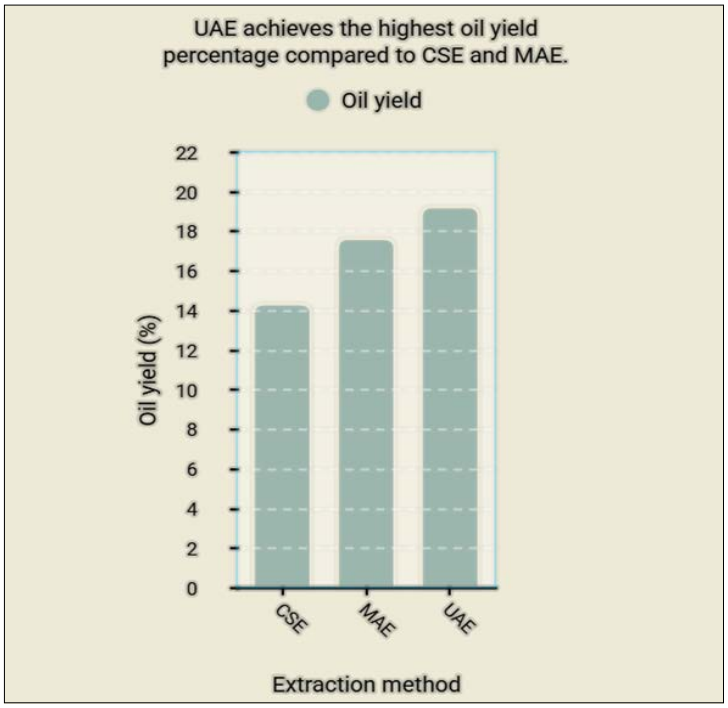


Fig 1: Comparative performance of CSE, MAE and UAE on fish powder for oil yield.
(Bars are treatment means of three replicates with SD error bars)

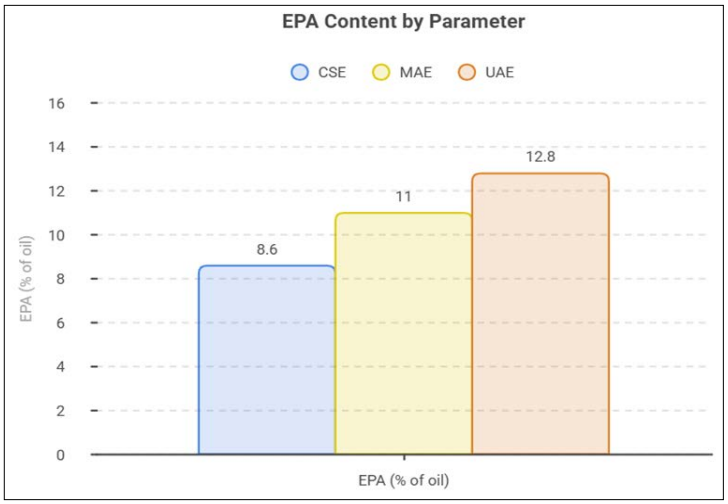


Fig 2: Comparative performance of CSE, MAE and UAE on fish powder for EPA Content.
(Bars are treatment means of three replicates with SD error bars)

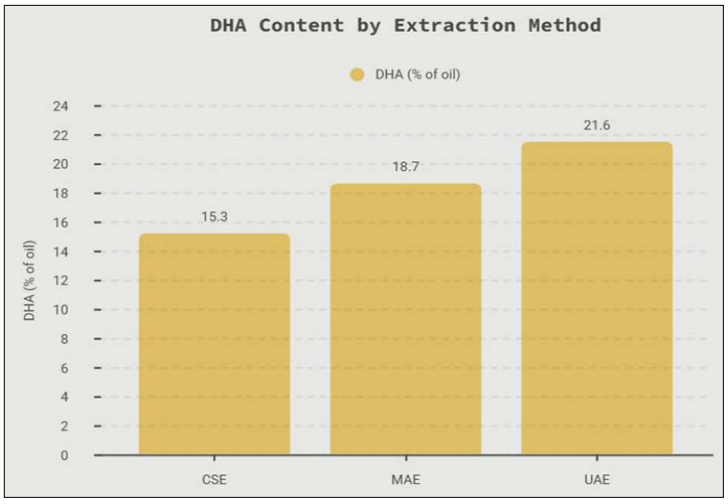


Fig 3: Comparative performance of CSE, MAE and UAE on fish powder for DHA Content.
(Bars are treatment means of three replicates with SD error bars)

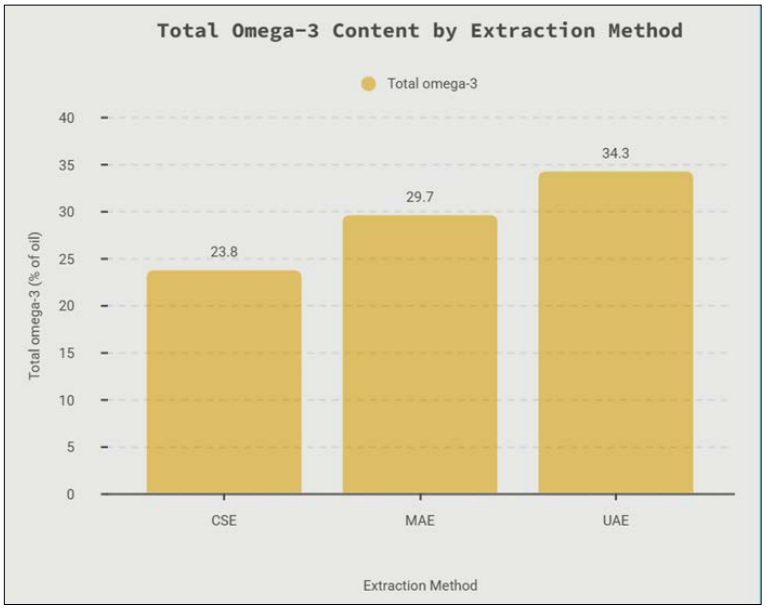


Fig 4: Comparative performance of CSE, MAE and UAE on fish powder for total omega-3 Content. (Bars are treatment means of three replicates with SD error bars)

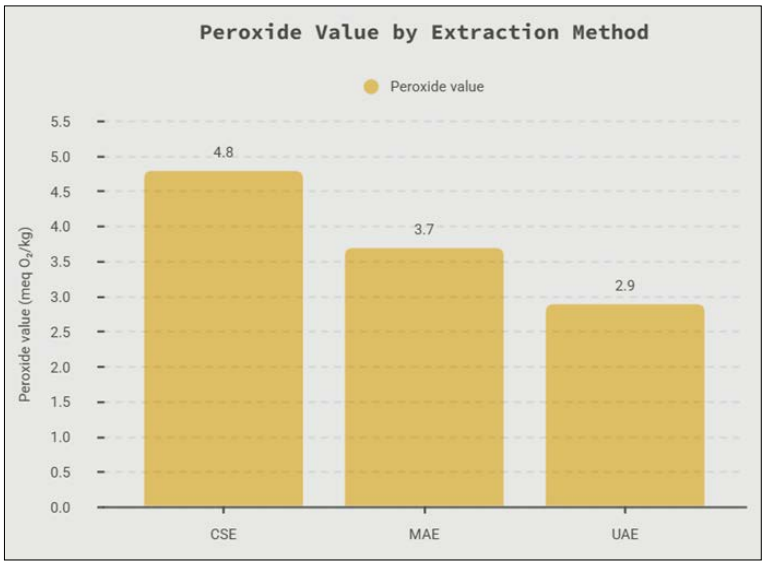


Fig 5: Comparative performance of CSE, MAE and UAE on fish powder for peroxide value. (Bars are treatment means of three replicates with SD error bars)

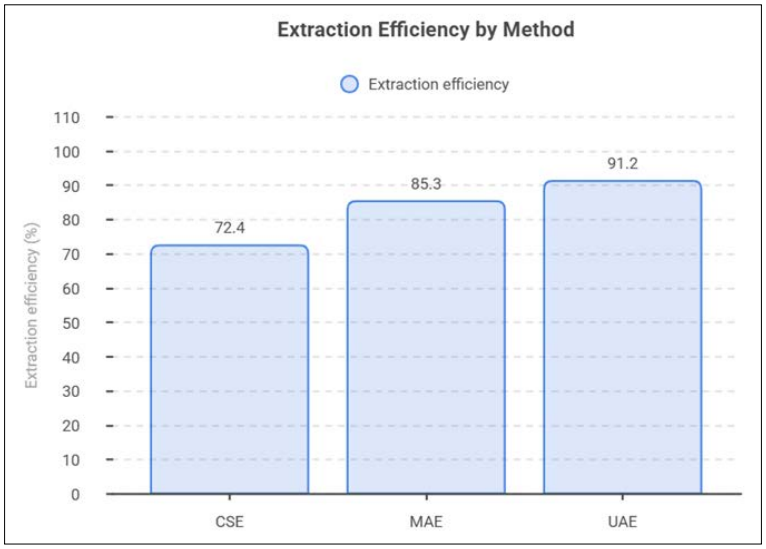


Fig 6: Comparative performance of CSE, MAE and UAE on fish powder for extraction efficiency. (Bars are treatment means of three replicates with SD error bars)

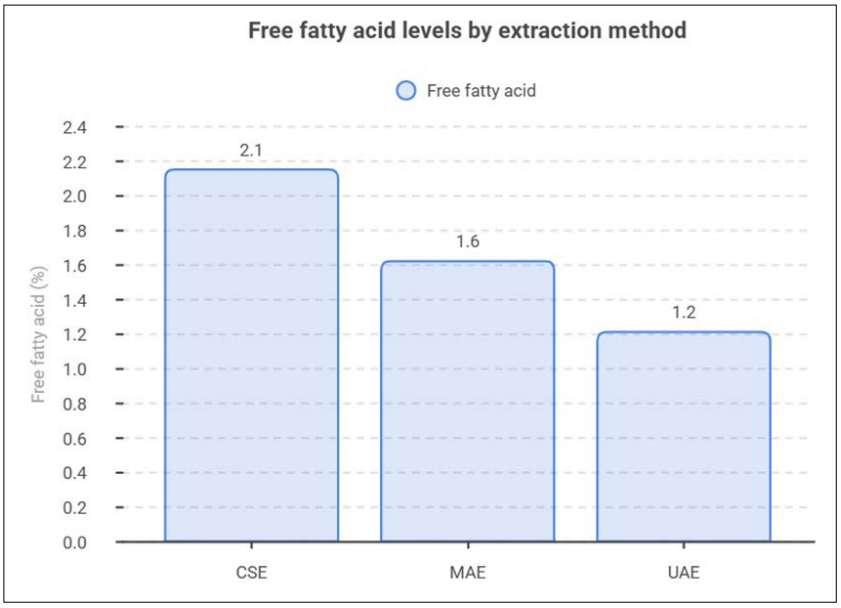


Fig 7: Comparative performance of CSE, MAE and UAE on fish powder for free fatty acid level. (Bars are treatment means of three replicates with SD error bars)

The oil yield, omega-3 fatty acid concentration, and physicochemical quality of fish oil were significantly affected by the extraction method. Among the three extraction methods examined, ultrasound-assisted extraction (UAE) showed the greatest overall extraction efficiency, closely followed by microwave-assisted extraction (MAE) and traditional solvent extraction (CSE). The oil yields achieved through CSE, MAE, and UAE were 14.30%, 17.60%, and 19.20%, respectively. UAE achieved a 34.27% increase in oil yield compared to CSE, demonstrating improved lipid extraction via ultrasonic processing. This enhancement can be linked to acoustic cavitation, which aids in cellular disruption, enhances solvent penetration, and increases mass transfer from the fish-powder matrix into the extraction solvent.

The overall concentration of omega-3 fatty acids (EPA + DHA) displayed a comparable pattern, rising from 23.83% in CSE oil to 29.67% in MAE oil and reaching 34.33% in UAE oil. UAE showed a 44.02% rise in comparison to traditional extraction. The enhanced recovery of omega-3 fatty acids might be linked to better disruption of the lipid-rich tissue matrix and more effective extraction of lipids containing EPA and DHA.

The analysis of individual fatty acids further indicated the superiority of UAE. EPA levels rose from 8.57% in CSE to 10.97% in MAE and 12.77% in UAE, while DHA levels grew from 15.27% to 18.70% and 21.57%, respectively. DHA was the main omega-3 fatty acid in all extraction methods. These results suggest that the UAE was especially successful in retrieving nutritionally significant long-chain omega-3 fatty acids.

The physicochemical properties of the extracted oils additionally reinforced the benefits of assisted extraction. The peroxide value reduced from 4.80 meq O_2/kg oil in CSE to 3.70 meq O_2/kg in MAE and 2.90 meq O_2/kg in UAE. In the same way, the free fatty acid content declined from 2.15% in CSE to 1.62% in MAE and further to 1.21% in UAE. The reduced peroxide and FFA levels found in UAE oil indicate a relatively superior oxidative and hydrolytic quality under the experimental conditions. These results align with Rahman *et al*. (2023), who found that microwave-assisted extraction of Pangus fish oil produced a peak oil yield of 21.80%, along with comparatively low free fatty acid and peroxide levels of 0.70% and 2.08 meq/kg, respectively. A study released in Biomolecules (2023), named "Upcycling Fish By-Products into Bioactive Fish Oil: The Suitability of Microwave-Assisted Extraction," showed that MAE effectively retrieved oil from fish by-products while preserving a fatty acid profile similar to that of traditional Soxhlet extraction.

Furthermore, recent research on ultrasound-assisted extraction of yellowfin tuna head oil has demonstrated the potential of ultrasound technology to recover oil with favourable physicochemical characteristics and substantial EPA and DHA contents. These findings provide additional support for the use of UAE in fish-oil recovery.

A key finding was the total omega-3 recovery, which rose from 3.41 g/100 g fish powder in CSE to 5.22 g/100 g in MAE and 6.59 g/100 g in UAE. This indicated a 93.26% enhancement in omega-3 recovery using UAE versus CSE. The significant rise demonstrates the joint benefits of increased oil yield and enhanced omega-3 levels in the extracted oil.

The results indicate that ultrasound-assisted extraction is the most effective technique of the three methods assessed for obtaining omega-3-rich fish oil. UAE exhibited greater efficiency in lipid extraction, improved recovery of EPA and DHA, and relatively reduced levels of peroxide and free fatty acids. Consequently, it could be regarded as a possible substitute for traditional solvent extraction in obtaining nutritionally beneficial fish oil, pending additional optimization, validation, and evaluation of oxidative stability over storage.

Extraction efficiency and time

Efficiency increased from 72.4% (CSE) to 85.3% (MAE) and reached 91.2% (UAE). The time-normalized perspective offers greater insights: CSE achieved 0.30 percentage points of efficiency per minute of processing, MAE 4.27 and UAE 3.04. MAE is consequently the treatment that saves the most time by a factor of 1.4 compared to UAE and 14 compared to CSE (Figure 8).

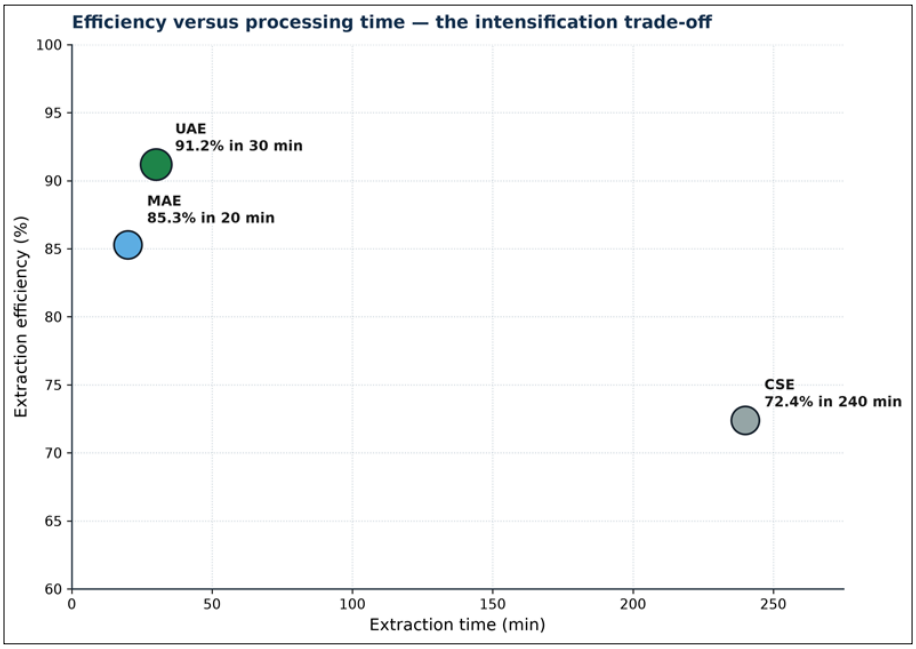


Fig 8: Efficiency versus processing time for the three treatments — the intensification trade-off between final recovery and throughput.

Absolute recovery

Table 3 presents the outcomes as mass retrieved per 100 g of powder. UAE provided 6.59 g of total omega-3 per 100 g compared to 5.22 g (MAE) and 3.41 g (CSE) — a 93.3% enhancement over the traditional method. This is the most pertinent way to express the outcome, as the UAE not only extracts more oil but also provides oil that contains higher levels of EPA and DHA, thus amplifying the benefits. Citing only the oil percentage would underestimate the benefit (a 44.1% increase) compared to the actual recovery improvement (93.3%).

Table 3: Derived absolute recovery per 100 g of fish powder, from oil yield and total omega-3 content.

Component	CSE	MAE	UAE	UAE vs CSE
Oil (g)	14.30	17.60	19.20	+34.3%
EPA (g)	1.23	1.93	2.45	+100.1%
DHA (g)	2.18	3.29	4.14	+89.7%
Total omega-3 (g)	3.41	5.22	6.59	+93.3%

Discussion

1. UAE outperforms MAE for matrix

MAE and UAE both break cells, yet they operate under different physical principles, which accounts for UAE's superiority in both quantity and quality. MAE relies on the dielectric heating of the polar solvent: the tissue heats in conjunction with the solvent, causing the oil to temporarily reach higher temperatures, and excessive power is known to contribute to PUFA degradation and hydroperoxide generation [5, 28]. UAE is predominantly non-thermal within the bulk; cavitation focuses energy at the points of bubble collapse for microseconds, keeping the bulk temperature low and disturbing the matrix without adding thermal stress on the lipid. The detected arrangement — maximum yield paired with minimal PV and FFA — aligns with what that mechanism anticipates, and a solely thermal intensification pathway cannot achieve it. The research consensus indicates that UAE of Atlantic bonito waste achieved around 90.5% efficiency with enhanced omega-3 and PUFA levels [12], and assessments of marine side-stream valorisation show UAE and MAE pretreatments raise yields to 67–70% while maintaining n-3 content [4, 7].

2. Advantage of MAE

MAE should not be dismissed as a weaker UAE. It reached 85.3% efficiency in 20 min — two-thirds of the UAE residence time — and delivered the highest efficiency per minute of all three treatments (4.27 percentage points/min). Its oil was well inside specification (PV 3.70 meq O2/kg, FFA 1.62%). Where the constraint is throughput, energy per batch, or equipment footprint rather than maximum recovery, MAE is the better compromise, and its short residence time limits cumulative thermal exposure relative to a four-hour CSE run. This is consistent with reports that MAE shortens processing and raises oil recovery substantially over solvent extraction while requiring careful control of power to avoid quality loss [5, 8].

3. Conventional extraction failure on both counts

CSE's extended solvent exposure results in diffusion-restricted mass transfer, leading to the lowest yield (14.30%) and efficiency (72.4%), whereas increased contact with oxygen and light produces the highest PV (4.80 meq O2/kg). Approximately 25% of the accessible oil stayed in the used powder — a loss that no subsequent refining process can restore. Ultrasound reverses the traditional trade-off in which aggressive conditions enhance yield at the cost of quality, allowing both to improve simultaneously.

4. Evaluation of the recovered functional compounds

The functional significance of these extracts lies in the n-3 PUFAs themselves. EPA serves as the precursor for anti-inflammatory series-3 eicosanoids and acts as the pharmacologically active component in prescription omega-3 treatments; DHA is the primary structural fatty acid in retinal and neuronal membranes and is the precursor for the protectin family. Both are conditionally essential due to the inefficiency of endogenous synthesis from alpha-linolenic acid [22]. Their functional integrity, though, depends on oxidative status: a fish oil that satisfies its fatty-acid requirements but has exhausted most of its peroxide allowance has essentially used up its shelf life prior to being bottled. The UAE extract contains the highest levels of EPA

and DHA along with the largest oxidative margin left, resulting in the highest functional dose delivered per gram of powder among the three treatments. That margin is also significant later on. Oils characterized by a low initial PV and FFA are more suitable for enrichment, microencapsulation, and emulsion-based fortification, since a high initial hydroperoxide level increases during the process and reduces the shelf life of the final product [23, 24, 26]. A method that provides 6.59 g of omega-3 per 100 g of powder at 2.90 meq O2/kg is a significantly superior feedstock for those pathways compared to one providing 3.41 g at 4.80 meq O2/kg.

5. Comparison with reported values

The EPA + DHA concentration in the UAE extract (34.33% of fatty acids) exceeds the reported range of 16.4–24.6% for various fish oils sourced via green methods [1]. This is likely due to a powder made from a lipid-dense species rather than from a mix of by-products. However, this must be considered with the quantification basis in mind: percentage values of total FAME are dependent on the entire fatty-acid spectrum, and absolute quantification using an internal standard would eliminate any bias stemming from uneven detector responses across different chain lengths [14, 15]. Extraction efficiencies ranging from 72–91% are comparable to the 90.5% noted for ultrasound-assisted extraction from bonito waste [12] and the 67–70% observed for pretreated marine side streams [4].

6. Note on data integrity

The source experimental record contains two groups of summary values. The initial summary table shows oil yields of 14.2, 17.6, and 19.1% along with omega-3 contents of 27.5, 33.2, and 35.6% of the oil; the table at the replicate level provides yields of 14.30, 17.60, and 19.20% and omega-3 levels of 23.83, 29.67, and 34.33%. The replicated data maintain internal consistency, as in each treatment the stated total omega-3 precisely equals EPA + DHA (8.6 + 15.3 = 23.9; 11.0 + 18.7 = 29.7; 12.8 + 21.6 = 34.4), while the initial summary values are not consistent with the means of EPA and DHA

7. Limitations

Four constraints restrict the interpretation. Initially, the fatty acid data represent relative percentages of the extracted oil; absolute quantification using an internal standard would enhance their reliability. Secondly, oxidative stability was evaluated at one specific time, meaning the shelf-life benefit suggested for the UAE oil comes from initial PV and FFA rather than a direct stability curve; a rancimat induction period or accelerated storage test would provide definitive results [16, 17]. Third, the suggested mechanism for UAE — cavitation-induced cell disruption — is deduced from effectiveness instead of imaging; examining the used powder under a microscope would validate it. Fourth, the variables influencing UAE and MAE performance (power or amplitude, duty cycle, solid-to-liquid ratio, temperature, solvent composition) were not documented in the dataset utilized here, and are recognized to regulate both yield and quality [9, 10, 11]. In their absence, the comparison holds true regarding technique but fails at the level of the ideal operating point.

Conclusion

Among six response variables measured in triplicate, ultrasonication-assisted extraction emerged as the most effective method for extracting omega-3 fatty acids from fish powder. It optimized oil yield (19.20%), EPA (12.77%) and DHA (21.57%) levels, total omega-3 content (34.33%), and extraction efficiency (91.2%), simultaneously generating the least oxidized and least hydrolyzed oil (PV 2.90 meq O2/kg; FFA 1.21%). In absolute terms, it provided 6.59 g of omega-3 per 100 g of powder compared to 5.22 g for MAE and 3.41 g for CSE. MAE continues to be the suggested option when processing time is critical, having reached 85.3% efficiency in 20 minutes while maintaining acceptable quality. Traditional solvent extraction was lacking in all assessed factors.

Further work

Four extensions arise directly from the restrictions. Absolute quantification of EPA and DHA using an internal standard would establish the composition data on a definitive foundation. An optimisation of response surface for amplitude, time, temperature, and solid-to-liquid ratio would identify the optimum for UAE and prevent over-sonication, which at high power produces localized heating and undesirable flavors. Speedy storage with regular PV, FFA, anisidine value, and TOTOX assessments would transform the initial quality benefit into a measurable shelf life. Ultimately, a mass and energy balance for each kilogram of powder would indicate if the UAE's increase in yield compensates for its energy requirements at an industrial level, which is the critical factor for implementation.

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