Review

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Statistical Optimization Strategies for the Extraction Process Parameters of Bioactive Molecules from Seafood Byproducts

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Abstract: Extraction techniques of important bioactive molecules from the major concern of Seafood byproducts are getting emphasis for better valorization. Employing green extraction technologies for efficient and quality production of these bioactive molecules is also strictly required. Hence, understanding the extraction process parameters to effectively design an applicable optimization strategy could make this real. In this review, statistical optimization strategies applied for the extraction process parameters of bioactive molecules from Seafood byproducts are focused. The type of experimental designs, and techniques applied to criticize and validate the effects of independent variables on the extraction output was addressed. Dominant parameters studied were enzyme/substrate ratio, pH, time, temperature and power of extraction instruments. The yield of bioactive compounds, chitin and chitosan, proteins and peptides, enzymes and carotenoids (Astaxanthins) were the most studied responses. Efficiency and/or economic and quality considerations and their selected optimization strategies that favour the production of potential bioactive molecules were also reviewed.

Keywords: optimization; extraction; parameters; bioactive molecules; seafoods byproducts; green extraction

1. Introduction

Studying the nature of the extraction process factors is critically important for an efficient optimization process and for saving cost and time. There are different optimization strategies of the extraction process used for bioactive compounds from seafood byproducts. These strategies can be grouped into classical (one factor at a time) and multivariate or more than one-variable-at-a-time optimization techniques.

Classical optimization strategies in bioactive compound extraction methods have been carried out by controlling the influence of one factor at a time to predict the experimental response commonly called univariate or one variable at a time [1]. This is by keeping other extraction variables at a constant level when one parameter is changed. The major disadvantages of one-variable-at-a-time optimization are the interactive effects between variables cannot be studied and use larger numbers of experimental works, which makes it less applicable by consuming time and resources. Classical optimization (univariate) methods are mostly applied for selecting suitable extraction parameters such as extraction mixture or solvent type for particular bioactive compounds. It can not have robust experimental conditions since they disregard the possible simultaneous interaction of extraction parameters such as the composition of the extraction solvent, solvent volumes used, solvent type,

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extraction times, and solid/solvent ratio [2]. Simultaneous interaction effects of extraction parameters should be investigated to obtain the required bioactive molecule. This is due to the effects of extraction parameters to achieve optimal conditions such as the effect of temperature on a particular solvent; the effect of temperature on extraction time; the effect of extraction time and type of solvent etc. are very critical. Studying the interaction effects of such extraction parameters strongly affects the quality and yield of the final bioactive molecule. Many bioactive molecule extraction methods as it will be shown later apply univariate statistical strategies for screening and optimizing bioactive molecule extractions from seafood byproducts whereas multivariate or more than one-variable-at-a-time optimization strategies are being applicable in the extraction process optimizations of bioactive compounds from seafood byproducts. Optimization strategies such as surface response methodology, mixing modelling, and factorial design enhance the quality and performance of bioactive compound extraction techniques. Response surface methodologies (RSM) used to determine the maximum and minimum values of the extraction factors employ statistical designs such as Central composite design (CCD), Box Behnken design, Doehlert matrix, three-level factorial design, and mixture design. Applying these methods enables to study of the simultaneous effects of extraction parameters which save resources, and time and even enhance extraction efficiency [2]. For instance, in a study conducted on the microwave-assisted extraction of bioactive fish oil from the heads and fins of fish, RSM coupled with CCRD was applied to optimize the effects of extraction factors (time, microwave power, and solid/liquid ratio) [3].

The extraction of bioactive compounds from seafood byproducts such as carotenoids, polyphenolic constituents, omega- 3 long-chain polyunsaturated fatty acids, peptides, enzymes, chitin, amino acids, gelatine, collagen, and vitamins from fishery byproducts apply for sustainable resource valorization concurrently to solving environmental problems [4]. Moreover, Roy, *et al.* [5] reviewed the demand for proper utilization of a large portion of bioactive molecules like lipids, chitosan, gelatin, carotenoids, fucoidan, collagen, chitin, proteins, amino acids, and enzymes present in seafood byproducts which negatively affect the environmental condition as well as the industries' economic value. These bioactive molecules have important health effects showing antiproliferative, dipeptidyl peptidase-IV inhibition, calcium-binding ability, immunomodulation, antimicrobial, antioxidant, and angiotensin-I converting enzyme inhibition activity [6]. Considering extraction yield, quality, energy, time and costs, optimized green technologies for the extraction of these bioactive molecules from seafood byproducts have been showing promising results [7].

Optimization plays a significant role in the extraction of bioactive molecules from seafood byproducts. It is applicable in determining the maximum or minimum values of extraction variables (power of extraction instrument, solid-to-solvent ratio, temperature, time, the composition of the extraction solvent, etc.) considering quality, yield and cost of the expected response (output). It is also used as a cornerstone in designing for larger manufacturing processes. Many extraction instruments are tested based on optimization techniques to validate their efficiencies, time and processing cost. During a solid-liquid extraction, the solvent plays a great role in selectivity in which its polarity directly affects the solute to be extracted [8]. Hence, optimizing the type of solvent for selecting the appropriate extracting liquid is very significant. Optimization of the extraction of protein hydrolysates from shrimp (*Metapenaeus dobson*) was carried out by head waste response surface methodology (RSM) in order to determine the optimum extraction pH, temperature and enzyme/substrate ratio for better antioxidant activity [9]. In another study conducted on the extraction of chitinase from shrimp shell waste, the chitinase activity was optimized using a particle swarm optimization algorithm and artificial neural network by controlling variables (colloidal chitin, glucose, Tween 80 (common surfactant micelles), and yeast extract) of the fermentation medium [10]. Sharayei, *et al.* [11] studied optimizing extraction variables using the ultrasonic method. First, they optimized the effect of solvent type and extraction time. Then, extraction temperature, extraction time, and ultrasound amplitude on astaxanthin extraction efficiency from shrimp shell (green tiger, *Penaeus semisulcatus*). In their study, they suggested that the green extraction method (applying ultrasonication) is safe and efficient compared to the nonpolar solvent (petroleum ether, n-hexane) extraction of astaxanthin pigment with higher antioxidant activity.

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The extraction methods of bioactive compounds from seafood byproducts are broadly applied using traditional (such as wet pressing and extraction using solvents or heat) and green, novel and sustainable methods (such as enzymatic hydrolysis, microwave-assisted extraction, and supercritical carbon dioxide (SC–CO_2)) extraction techniques. These green and novel methods are more applicable in quality production, and saving extraction energy, resources, time, and reducing associated environmental problems [4]. Moreover, non-thermal extraction methods of bioactive compounds such as membrane technology, pulsed electric field, high hydrostatic pressure, microwave-assisted extraction, cold atmospheric plasma extraction, and dense phase carbon dioxide are promising to recover extraneous chemical free bioactive compounds [7, 12, 13]. Other non-thermal extraction techniques employed combined extraction methods for comparison as well as for purification of the required bioactive molecule [14]. Membrane ultrafiltration was applied for the purification of bioactive peptides from Codfish blood and Sardine cooking wastewaters. Membrane sizes and appropriate pressure that achieve larger molecules of protein/peptides were the main factors considered for quality production [15]. Extraction methods using the traditional, green and novel methods with their controlling parameters are summarized accordingly and presented later.

The six principles of green extraction for natural products are the application of selective varieties and use of renewable plant resources, water or agro-solvents, innovative technologies that optimize energy consumption, bio- and agro-refining industry to produce co-products, minimize number of unit operations lead to convenient, robust and controlled process, and preserve extracted bioactive compounds from contamination and biodegradation [16]. Green extraction methods of bioactive compounds are designed to apply non-thermal/modern extraction techniques and use green solvents. This aims in reduction of energy consumption, allowance of the use of new-generation solvents, limitation of waste (conversion into co-products) to minimize environmental pollution, high quality of the required product, and result in non-hazardous extraction processes. Most of these non-thermal extraction methods and greener extraction procedures demand optimized processes for quality and better future production [12]. Green extraction processes use alternative solvents such as natural deep eutectic solvents, deep eutectic solvents and Ionic liquids to those organic/non-polar solvents. These green solvents are efficient for the extraction of organic, polymeric bio-compounds and inorganic compounds containing bioactive molecules which can be applied to food and pharmaceutical formulations [17]. From our review, studies are limited in clearly specifying which of these green solvents can be suitable to every bioactive compound to be extracted from seafood byproducts except the application of deep eutectic solvents and natural deep eutectic solvents such as malonic acid, thiourea, glycerol, and urea for the extraction of chitin from Lobster shells and Shrimp shells [18].

Efficiency and/or economic and quality considerations to apply statistical optimization methods considering the production of potential bioactive molecules are other important issues emphasized by researchers. An efficient, exploration of eco-friendly, and cost-effective extraction methods, which maximize the recovery of valuable bioactive compounds is the example of unconventional and/or green solvent extraction methods employing best optimization strategies [19]. Efficiency and/or economic and quality considerations for the extraction of bioactive compounds from seafood byproducts are not limited to the application of unconventional and/or green solvent extraction methods but rather focus on the employment of best statistical optimization methods which ensure for the production of potentially bioactive molecules. Response surface methodology (RSM) for the optimization of extraction parameters of bioactive molecules is dominantly applicable which optimizes the utilization of processing materials, extraction time, and proper solvent. Response surface methodology (RSM) for the optimization of extraction parameters of bioactive molecules is dominantly applicable which optimizes the utilization of processing materials, extraction time, and proper solvent. In particular, the Box-Behnken design of response surface methodology (RSM) is efficient and economical to optimize the enzymatic hydrolysis variables which maximize the degree of deproteinization of carotenoprotein production from shrimp head waste and shrimp shell waste. The produced carotenoproteins have shown attractive amino acid composition, color, and functional properties [20]. Moreover, An extraction method applied supercritical extraction combined with co-

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solvents for the efficient recovery of astaxanthin and lipids from Atlantic shrimp by-products (*Pandalus borealis*) to maximize astaxanthin yield and total carotenoid content employed RSM [21].

This review aimed at providing overview of statistical optimization strategies applied for the extraction process parameters of bioactive molecules from seafood byproducts. We reviewed optimization strategies used to extract bioactive molecules from seafood byproducts. Parameters considered for bioactive extraction techniques and types of seafood byproducts were identified and their method of optimizations was reviewed. Limitations on the statistical optimization strategies were also analyzed and best options were forwarded.

2. Statistical Optimization Strategies Applied for the Extraction of Bioactive Molecules from Seafood Byproducts

To study the effects of more than one treatment of an experiment, the experiment should be designed considering the following stages: 1) choosing and understanding the measuring instruments; 2) selecting the experimental subject; 3) selecting procedures and operations of the expected measurement. These stages are incorporated into the basic steps in designing the experiments. First, defining the problem expected to be solved; second, listing and understanding the factors that affect the extraction process; third, screening the factors that affect interactively by experimentation; at last, optimizing the extraction process using the chosen factors. The optimized extraction condition should show efficient and quality yield at lower extraction processing cost and time [22, 23]. Therefore, statistical experimental design (DOE) methodologies are very important to get the required efficient amount of information at the lowest number, cost and time of experimental analysis. This can be achieved by planning the testing method, applying appropriate data analysis, analyzing of interactive variability of factors, and reporting data in a scientific approach [22].

2.1. Classical versus Multivariate Optimization Techniques Applied for the Extraction of Bioactive Molecules from Seafood Byproducts

Classical/univariate statistical optimization approaches are applied for comparing the means between two groups of analysis or to discriminate the effect of extraction variables using statistical analysis such as variance (ANOVA), t-tests, and Fisher's multiple comparisons test. Moreover, statistical methods have been applied for the determination of the normality of the data and to detect outlier values during parameter testing and optimization [24, 25]. Kumar, *et al.* [1] studied chitinase production from shrimp waste using submerged fermentation. They applied one variable at a time optimization method for chitinase production from shrimp waste using submerged fermentation. In this study, fermentation variables screened using Plackett-Burman were incubation time, different media, pH, temperature, carbon source, nitrogen source and metal ions. To study the effect of enzyme activities of every factor determination was carried out using Equation 1. Univariate statistical optimization techniques are dominantly applied for screening of extraction affecting variables.

$$E_i \ \frac{\sum P_{i+} \sum P_{i-}}{N} \qquad (1)$$

where E_i represents the effect of parameter i studied, P_{i+} and P_{i-} correspond to the responses of trails at which the parameter was at its high and low level correspondingly, and N refers to the total number of trails. Although one factor at a time optimization approach has drawbacks such as time and cost consumption to conduct a large number of variables and limited knowledge on the interaction effects of variables on the responses, it has been applied for optimizing bioprocesses to extract different active secondary metabolites [26].

Multivariate statistical optimization methods like response surface optimization (RSM), nonlinear least-squares (quasi-Newton) method, Particle Swarm optimization algorithm, and artificial neural networks (ANN) have been employed for optimizing the extraction processing parameters of bioactive compounds from seafood byproducts. In particular, the response surface optimization (RSM) is coupled with statistical experimental designing such as Doehlert design, full factorial design, Box-Behnken design, and Central Composite design. Some of the multivariate

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statistical optimization methods apply to the screening of independent factors, selecting appropriate regression model, coding and defining the level of variables, verifying the fitted model, visualization of the predicted model equation, determining the optimal extraction condition, and validating the model equation by measuring the response at the predicted optimal conditions.

2.2. Screening Extraction Parameters Used for the Extraction of Bioactive Compounds from Seafood Byproducts

During the definition of the problem expected to be solved and understanding and listing of the factors that affect the extraction process, intensive potential factors which affect the desired response may be present. These factors should be reduced by eliminating less important ones to save processing time and cost. Moreover, the level of complexity of the experimental designs can cause difficulties and experimental errors in understanding the interactive effects of the independent variables on the expected response. Selecting influencing factors by minimizing the number of experiments helps to collect the maximized information [22, 27]. In the parameter screening the experiment should be based on: first, the need for the screening design should be identified; second, a specific number of the runs considering the range between the information gained and the extraction cost should be identified; last, feasibility and listing of the variables should be performed [22]. Some statistical software packages could give the screening outputs with the researcher's existing knowledge of the system and extraction cost of factors. Extraction factors screening can be applied using Plackett-Burman design or fractional factorial if the factors are more than 5 and full or fractional factorial designs for a lower number of factors (2–4) [23]. To develop the fractional factorial design, the quantity of experimental points is calculated as j^{k-1}, where j represents the number of factors to be tested and k for the number of levels. Multiple linear regression analysis should be applied to model the interaction between responses and the tested variables [28]. The readers can get details of the Plackett-Burman screening design from Vanaja and Shobha Rani [22]. The Plackett and Burman (PB) design is effective for screening n factors with $n+1$ experiments (i,e. for screening 7 variables 8 experiments should be conducted). It has been applied in designing chemometric tools combined with Box-Behnken design. This screening design is effectively applied only with expected linear (main) effects which do not consider factors with interaction. Factors that significantly affect the response values are depicted using the Pareto chart of standardization effects as quality tools [29]. Nidheesh and Suresh [30] studied the optimization of chitin extraction from shrimp processing raw byproducts. They employed fractional factorial design as a factor screening technique and in their optimization method (for screened variables), CCD coupled with RSM was applied to optimize the screened interaction effects of two variables. Variables such as concentration of HCl (%, v/v), reaction time (h), solid-liquid ratio of HCl solution, w/v), and number of treatments were used for studying demineralization effects on shrimp byproducts. After screening the variables, they optimized the effect of two significant factors (concentration of HCl (%, v/v) and solid-liquid ratio of HCl solution, w/v) on the demineralization. The one-variable-at-a-time approach is applied to screen factors that affect the extraction of bioactive molecules. In particular, factors were screened that affect the deproteinization and demineralization during the extraction of chitin and chitosan from Shrimp (*Parapenaeus longirostris*) shell waste. To achieve the highest deproteinization and demineralization degree, one-variable-at-a-time screening on the effects of carbon source (sucrose, glucose, or fructose) type, carbon source concentration, shrimp shell waste concentration, and incubation time were conducted before the optimization of the selected variables. Factors such as sucrose concentration, shrimp shell waste concentration, inoculum size, and fermentation time were selected for the optimized deproteinization and demineralization of the best chitin and chitosan extraction yields [31]. Ismail [32] applied a two-phase optimization model. The Plackett-Burman design was employed as the first phase to screen multiple fermentation parameters which have the highest influence on the extraction of thermostable chitosanase and chitooligosaccharides from marine shrimp processing raw byproducts. Seven independent factors named fermentation time temperature, period of microwave pretreatment of SPB, K_2HPO_4, $MgSO_4$, KCl and $FeSO_4{\cdot}7H_2O$ in eight experimental runs were screened

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in this study. The linear effect of the variables on chitosanase production was calculated using Equation 2.

$$Y = B_0 + \sum B_i X_i \qquad (2)$$

where Y refers to the response value or chitosanase production, B_0 represents the model intercept B_i for the linear coefficient and X_i represents the level of the independent factor.

Taguchi design is also effective to screen significant extraction factors which affect the quality and yield of bioactive molecules. Many of these factors are screened and optimized for the best extraction of phytochemicals, total phenolic content, and antioxidant activity [33]. Moreover, Taguchi analysis has been applied to screen suitable and efficient extraction methodologies such as maceration, decoction, and microwave-assisted extraction [34]. Jabeur, *et al.* [35] employed Taguchi experimental design to screen the most influencing factors named temperature, inoculum size of strain, and culture volume from 9 factors to develop an optimized protease production. Similarly, a two-factor Taguchi orthogonal array was employed to optimize the oil extraction process from catfish heads. In this study, extraction temperature and time were screened as influential variables for better oil recovery and yield [36].

2.3. Screening Used for Selecting Potential Extraction Solvents and Hydrolyzing Enzymes

Different extraction capacities to the bioactive molecules are presented for individual polar and non-polar solvents. However, the mixtures of the polar and non-polar show better extraction. Hence, screening of these appropriate solvents for selecting potential extraction solvents is very important. Moreover, solvents like microemulsion (containing tributyloctylphosphonium bromide, tributyloctylphosphonium trifluoroacetate, or tetrabutylphosphonium trifluoroacetate) have strong electrostatic and hydrogen bonding interactions than the less polar solvents (ethanol, acetone, and dimethyl sulfoxide) which can enhance the extraction of bioactive compounds such as astaxanthin [37]. In a study conducted to extract astaxanthin from shrimp (green tiger, *Penaeus semisulcatus*) shell using ultrasonic-assisted extraction, individual effects of solvents (petroleum ether, n-hexane, ethanol, acetone) and ternary mixtures of petroleum ether, acetone, and water were screened. The solvents having higher polarity were reported the most effective for astaxanthin extraction. Moreover, the effect of different ternary mixtures of petroleum ether/ acetone/water solvents has shown larger extraction of astaxanthin. This is due to the reason that, during the extraction of bioactive molecules the solvents can diffuse into the material substrate and dissolve molecules that have relative polarity however, the non-polar solvents withhold to diffuse into the hydrophilic layer [11]. During the production of protein hydrolysates from undersized hakes (fish by-catch), enzymatic activities of broadspectrum endoprotease, serine-type endoprotease, trypsin-specific protease, chymotrypsin-like protease, blend of endo- and exopeptidases and glutamic acid-specific protease were screened to select the best hydrolyzing enzyme [38].

2.4. Multivariate Regression Model Selection and Optimization of Screened Extraction Parameters of Bioactive Compounds

Once the determining extraction parameters/variables are screened, selecting an appropriate statistical regression methodology to study their interaction with the dependent variables is crucial [23, 39]. From the study of the relationship between independent and dependent variables, it is possible to show if the model can be linear, quadratic or cubic with coefficients which indicate values and signals that help to interpret the influence of the factors [40]. To optimize the extraction parameters of bioactive compounds from seafood byproducts statistical regression methodologies such as response surface optimization (RSM), nonlinear least-squares (quasi-Newton) method [41-43], particle swarm optimization algorithm [10, 44], and artificial neural networks (ANN) [10] were employed. Some of the statistical regression methodologies were applied in combination with two or three methods such as particle swarm optimization algorithm with artificial neural network [10] and RSM with Genetic algorithm and particle Swarm [44]. From our reviewed multivariate statistical optimizations applied for optimizing extraction variables/parameters of the bioactive compounds from seafood byproducts RSM was dominantly applied.

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Nonlinear least-squares (quasi-Newton), particle swarm optimization algorithm, and Artificial neural networks (ANN) optimization methods have been rarely employed to optimize the extraction parameters of bioactive compounds from seafood byproducts. Vázquez *et al.* [41] optimized proteolytic digestion independent variables (pH, temperature, and protease concentration) for the protein hydrolysates production from monkfish (*Lophius piscatorius)* heads and viscera using nonlinear least-squares (quasi-Newton) method. From the calculated individual percentage contributions (PC) of independent variables using Equation 7, the quadratic terms (pH and temperature) of the developed models have shown a significant effect on the enzyme proteolysis of monkfish. Moreover, an enzymatic hydrolysis optimization study was conducted by controlling temperature and pH as critical factors to produce protein hydrolysates from *Scyliorhinus canicula* discards (muscle) [42]. From the developed equations quadratic term for the alcalde enzyme hydrolysis (95.8%) and linear effect (Temperature, 97.2%) of esterase enzyme hydrolysis have shown the highest percentage contributions than the other terms.

The particle swarm optimization method is applicable for optimizing complex optimization problems, such as fermentation process parameters that were developed by Kennedy and Eberhart [45]. This method is applicable for searching the best values by linking and exchanging knowledge among swarm individuals. In particular, Suryawanshi and Eswari [10] studied the production of chitin from seafood byproducts like shells, tails, heads and bones by enzyme hydrolysis optimized using particle swarm optimization algorithm and artificial neural network optimizations considering colloidal chitin, glucose, Tween 80 (common surfactant micelles), yeast extract as basic fermentation medium factors.

Genetic algorithm as part of randomized search optimizations (natural evolution studies) is applicable for presenting initial conditions at previously developed process mathematical model. It has been applied for optimizing protein extraction by aqueous two-phase system [44, 46]. Saravana Pandian, *et al.* [44] conducted an aqueous two-phase system protein extraction yield from Shrimp (*Litopenaeus vannamei*) waste. They optimized the process condition considering polyethylene glycol concentration, trisodium citrate concentration, pH and temperature as determining factors. In their study, they employed RSM coupling Genetic algorithm (GA) and Particle Swarm. The RSM optimized parameters were used as initial conditions for the Genetic algorithm partitioning study of the recovered proteins. Moreover, the initial conditioning of the RSM regression equation was utilized for studying parameter influences over the process using Particle Swarm optimization. From the developed optimization models of the top phase protein yield response, the calculated maximum percentage contribution of the terms are from the linear (59.5%) and the quadratic (40.1%).

2.4.1. Response Surface Optimization (RSM) as a Tool to Optimize the Extraction Parameters of Bioactive Compounds

Response surface methodology (RSM) is a collection of mathematical and statistical techniques where experimental data are fitted on a polynomial equation. It is applicable to show the effect of independent factors on the dependent (response) variables with a generated empirical model. Moreover, it is a more suitable methodology to select if the extraction processing data favours a linear or square polynomial function. RSM is applied by coupling with different experimental designs such as Doehlert design, full factorial design, Box-Behnken design, and central composite design [23, 26, 39]. Most of the multivariate statistical optimizations employed for optimizing the extraction parameters of bioactive compounds from seafood byproducts were RSM coupling Box-Behnken and central composite designs.

Choice of the RSM Experimental Design

The choice in applying RSM coupling experimental designs (Doehlert design, full factorial design, Box-Behnken design, central composite design) is the applicability (efficiency of parameters) for a larger number of experiments, number of experiments/runs and blocks, required, number of variables/factor level used, the centre point used, selection of experimental points, and axial points

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used. The three-level factorial design is not efficient if the number of factors that can be greater than 2 [23, 47].

Suitable models starting from linear function (simplest model) as shown inEquation 3, should be tested to the obtained responses. In this linear model, the responses should not show any curvature. Any curvature observed should be evaluated using a second-order model which has central points. Interaction effects between experimental variables are evaluated by applying polynomial models (Equation 4) which have additional terms. Critical points (maximum, minimum, or saddle) of the variables are evaluated using a polynomial function (Equation 5) which contains quadratic terms. Moreover, this polynomial function should be performed using at least three-factor levels. Box–Behnken design, three-level factorial design, central composite design, and Doehlert design are commonly applicable second-order symmetrical designs [23, 39].

$$y = \beta_0 \sum_{i=1}^{k} \beta_i x_i + \varepsilon \quad (3)$$

where k represents the number of variables, β_0 the constant term, β_i the the coefficients of the linear parameters, x_i the variables, and ε refers to the residual associated with the experiments.

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{1 \le i \le j}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (4)$$

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{1 \le i \le j}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (5)$$

where β_{ij} and β_{ii} refer to the regression coefficients of interactive parameters and quadratic parameters respectively.

2.4.2. Coding the Factor Levels

Factors having different units and levels must be coded by converting their real value into ranges by keeping their dimensions (-1 to +1) when the design is developed based on the coded value. The real Z_i value can be changed to coded values x_i using Equation 6. Defining the level of factors is very critical for the achievement of process optimization of the screened variables before conducting the regression analysis which also helps for the codification [39].

$$x_i = \frac{Z_i - Z_i^0}{\Delta Z_i}, \mathrm{I} = 1, 2\ldots\ldots, \mathrm{k} \quad (6)$$

where x_i refers to the dimensionless coded value of the independent factor, Z_i corresponds to the actual value of the independent factor i, Z^0_i refers to the real value of the independent factor at the centre point and ΔZ_i refers to the step change of the real value in the center point. Some studies on optimization of the extraction of bioactive compounds from seafood byproducts have been applied coding the factor levels but others use the coding and actual values for better understanding of the readers [44, 48, 49].

2.4.3. Central Composite Design

Many studies applied the central composite design coupled with RSM for optimizing the extraction parameters of bioactive compounds from seafood byproducts. The central composite design is applicable for sequential experimentations with reasonable evidence since it contains three-point types such as (1) full factorial or fractional factorial design (2) a central point, and (3) axial points. The axial points are additional designs to show if the experimental points are at some distance from the centre point. A complete routable central composite designs are characterized as (1) experimental numbers should be calculated as $N = 2^k + 2k + c_p$, where k is for the number of extraction process factors, 2^k is for the number of designed factorial points, $2k$ the number of axial points at a distance of $\pm\alpha$, and c_p for the replicate number of the central point; (2) considering the number of variables the α/axial points should be calculated as $\alpha = 2^{(k)1/4}$; and (3) factors should be investigated at five levels ($-\alpha$, -1, 0, +1, $+\alpha$) [39, 50].

Nidheesh and Suresh [30] studied the optimization of isolation conditions of high-quality chitin from shrimp byproduct. In their optimization, they applied a 2-level with centre points fractional

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factorial design (FFD) for identifying influential shrimp byproduct demineralization variables (concentration of HCl solution, reaction time, solid-liquid ratio of HCl solution, and number of treatments). Similarly, for the deproteinization of demineralized shrimp byproducts they screened from five variables (reaction time, solid liquid ratio of NaOH solution, and number of treatments as before and adding 2 new variables reaction temperature and concentration of NaOH solution). Then, they optimized the screened variables for demineralization (concentration of HCl solution and solid-liquid ratio of HCl solution) and deproteinization of demineralized (concentration of NaOH solution, reaction temperature, and solid liquid ratio of NaOH solution) of shrimp byproducts using CCRD. Fitted/developed models of RSM coupled with CCD optimizations from experimental studies conducted on the extraction of bioactive compounds from seafood byproducts were selected to see their effect on the response or extraction yields. Individual percentage contributions (PC) of extraction variables from RSM coupling CCRD equations can be calculated using the Equation 7 [50, 51, 52] Total percentage contributions (TPC) of linear, quadratic and interactive terms of the selected independent variables were calculated using Equations 8-10 for better understanding of the effect of extraction variables on yield/response [53, 54].

$$PC_i = \left(\frac{\beta_i^2}{\sum \beta_i^2}\right) x\ 100\ (i \neq 0) \qquad (7)$$

where β_i represents for the regression coefficients of each individual extraction process. This equation is preferable to apply it for screening extraction variables which can be visualized using a Pareto chart [51, 52].

Total percent contribution of the linear, quadratic and interactive terms of extraction variables can be calculated using the following equations [53]:

$$TPC_i = \frac{\sum_{i=1}^{n} SS_i}{\sum_{i=1}^{n}\sum_{i=1}^{n} SS_i + SS_{ii} + SS_{ij}}\ x\ 100 \qquad (8)$$

$$TPC_{ii} = \frac{\sum_{i=1}^{n} SS_{ii}}{\sum_{i=1}^{n}\sum_{i=1}^{n} SS_i + SS_{ii} + SS_{ij}}\ x\ 100 \qquad (9)$$

$$TPC_{ij} = \frac{\sum_{i=1}^{n} SS_{ii}}{\sum_{i=1}^{n}\sum_{i=1}^{n} SS_i + SS_{ii} + SS_{ij}}\ x\ 100 \qquad (10)$$

where TPC_i, TPC_{ii}, and TPC_{ij} refers for total percentage contributions (TPC) of linear, quadratic and interactive terms; SS_i, SS_{ii}, and SS_{ij} represents for computed sum of square (SS) of the linear, interactive and quadratic terms correspondingly.

The calculated total percentage contributions (TPC) of linear (88.8%), quadratic (16.2%) and interactive (3%) terms of the variables (concentration NaOH solution, reaction temperature and solid liquid ratio of NaOH solution) for the deproteinization of demineralized of shrimp byproducts show that their individual activities are more influential than their quadratic and interactive terms. In another optimization study on microwave-assisted extraction of nutritional oil yield from fish heads and fins, the linear (88.7, 51.2%) terms dominantly affected the extraction yield than the quadratic (6.8, 47.6%) and interactive (4.5, 1.2%) terms of the total percentage contributions (TPC) of variables (time, microwave power, and solid/liquid ratio) [3]. Similarly, Blanco, *et al.* [49] studied the optimization of collagen recovery using chemical digestion from skin of the small-spotted catshark considering the effect of temperature, time and concentration of acetic acid. In this study the linear (86.5%) effect of the variables is more significant than their quadratic (13.5%) effects. As can be observed, the calculated total percentage contributions of the linear and quadratic terms of the developed model are more influential than their interactive terms.

Optimizations models of fish bioactive oil extraction yield considering time, microwave power, and solid/liquid ratio from salmon viscera, salmon backbones, and salmon heads using microwave-assisted extraction was developed by de la Fuente, *et al.* [55]. In this study the effect of quadratic terms (time and ratio) are more influential than their interactive terms for the better oil extraction yield from salmon viscera, backbones and heads (Figure 1A). However, linear term of the effect of microwave power show greater influence on the extraction of oil yield from salmon heads.

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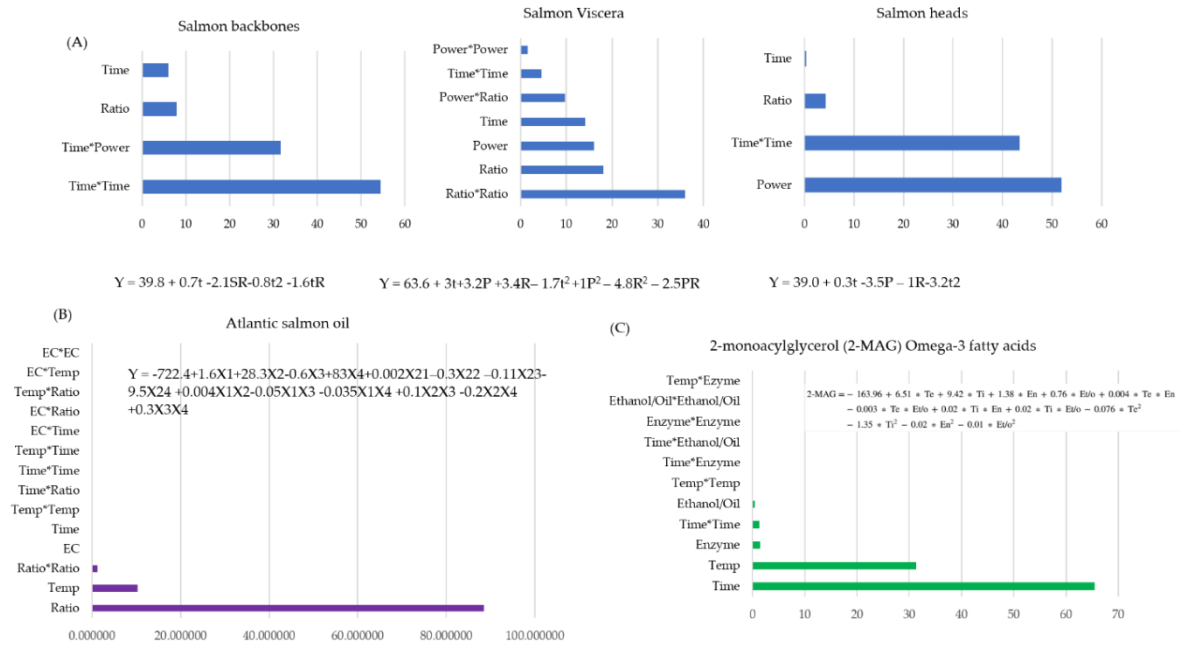

Figure 1. Individual percentage contributions (PC) of independent variables on optimization of bioactive compound extraction from Seafood byproduct of A) salmon viscera, backbones and heads using CCD, B) Atlantic salmon waste employed FFD, and C) Atlantic salmon (*Salmo salar*) bone using BBD [55-57].

2.4.4. Box-Behnken Design

Box–Behnken design is a three-level arrangement of factors which can be applicable to estimate coefficients of the first- and second-order mathematical models. Mainly, it contains a particular subset that originates from factorial combinations of the 3^k factorial design. It is more efficient and economical experimental design utilized for designing larger variables. In this design the experimental points are equally distant from the centre point which requires (1) experimental numbers calculated using the equation $N = 2k(k-1) + c_p$, where k represents the number of factors and (c_p) the number of centre points; and (2) factor levels should be with equally spaced intervals and arranged only to three-levels (-1, 0, 1). The three levels are low (−), high (+) and control or basal points in which the extreme points between factors or their high-level factors involved in the process can be evaluated. [23, 39].

The most influential independent variables selected to optimize the production of chitosanase and chitooligosaccharides from marine shrimp processing raw byproducts using a solid-state fermentation of enzyme production were fermentation period; concentration of $MgSO_4$, and concentration of KCl [32]. From this study, the total percentage contribution of the linear and quadratic terms of the developed model is the most influential. Moreover, to study the improvement of chitosan production from Indian white shrimp waste using chemical and microwave methods, the basic parameters optimized were temperature, concentration of alkaline, time of reaction for chemical and power of microwave, Irradiation time, and concentration of alkaline for microwave method. From the regression models developed to predict the effect of the variables, the linear and quadratic terms of both models are the most total percentage contributors on the chitosan yield. But, at an elevated microwave power and longer heating times the yield may decrease due to the reason that inhibition of the deacetylation reaction of chitosan [58]. Chandra Roy *et al.* [59] investigated the extraction of astaxanthin from shrimp (*Penaeus monodon*) shells using ultrasound-assisted natural deep eutectic solvents. The extraction process was optimized considering the natural deep eutectic solvent molar ratio, ultrasound amplitude, and extraction time as basic independent variables. From their developed model the extraction yield of astaxanthin was affected dominantly by linear and

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quadratic terms. The ultrasonication power and sonication time are very determinant factors for the extraction yield which could inhibit at an elevated level. Optimization of enzymatic hydrolysis reaction for the production of protein hydrolysate from scallops (*Argopecten purpuratus*) visceral meal and defatted meal was conducted to study the effect of process variables such as temperature, time, and enzyme concentration (enzyme/substrate level). The total contribution of factors in linear terms was the most influential than the quadratic and interactive terms [60]. In a supercritical carbon dioxide extraction of oil enriched with Eicosapentaenoic acid and Docosahexaenoic acid from Atlantic salmon frame bone, the effects of important variables such as urea/fatty acids ratio, crystallization temperature and crystallization time were optimized [61]. From the prediction regression model, the linear term has shown the most total percentage contribution than its quadratic and interactive terms. From this study, it is reported that the urea to fatty acid ratio is the most influential factor due to its contribution to the urea complexation process. In another study conducted on the production of carotenoids from red shrimp (*A. antennatus)* head using ultrasound-assisted and microwave-assisted extractions, the basic processing variables optimized were extraction time, ultrasound and microwave power and solvent/material ratio. The carotenoid extraction yield using the two modern extraction methods was affected by the quadratic linear and interaction terms. In particular, the total percentage contribution of the quadratic term dominantly contributed to the ultrasound-assisted extraction whereas the microwave-assisted extraction is affected by the interactive terms rather than the linear terms [48]. The summarized total percentage contribution of variables which affect the production of bioactive compounds from seafood byproducts that are optimized using RSM coupling BBD are presented in Table 1. The extraction yield (2-monoacylglycerol) of 2-monoacylglycerol (2-MAG) Omega-3 fatty acids from Atlantic salmon (*Salmo salar*) bone using supercritical carbon dioxide method was optimized using RSM coupled BBD considering extraction variables such as reaction temperature, time, enzyme load, and ethanol: oil molar ratio [57]. Individual percentage contributions of linear terms of the model equation developed show more influence than the interactive and quadratic terms which is depicted in Figure 1 (C).

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Table 1. Some RSM equations to depict total percentage contributions (TPC) of extraction variables for bioactive compound extraction responses.

DoE	Developed Equation	Number of factors	p	Percentage contribution of variables (%)			Reference
				TPC_i	TPC_{ii}	TPC_{ij}	
CCD	$Y = 39.2 + 9.3X_1 + 3.1X_2 + 4.1X_3 – 3.4X^2_3$	3	5	86.5	13.5		[49]
	$Y = 82.46-2.43X_1 +5.23X_2 +7.02X_3 + 0.64X_4 +0.31X_1X_2 +0.35X_1X_3 +0.33X_1X_4 -0.16X_2X_3 +0.1X_2X_4 – 0.5X_3X_4–10.6X^2_1 +0.4X^2_2 –0.051X^2_3 +16.62X^2_4$	4	15	59.5	40.1	0.4	[44]
	$Y = -120.3 + 416X_1 + 2.8X_2 + 9.2X_3 -3.6X^2_1 – 0.01X^2_2 -0.2X^2_3 – 0.14X_1X_2 -0.8X_1X_3 – -0.04X_2X_3$	3	10	80.8	16.2	3.0	[30]
	$Y = 14.4 + 0.8X_1 +0.04X_2 -1.5X_3–0.45X^2_3 – 0.3X_1X_3 +0.4X_2X_3$	3	7	88.7	6.8	4.5	[3]
	$Y = 19.5 + 0.52X_1 +1.2X_2 -1X_3–1.25X^2_1 -1\ X^2_2–0.3X_2X_3$	3	7	51.2	47.6	1.2	
BBD	$Y = 39.2 + 21.2X_1 - 3.7X_2 -0.066X_3 +0.154X_1X_2 + 0.045\ X_1X_3 + 0.003\ X_2X_3 – 0.64\ X^2_1 – 0.1\ X^2_2 + 0.004X^2_3$	3	10	86.2	13.5	0.3	[61]
	$Y = -33.1+0.81X_1 + 0.6X_2 + 85.3X_3 – 0.008X^2_1 – 0.003X^2_2 -91.2X^2_3 – 0.003X_1X_2 – 0.3X_1X_3$	3	9	51.1	44.3	4.6	[60]
	$Y = -9.9 + 11.5X_1 +1.7X_2 +1.7X_3 -0.09X_1X_2 -0.32X_1X_3 -0.006X_2X_3 – 0.7X^2_1 – 0.01X^2_2 - 0.1X^2_3$	3	10	64.2	19.7	16.1	[59]
	$Y = 4.9+0.9X_1 +0.5X_2 -0.4X_3– 0.3X^2_1 – 1X^2_2 - 0.4X^2_3$	3	7	68.2	30.9	0.9	[58]
	$Y = 7.1+0.9X_1 +0.5X_2 -0.4X_3– 0.5X^2_1 – 1.2X^2_2 - 0.5X^2_3$	3	7	52.2	43.0	4.8	
	$Y = -18.1+3.2X_1 -580.2X_2 +0.02X_3 –0.05X^2_1 +49269.7X^2_2 +0.27X^2_3 -16.6X_1X_2 +0.13X_1X_3 -47.5X_2X_3$	3	10	72.9	26.2	0.9	[32]
	$Y = 63.7-63.7X_1 -5.8X_2 -3X_3 + 16.6X_4 +5.8X_1X_2 +6.14X_1X_3 -2.9X_1X_4 -0.24X_2X_4 -0.3X_3X_4–1.4X^2_1– 4.7X^2_2 – 4.34X^2_3 + 1.8X^2_4$	4	14	86.2	6.6	7.2	[31]
	$Y = 10.7+1.3X_1 +0.1X_2 +2.2X_3 +0.4X^2_1 -0.6X^2_2 +1X^2_3 -0.8X_1X_2 +0.25X_1\ X^2_2 +0.8X^2_1X_2 +0.7X_1X_3 – 0.3X^2_1X_3 +0.4X_2X_3$	3	13	18.1	68.0	13.9	[48]
	$Y=15.9+0.6X_1+0.5X_2+0.7X_3-0.9X^2_1-0.4X^2_2+0.4X^2_3+0.23X_1X_2-1.55X_1X^2_2+0.5X^2_1X_2+1X_1X_3+1X^2_1X_3 -0.6X_2X_3$	3	13	30.6	14.0	55.4	
Full Factorial Design	1. $Y = 528.9 –29.04X1+0.87X^2_1 -164.8X_3 +23.2\ X^2_3$	3	5	98.1	0.003	1.9	[62]
	2. $Y = 28.8 –0.0013X^2_1 – 0.1X_2 -12.7X_3 + 1.8X^2_3$		5	98.0	0.006	2.0	
	3. $Y = 121.1 –78.4X1+49.3X^2_1 -44.2X_3 +31.9X^2_3$		5	70.1	21.0	8.8	
	$Y = -722.4+1.6X_1+28.3X_2-0.6X_3+83X_4+0.002X^2_1–0.3X^2_2 –0.11X^2_3-9.5X^2_4 +0.004X_1X_2-0.05X_1X_3 -0.035X_1X_4 +0.1X_2X_3 - 0.2X_2X_4 +0.3X_3X_4$	4	15	10.3	1.2	88.5	[56]

p- Number of coefficients of the developed model, Y = dependent variable/response/yield of the focused bioactive compound extracted from seafood byproduct; X_1, X_2, X_3, X_4 = optimized independent variable/factors/parameters which influence/affect the dependent variable/response/yield. TPC_i for total percentage contributions (%) of linear terms, TPC_{ii} for total percentage contributions (%) of quadratic terms, and TPC_{ij} for the interaction terms.

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2.4.5. Full Factorial Design

Full three-level factorial design is rarely applied in RSM optimization of bioactive molecules from seafoods byproducts compared to Box–Behnken, central composite, and Doehlert designs at factor numbers greater than two. This is due to the experiment numbers (N) required to conduct (which can be calculated as $N = 3k$, where k is several factors) being very high, so modelling of the quadratic functions can be inefficient. Fractional factorial design is preferable when the number of variables is greater than two which is mostly applied for screening larger variables [23, 39].

Some studies for the extraction of bioactive compounds from seafood byproducts have applied the full three-level factorial design coupling with RSM. In a study conducted on the Natural Deep Eutectic Solvent (choline chloride and L(+)-tartaric acid) based ultrasound and microwave-assisted extraction of carotenoids from shrimp waste, RSM coupled two-and three-level fractional factorial experimental design were applied to study the effects of extraction variables such as extraction time, solvent-to-propolis and Choline Chloride: Tartaric Acid-to-H_2O ratio on the carotenoid yield [62]. Moreover, Ramakrishnan, *et al.* [56] studied the enzymatic transesterification optimization of biodiesel yield from Atlantic salmon (*Salmo salar*) considering crucial factors such as enzyme concentration, temperature, oil/alcohol molar ratio and time. The individual percentage contributions of the model linear terms (temperature and oil/alcohol molar ratio) are more significant than the quadratic and interactive terms (Figure 1, B). They suggested that incorporating these terms into the developed model can make it unstable and can be difficult to interpret.

2.4.6. Doehlert Design

Doehlert design is considered as practical and economical compared to other second-order experimental designs which also require small experimental points that make it applicable and efficient. It is mainly characterized as 1) experiment number should be calculated using $N = k^2 + k + cp$, where k refers to the number of factors, cp for the number of centre point replication; 2) Important considerations such as cost and/or instrumental constraints of variables can be studied at a major or minor number of levels; 3) intervals can be uniformly distributed among levels; and 4) previous adjacent points can be used to displace the experimental matrix from another experimental region [39]. From our current study on the statistical optimization strategies, none of the bioactive compound extraction from seafood byproducts has applied RSM coupling Doehlert design. However, other studies on bioactive component extraction from other sources have been applied. For instance, da Silva Bambirra Alves, *et al.* [63] studied the production of protein hydrolysates from chicken blood meal using enzymatic hydrolysis by optimizing critical factors such as Temperature, pH and enzyme-to-substrate ratio employing the Doehlert design matrix.

2.4.7. Presentation of the Model and Determination of Optimal Conditions

Surface and response contour plots are theoretical two-and three-dimensional outputs mostly utilized methods to visualize the predicted model equations to depict the relationship among the dependent and independent variables. These are also applied to show any changes in the independent factors which lead to changes in the response values. Where contour plot works on a two-dimensional surface plot this improves understanding of the plots of the response surface. When the contour plot shows ellipses or circles then the experimental region is in the maximum, minimum or ranged point, however, if the contour plot depicts parabolic or hyperbolic shapes the target point is saddle point or not maximum nor minimum [23, 39, 64]. The ellipses shape contour plots developed from an optimization process of ultrasound-assisted astaxanthin extraction yield from shrimp shells are shown in Figure 2. A three-dimensional plot of two-dimensional representation is plotted using statistical software packages such as Design Expert and Sigma Plot for graphical representation/visualization of fitted model equations [58, 65]. So, for more than three independent variables the plot visualization is only applicable when one or more variables will be set at a constant value. There should be a consideration that, the response surface and contour plots only show

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estimated response and the general nature of the optimization system from the fitted model, but not the true structure. Although limited multivariate optimization strategies listed have used response surface polynomials to locate the maximum or minimum effects of independent variables, many of them have demonstrated response surface plots. The response surface graphs are not sufficient to locate the maximum or minimum value. On the contrary, one must work directly with the response surface polynomials and find the maximizing or minimizing factors. Hence, other methods like computing the first derivative of the fitted model function equal to zero then finding the stationary point by solving the linear equations. If the fitted model equation is like the Equation 11 [23, 64]:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_{22}^2 + \beta_{12} x_1 x_2 \qquad (11)$$

by computing the first derivative ($\partial_Y/\partial_{x_1}$ and $\partial_Y/\partial_{x_2}$) of this equation and setting zero, one can find the stationary point from Equation 12 and 13. These equations can be solved using Excel Solver tool.

$$\frac{\partial_Y}{\partial_{x_1}} = \beta_1 + 2\beta_{11} x_1 + \beta_{12} x_2 = 0 \qquad (12)$$

$$\frac{\partial_Y}{\partial_{x_2}} = \beta_2 + 2\beta_{22} x_2 + \beta_{12} x_1 = 0 \qquad (13)$$

where x_1 and x_2 refer for the coded value of the independent variables that give the highest or lowest response. Generally, the stationary point (minimum or maximum point) should be identified in the ranges of the tested independent parameters from the fitted second-order equation [64].

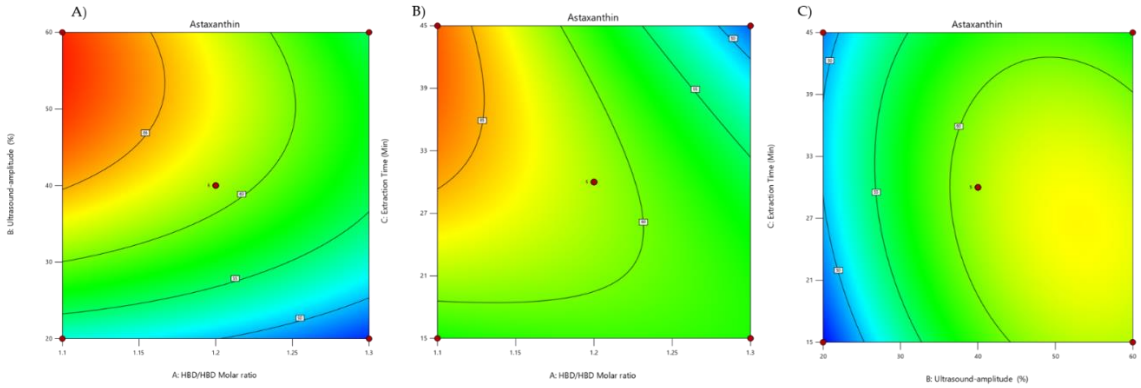

Figure 2. Contour plots of two-dimensional plots representing the interaction effect of natural deep eutectic solvents molar ratio (hydrogen bond donor HBD/acceptor HBA), ultrasound-amplitude, and extraction time on the ultrasound-assisted astaxanthin extraction yield from shrimp shells [59].

2.4.8. Robustness, Validation and Verification of Predicted Models/Optimized Extraction Conditions

Residual plots are valuable criteria to evaluate if the observed error (residuals) and stochastic error are consistent in which the residuals should be centred on zero within the fitted values and should not be systematically high or low. Undesirable residual plotting (residual analysis) shows a non-random pattern in which the predictor variables in the fitted model indicate the possibilities of missing variables and/or the presence of curvature due to higher-order terms of variables [23]. Figure 3 shows a residual plot of an ultrasound-assisted astaxanthin extraction yield from shrimp shells in which the residuals are slightly scattered from the centre point and the residuals are constantly spread throughout the range. Moreover, model adequacy can be evaluated by plotting predicted versus actual values (Figure 3 (B)) and Cook's distance values versus run number (Figure 3 (C)) [53]. The plot for predicted versus actual values shows the points of all predicted and experimental response values present very close to the 45◦ line to give information as there is a correlation between the process variables on the response of the developed model. Similarly, Cook's distance values fall in the determined range indicating the experimental data have no strong evidence of influential error observations. Studies conducted on the optimization of oil from aqueous two-phase protein extraction from *Litopenaeus vannamei* waste [44], oil enriched with eicosapentaenoic acid and docosahexaenoic acid extraction from Atlantic salmon by-product oil [61], production of omega-3

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fatty acids (rich 2-Monoacylglycerol) from Atlantic salmon oil byproduct [57], chitosan production from Persian Gulf shrimp waste [58], and extraction of high-energy carotenoid from *Aristeus antennatus* shrimp [48] used residual plots to check the models for any undesirable residuals.

Analysis of variance (ANOVA is more reliable way to evaluate the statistical significance of developed model by applying descriptive statistical analysis such as the standard deviation, prediction error sum of squares (PRESS) residuals, the lack-of-fit test, the coefficient of variation, the coefficient of determination (R^2), the adjusted determination coefficient (R^2), Adequacy precision, the F-value, and the p-value. The correctness of the model with experimental data can be evaluated by the adequacy of precision, determination coefficient R^2, and adjusted R^2. An adequate model is explained showing that the difference between the adjusted R^2 and predicted R^2 (Adj-*R*2–Pre-*R*2) should be less than 0.2, with maximum PRESS, and with a predicted R^2 value greater than 0.7. Adequacy precision measures the signal-to-noise ratio in which a ratio greater than 4 is desirable [39]. However, verification of the adequacy of the fitted model using the above statistical analysis only is not sufficient. There are two reasons for the coefficient of determination (R^2) alone cannot show the accuracy of the model. First, it will increase when the number of contributing variables to the model increases neglecting the statistical significance of the added variable. Second, measurement of the decreasing changeability of the achieved responses applying the affecting variables in the model is depicted by R^2 index. Hence, the accuracy of the model should also be checked using absolute average deviation (AAD) (Equation 14) showing statistical dispersion or variability or central point's absolute deviations [66]. From the analysis of R^2 and AAD, it is expected that the R^2 must be near to 1 and the range of estimated and observed AAD must be as low as possible [67].

$$AAD\ (\%) = \left\{\left[\sum_{i=1}^{p}\left(\frac{|yi_{exp} - yi_{cal}|}{yi_{exp}}\right)\right]/p\right\}\ x\ 100 \qquad (14)$$

where p indicates the number of experiments as well as yi_{exp}, and yi_{cal} for experimental, and calculated outputs of the experimental results respectively. The Analysis of variance (ANOVA) for the regression model developed and the calculated AAD for the extraction of astaxanthin yield from shrimp shells is presented in Table 2. The calculated AAD (%) presented in Table 2 gives additional adequacy information to the developed response. Most of the studies reviewed here considered ANOVA to discriminate the model developed. Although the AAD is a very important criterion to evaluate the adequacy/suitability of fitting the response surface of a model, no statistical optimization strategies in the current review were applied to verify the adequacy of the developed models for the extraction of bioactive molecules from seafood byproducts.

Fitting experimental data, analyzing the data, checking the validity of the fitted model, and determining the optimum extraction conditions are not enough to publish the adequacy of the developed model. Conducting confirmation experimental works at the optimized factor values and comparing the mean data with predicted values is very important for checking the reliability of the process. Unfortunately, none of the papers reviewed here considered conducting two or more confirmation experimental works at the selected optimum conditions.

Table 2. Model adequacy evaluation statistical parameters for a developed model to predict the extraction of astaxanthin yield from shrimp shells.

Statistical parameter	Value
Std.dev.	1.19
C.V. %	2.09
R^2	0.9870
Adjusted R^2	0.9702
Predicted R^2	0.8990
Adeq Precision	24.6656
PRESS	77.19
AAD (%)	1.07

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Std.de. = standard deviation, C.V. = coefficient of variance, PRESS = predicted error sum of square, and AAD = absolute average deviation.

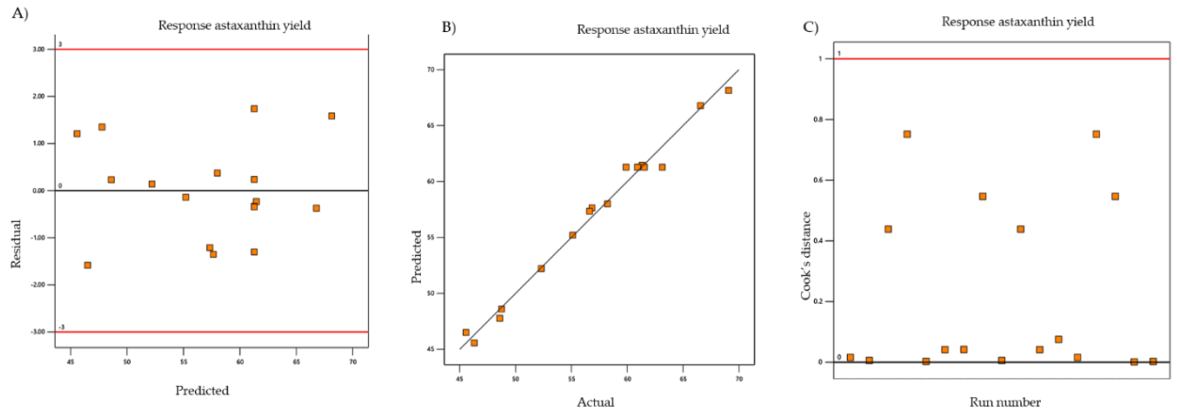

Figure 3. Diagnostic plots for the model adequacy developed for the extraction of astaxanthin yield from shrimp shells A) residual versus predicted values, B) predicted versus actual values, and C) Cook's distance versus run number.

Table 3 shows the application of univariate statistical strategies for screening and optimizing bioactive molecule extractions from seafood byproducts whereas Table 4 **shows** multivariate techniques of statistical optimization strategies applied for the extraction of bioactive molecules from seafood byproducts.

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Table 3. Classical (One-variable-at-a-time) methods of statistical optimization strategies applied for the extraction of bioactive molecules from seafood byproducts.

Seafood byproduct type	Design method of experiments (DoE)	Employed software	Extraction method	Targeted Bioactive molecule	Considered Extraction parameter/s	Reference
Shrimp chitinaceous waste	Central composite design	Design Expert 8.0.7.1	Enzymatic Digestion	Chitinase and chitin oligosaccharides	Incubation time, different media, pH, temperature, carbon source, nitrogen source and metal ions	[1]
Red Shrimp (Aristeus alcocki) shell waste	Analysis of variance technique	SPSS 15	Non-deproteinization of enzymatic digestion	Carotenoids	Different organic solvents Three different vegetable oils	[68]
Fish scales and feather wastes	Analysis of variance technique		*Bacillus sp*. CL18 as a bioconverter	Protease, Bioactive hydrolysates	Twelve substrates and co-substrates	[69]
Sea bass skinhead, tail, thorns, and backbone)	Analysis of variance technique	InfoStatfi and StatAdvisorfi version 2018	Bacterial fermentation	Phenolic acids	Fermentation time (in hours)	[70]
Comb penshell (Atrina pectinata)	One-way analysis of variance	SPSS version 23	Subcritical Water Hydrolysis	Amino acids and marine bioactive peptides	Extraction temperatures	[71]
Crustacean shell waste	One-way analysis of variance	Sigma Plot 14.0	Submerged fermentation	Chitinase, protease	fermentation time, pH, and temperature	[72]
Speckled shrimp *Metapenaeus monoceros* shells	One-way analysis of variance	SPSS Version 11.0.1.2001	Flask based hydrolysis	Protease	Concentrations of shrimp, sugar	[35]
Speckled shrimp *Metapenaeus monoceros* shells	One-way analysis of variance	SPSS ver.17.0	Deproteinization of enzymatic digestion	Deproteinized bioactive hydrolysate	enzyme/substrate ratios	[73]
shrimp (P. kerathurus) shells and blue crabs (P. segnis) Viscera	One-way analysis of variance	SPSS ver.17.0	Deproteinization of enzymatic digestion	Chitin	pH and temperature	[74]
Shrimp (*Parapenaeus longirostris*) heads, thorax, appendix cephalothorax and abdominal parts	One-way analysis of variance	SPSS version 20.0	Supercritical CO_2 Extraction	Astaxanthin and Peptides Carotenoid astaxanthin	Extraction rate	[75, 76]
Shrimp (*Penaeus merguiensis*) shells	One-way analysis of variance	SPSS version 19.0	Fermentation	Chitin, chitosan	Differences in bacterial strains	[77]
Shrimp shells powders	One-way analysis of variance	SPSS version 19.0	Submerged fermentation	Chitin	Time, Dilution, 2% diethyl sulfate, UV-irradiation, microwave heating treatments	[78]
Head, skins and viscera of Rainbow Trout (*Oncorhynchus mykiss*) and Sole (*Dover sole*)	One-way analysis of variance	SPSS	Accelerated solvent extraction and pulsed electric fields	Protein content	Temperature, time, pH and pressure	[79]
Blue crab (*Portunus segnis*) shells	One-way analysis of variance	SPSS ver. 17.0	Enzymatic pretreatment combined with solvent maceration	Carotenoproteins	Time intervals and concentration *Portunus segnis* proteases	[80]

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Table 4. Multivariate techniques of statistical optimization strategies applied for the extraction of bioactive molecules from seafood byproducts.

Seafood byproduct type	Statistical Methodology	Design method of experiments (DoE)	Employed software	Extraction method	Targeted Bioactive molecule	Considered Extraction parameters	Reference
Cod fish liver	RSM			Conventional hexane and supercritical carbon dioxide	Cod liver oil	temperature, pressure, and CO_2 flow rate	[81]
Shrimp shell waste	Particle swarm optimization algorithm and artificial neural network	Central composite design	MATLAB R2016a	Fermentation	Chitinase	Colloidal chitin, glucose, Tween 80 (common surfactant micelles), yeast extract	[10]
Shrimp (*Penaeus sp.*) cephalothoraxes and carapaces	RSM	Fractional factorial design (FFD) CCD	Statsoft 1997	Thermochemical treatments	Chitin	Concentration of HCl solution, solid liquid ratio of HCl solution, number of treatments, Concentration of NaOH solution, reaction time, reaction temperature, solid liquid ratio of NaOH solution	[30]
Shrimp *Litopenaeus vannamei* waste	RSM Genetic algorithm and Particle Swarm	Central composite design	Design-Expert software (version 10.0.1.0	Aqueous two-phase system	Protein recovery	Polyethylene glycol concentration, trisodium citrate concentration, pH and temperature	[44]
Speckled shrimp *Metapenaeus monoceros* shells	RSM	Taguchi's L27, Box–Behnken Design	SPSS Version 11.0.1.2001	Flask based hydrolysis	Chitin	Temperature, Inoculum size of strain, Culture volume	[35]
Shrimp heads	RSM	3-level fractional factorial	Statistica software Version 10	Ultrasound and Microwave Assisted Extraction	Phenolic and Carotenoids	Extraction time, solvent-to-propolis and Choline Chloride: Tartaric Acid-to-H_2O ratio	[62]
Atlantic salmon frame bone	RSM	Box-Behnken Design (BBD)	Design-Expert v. 7 Trail	Supercritical carbon dioxide (SC-CO_2)	Oil	Urea/ fatty acids ratio, crystallization temperature and crystallization time	[61]
Small-Spotted Catshark (*S. canicula*) skin	RSM	CCRD	Microsoft Excel spreadsheet	Alkaline pre-treatment, Acid-soluble collagen extraction	Collagen	Chemical treatment (NaOH) concentration, temperature and time, concentration of acetic acid	[49]
Scallops (*Argopecten purpuratus*) byproducts	RSM	Box–Behnken Design	Minitab 19	Enzymatic Hydrolysis	Protein Hydrolysate	Temperature, time, and enzyme concentration (enzyme/substrate level)	[60]
Shrimp (*Penaeus monodon*) shells	RSM	Box-Behnken design	Design-Expert software (version 7.0.0)	Ultrasound-assisted natural deep eutectic solvents	Astaxanthin	Natural deep eutectic solvents molar ratio, Ultrasound-amplitude, Extraction Time	[59]
Indian white shrimp waste	RSM	BoxBehnken Design	Design Expert 7.1.6 and Minitab 16 statistical software	Chemical and Microwave method	Chitosan	Temperature, concentration of alkaline, time of reaction, power of microwave, Irradiation time	[58]
Marine shrimp processing raw byproducts		Plackett-Burman and BBD		Fermentation	Chitosanase	fermentation period, temperature, period of microwave pretreatment, K2HPO4 (%), MgSO4 (%), KCl (%), FeSO4·7H2O	[32]

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Seafood byproduct type	Statistical Methodology	Design method of experiments (DoE)	Employed software	Extraction method	Targeted Bioactive molecule	Considered Extraction parameters	Reference
Salmon (*Salmo salar*) backbones, heads, and viscera	RSM	Central composite rotatable design	Design-Expert Version 11	Soxhlet and microwave-assisted extraction	Bioactive oils	Time, microwave power, and solid/liquid ratio	[55]
Monkfish (*Lophius piscatorius*) heads and viscera	Non-linear least-squares (quasi-Newton) method	Data-fitting and parametric estimations	Solver of Excel spreadsheet	Proteolytic digestion	Protein hydrolysates	pH, temperature, and protease concentration	[41]
Shrimp (*Parapenaeus longirostris*) shells waste	RSM	Box-Behnken Design	STATISTICA	Fermentation	Chitin and chitosan	Sucrose concentration, Shrimp shells waste concentration, inoculum size, incubation period	[31]
Undersized hakes (fish by-catch)	RSM	Box–Behnken Design	Statgraphics Centurion XVI	Enzymatic Hydrolysis	Protein hydrolysates	Enzyme/substrate (protein) ratio, %solids, time	[38]
Black tiger shrimp (*Penaeus monodon*) shells	RSM	Box–Behnken Design	Sigmaplot-11 Excel	Enzymatic Hydrolysis	chitin	pH, temperature, agitation speed, enzyme substrate ratio, incubation time	[65]
Scyliorhinus canicula Discards	Non-linear least-squares (quasi-Newton) method	Rotatable second order design	SolverAid, Microsoft Excel spreadsheet	Enzymatic Hydrolysis	Protein Hydrolysates	Temperature and pH	[42]
Red shrimps, *A. antennatus* head	RSM	Box-Behnken Design	Statistica Version 10	ultrasound assisted, microwave assisted extraction	Carotenoids	extraction time, ultrasound, microwave power and solvent/material ratio	[48]
Atlantic salmon (*Salmo salar*) heads, frames, viscera	RSM	Factorial design	Minitab 17.1	Enzymatic Transesterification	Oil, biodiesel	Enzyme concentration, oil/alcohol molar ratio, time, and temperature	[56]
Fish by-product: heads, fins	RSM	CCRD	Design-Expert, Version 11	Microwave-Assisted Extraction	Bioactive Fish Oil	Time, microwave power, and solid/liquid ratio	[3]
Salmonids (rainbow trout and salmon) heads, trimmings, frames	Nonlinear least-squares (quasi-Newton) method	Second order rotatable design	Solver, Microsoft Excel spreadsheet	Enzymatic Hydrolysis	Protein hydrolysates	Enzyme concentration, pH, ratio (Solid:Liquid, time of hydrolysis, agitation speed	[43]

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3. Extraction Process Parameters Considered for Bioactive Molecules from Seafood Byproducts

3.1. Chitin and Chitosan

Extracting chitin and chitosan from discarded seafood parts requires particular attention to multiple factors to ensure maximum output and effectiveness. Determining the suitable extraction method—be it chemical, physical, or biological—is crucial, each method carrying its own set of advantages and drawbacks [82, 83].

Variables like the type of seafood wastes (like shrimp or crab shells), their size, and composition significantly impact the extraction process. Parameters like temperature, pH, and duration of reaction are pivotal in chemical and enzymatic extraction techniques, affecting both the rate of chitin breakdown and impurity elimination.

Moreover, careful selection of demineralization and deproteinization agents—whether solvents, acids, or alkalis—is imperative to achieve chitin of high purity. The selection of a demineralization agent significantly impacts the effectiveness of mineral removal, whereas the deproteinization agent plays a crucial role in eliminating proteins without compromising chitin integrity. Moreover, variables such as the ratio of waste material to extraction solvent, agitation speed during processing, and the incorporation of co-solvents can also influence both the efficiency and quality of chitin and chitosan extracted from seafood byproducts.

Kumari et al. detailed the process of chitin and chitosan extraction from Fish Scales, *Labeo rohita*. For demineralization, they employed a 1% HCl solution for 36 h, followed by deproteinization using 0.5 N NaOH solution for 18 h. The chitin underwent treatment with 50% NaOH solution for 2 h at 80°C [84].

Srinivasan et al. outlined the process of chitin and chitosan extraction from shrimp shells, *Penaeus monodon*. In the demineralization step, they utilized a 1.0 M HCl solution for 75 min at room temperature, while in the deproteinization step, they employed a 3.0 M NaOH solution for the same duration and temperature. The deacetylation of chitin was conducted using a 50% sodium hydroxide solution at a ratio of 1:50 at 90°C for 50 min [85].

On the other hand, there is a growing emphasis on sustainable extraction methods, prompting innovative approaches such as enzymatic hydrolysis and microbial fermentation.

Arancibia et al. [86] detailed the extraction process of chitin and chitosan from shrimp *Litopenaeus vannamei* wastes. Demineralization of the material was conducted using lactic acid for 36 h at 21°C. For protein removal, enzymatic hydrolysis with Viscozyme and Alcalase was employed. The remaining solid material underwent deacetylation treatment with a 40% NaOH solution for 4 h at 110°C.

Regarding utilizing fermentation for deproteinization, microbes can naturally occur within the chitosan source (autofermentation) or be introduced into the source for deproteinization and/or demineralization. In these fermentation stages, deproteinization is achieved through proteolytic enzymes, while demineralization is facilitated by the organic acids generated by the microorganisms. For instance, Zhang et al. [87] conducted a study on successive co-fermentation with *Bacillus subtilis* and *Acetobacter pasteurianus* for chitin extraction from shrimp shells, achieving depolymerization efficiency (DP) of 94.5% and demineralization efficiency (DM) of 92.0%. Bahasan et al. [88] used *Kurthia gibsonii* as the demineralization microbe and *Aspergillus* spp. as the deproteination microbe. They inoculated these microbes into two shrimp species, *Fenneropenaeus semisulcatus* and *Fenneropenaeus indicus,* for chitin extraction. Additionally, Liu et al. [89] utilized successive fermentation with *Lactobacillus rhamnoides* and *Bacillus amyloliquefaciens* (BA01) strain for chitin extraction.

Arbia et al. conducted a series of investigations on the demineralization and deproteination of shrimp shell *Parapenaeus longirostris* employing *Lactobacillus helveticus* [90, 91].

Sedaghat et al. [77] elucidated the extraction process of chitosan from shrimp *Penaeus merguiensis* wastes utilizing a biological approach. Lactic acid fermentation by *Pseudomonas aeruginosa* bacterium was employed for fermentation, subsequently facilitating demineralization and deproteinization of

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shrimp shells over 4 and 6 days, respectively. Chemical deacetylation was accomplished by treating the extracted chitin with a 50% NaOH solution [77].

In Aranday-García et al. [92] study Chitins produced from the fermentation of shrimp waste by *Lactobacillus brevis*, both with and without additional *Rhizopus oligosporus* inoculations, had a greater molecular weight than commercial biopolymers.

Therefore, microbial fermentation offers a cost-effective solution by incorporating specific microbial strains and indigenous microorganisms, thereby reducing the need for expensive enzymes.

3.2. Proteins and Peptides

A blend of fragmented proteins derived from the hydrolysis of fish proteins or proteins found in fish by-products, resulting in peptides and amino acids is defined as fish protein hydrolysates. Protein hydrolysis has garnered significant interest recently due to its capacity to enhance protein retrieval and the growing exploration of potential industrial uses for the recovered hydrolysates [93-95]. These hydrolysates can be generated through chemical means (using acids or alkalis), enzymatic processes, or bacterial fermentation.

Research into marine byproducts has revealed significant concentrations of functional peptides and amino acids. Enzymatic protein hydrolysis (EPH) of marine by-products has garnered considerable attention as a promising method. In this approach, either naturally occurring or commercial enzymes (such as alcalase, trypsin, pepsin, papain, pancreatin, and thermolysin) are utilized to break peptide bonds between amino acids [4].

Enzymatic hydrolysis via endogenous enzymes (autolysis) present in the fish's digestive system typically requires extended periods to generate substantial amounts of cleaved peptides and can prove challenging to standardize and regulate due to various factors such as age, season, sex, fish species, environment, and diet. Autolysis has traditionally been employed in the preparation of fish sauce and silage [93, 94].

Siddik et al. [93] highlighted the challenges in standardizing and controlling the autolysis process, as enzyme production depends on various factors like age, season, species, diet, and environment. Conversely, the utilization of commercial enzymes in enzymatic protein hydrolysis offers numerous advantages over autolysis or chemical hydrolysis. This might lead to improved functionalities and bioactivities whereas autolysis might cause the accumulation of undesirable metabolites, nitrogenous compounds, and loss of freshness, particularly under conditions of inadequate handling and storage. Minimization and mitigation of environmental pollution might arise from endogenous and exogenous enzymes in the fish processing industry. Production of various fish products with industrial applications might be derived from the valorization of fish waste and discards [96].

Additionally, the concentrations of enzymes, as well as the pH and temperature, are dependent on the specific type of enzyme employed. Reported enzyme concentrations typically range from 0.01% to 5.00% (w/w), while the pH can vary within a range of 1.5 to 11, depending on the enzymatic activity and substrate requirements [97].

Dinakarkumar et al. [98] conducted an extraction of Fish Protein Hydrolysate from *Secutor insidiator* using papain and proteinase K enzymes. The degree of hydrolysis was found to be 0.8 and 0.9 for proteinase and papain respectively.

While the Chemical hydrolysis involves the utilization of chemical agents (such as acids or alkalis) under extreme conditions (including high temperature and/or pressure) to break the bonds between amino groups in the protein sequence. Acid hydrolysis is more prevalent in the marine industry compared to alkaline hydrolysis [94]. Chemically hydrolyzed proteins offer several advantages, including simplicity and cost-effectiveness. However, controlling the process proves challenging, resulting in protein hydrolysates of inferior nutritional and functional qualities. This can be attributed to the harsh, nonspecific cleavage of peptide bonds and the partial or complete degradation of valuable amino acids like cysteine, serine, and threonine. Alkaline hydrolysis may further lead to the formation of potentially toxic substances such as lysinoalanine, ornithinoalanine, and lanthionine [95].

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By systematically optimizing these extraction parameters, the full potential of seafood byproducts as valuable sources of proteins, enzymes, and peptides can be unlocked for diverse applications in food, pharmaceuticals, nutraceuticals, and biotechnology, contributing to the shift toward a more circular and sustainable economy [4].

3.3. Enzymes

Secondary raw materials derived from seafood processing encompass enzymes sourced from various parts such as the gut, liver, head, shell, and visceral organs, serving as valuable processing aids in the food industry to enhance functional and nutritive qualities [99].

Saranya et al. [100] isolated an alkaline protease from fish processing waste using a combination of methods including ammonium sulfate fractionation, ion-exchange chromatography on Sephadex G-25, and DEAE column chromatography. These purification steps resulted in a 4.0-fold increase in the purity of the protease, with a yield of 7.7%. SDS-PAGE analysis determined the molecular weight of the purified protease and estimated it to be equal to 33 kDa. The optimal temperature for enzyme activity was found to be 30°C at pH 8.

Murthy et al. [101], sourced visceral proteases from little tuna (*Euthynnus affinis*), catla (*Catla catla*), and tilapia (*Oreochromis mossambicus*) originating from different habitats and isolated and characterized them using acetone, ethanol, and ammonium sulfate fractionation precipitation methods. Proteases obtained from little tuna and tilapia displayed enhanced specific activity when precipitated at 40% saturation during ammonium sulfate fractionation, with specific activities of 18.19 and 13.67 U/mg, respectively. Conversely, catla-derived enzymes exhibited the highest specific activity of 8.32 U/mg when precipitated at 60% saturation during ammonium sulfate fractionation. Acetone precipitation demonstrated superior recovery for all crude enzymes analyzed in this study.

The chitinase-derived *Achromobacter xylosoxidans*, which was isolated from shrimp waste, exhibited full activity at an optimal temperature of 45°C, withstanding temperatures up to 55°C, and a pH of 8, demonstrating 80% stability [102].

A digestive chitosanase sourced from blue crab (*Portunus segnis*) viscera was isolated, characterized, and applied. The crude chitosanase displayed peak activity at pH 4.0 and a temperature of 60°C. Moreover, it retained over 80% of its activity across a pH range spanning from 3.0 to 10.0 [103].

The maximum chitosanase production occurred when utilizing a medium containing 2% (w/v) squid pens waste powder as the sole carbon and nitrogen (C/N) source, resulting in a yield of 0.60 U/mL. The chitosanase exhibited its highest activity at a temperature of 60°C and pH 7. Furthermore, it demonstrated enhanced activity towards chitosan solutions with higher degrees of deacetylation (DDA) values. Additionally, the hydrolysis products obtained from 98% DDA chitosan, catalyzed by TKU047 chitosanase, revealed a degree of polymerization (DP) ranging from 2 to 9, indicating an endo-type activity for the chitosanase[104].

3.4. Carotenoids: Astaxanthins

The extraction of astaxanthin from pink shrimp waste (*Farfantepenaeus subtilis*) was carried out using palm olein at three different temperatures (50, 60, and 70 °C) [105]. Under these conditions, the maximum extraction of astaxanthin reached 29.814 μg/g of dried waste. The extraction kinetics were modeled using a simplified mass transfer kinetic model, demonstrating a strong agreement ($0.9685 < r^2 < 0.9912$) between the experimental and calculated data.

Liu et al. [106] carried out solvent extraction method using dichloromethane: methanol (1:3, v/v), of Shrimps and prawns (from Head, shell, and tail) and presnted an astaxanthin content varied from 19.2 to 7.1 μg/g.

Hu et al. [107] mentioned the optimal experimental conditions, including a solid-liquid ratio of 1:7, an extraction time of 20 minutes, and a temperature of 50 °C, resulting in the highest extraction yield of astaxanthin. Thus, the analysis revealed that the astaxanthin content in the *Procambarus clarkia* shell was measured at 239.96 μg/g.

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Li et al. [108] reported on high-pressure extraction of astaxanthin from shrimp byproducts. Solvents' (such as ethanol, acetone, and dichloromethane) solvation properties and pressure levels (ranging from 0 to 600 MPa) were found to significantly influence astaxanthin extraction. High pressure was observed to disrupt cellular membranes and alter fiber structure, facilitating solvent diffusion and improving astaxanthin extraction. However, pressures exceeding 300 MPa had a detrimental effect on astaxanthin recovery.

Ultrasound application (using parameters like 23.6% amplitude, 26.3°C for 13.9 min) was found to enhance astaxanthin extraction from shrimp shells [11]. Fragmentation of the shell matrix was the result by cavitation induced by ultrasound, leading to increased solubility of bioactive compounds and their extraction by solvents. Solvent polarity and extraction time were identified as significant factors affecting astaxanthin yield.

An effective technique for astaxanthin extraction from crustacean byproducts was supercritical fluid extraction with the use of different solvents. Optimized conditions (including 56.88°C temperature, 215.68 bar pressure, and a flow rate of 1.89 mL/min) yielded both free (12.20 μg/g) and conjugated (58.50 μg/g) astaxanthin [109]. Temperature and pressure affected the solubility of the solute in the supercritical fluid, while extraction efficiency was greatly affected by solvent selection. Higher concentrations of ethanol (5%, 10%, and 15%) led to a significant increase in astaxanthin yield (from 26.0 to 34.8 μg/g) [110]. However, astaxanthin extraction could be hindered by application of high pressures (>400 bar) in supercritical fluid extraction.

Recently, microbial fermentation followed by supercritical extraction from shrimp waste liquid fraction was optimized [111]. Fermentation of the raw material by lactic acid bacteria was found to enhance astaxanthin extraction compared to common supercritical extraction methods. The extraction of lipophilic compounds in the liquor and enzymolysis of shrimp shells were increased by this fermentation, resulting in a 3.7-fold higher astaxanthin concentration (134.20 μg/g) [112].

Gulzar and Benjakul [113] investigated the combined effects of ultrasound- and pulsed electric field-assisted treatment on astaxanthin extraction from shrimp byproducts. They observed that disintegration, particularly in the cephalothorax, increased with higher electric field strengths. Additionally, ultrasound-induced electroporation enhances mass transfer and consequently improves astaxanthin recovery. Figure 4 summarizes the optimizing extraction parameters of some major seafood by products.

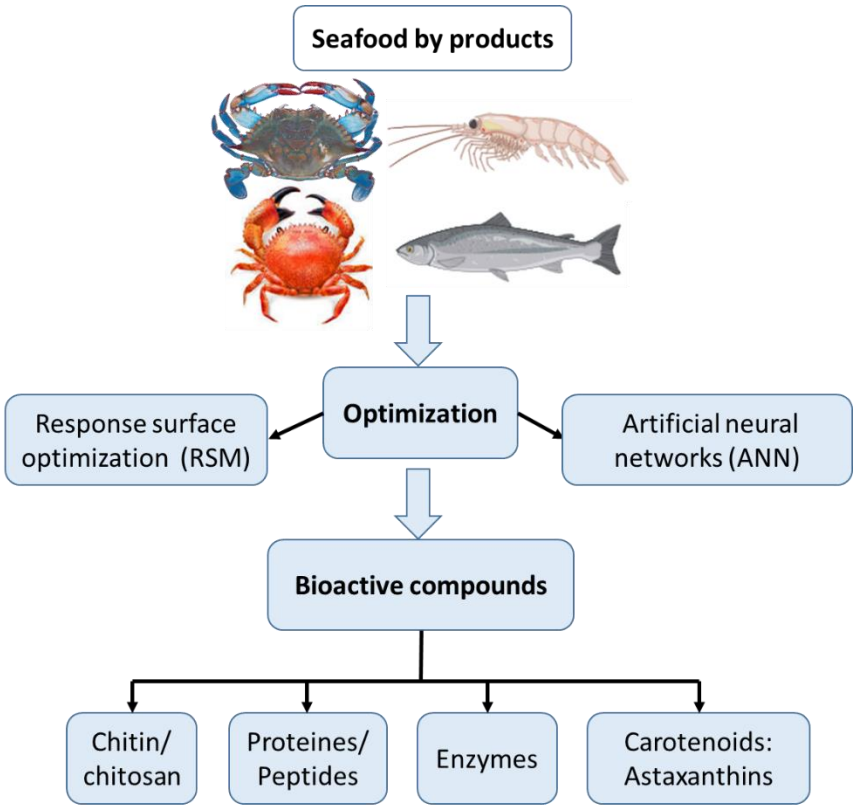

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Figure 4. Optimisation of extraction parameters of seafood by-products.

4. Economic and Quality Considered Statistical Optimization Methods

For economic and quality product development, all the innovative methods of bioactive extraction employing biological, physical, mechanical, microbial and enzymatic processes require optimum conditions such as the concentration of solvent (solvent to substrate ratio), temperature, time, power of microwave or ultrasound, etc. In this subheading, studies focused on efficiency, quality and processing cost optimization strategies for the extraction of bioactive substances from seafood byproducts were considered. These optimization studies were conducted to choose the best extraction technologies, check the efficiency of processing technology, select the best green extraction solvent and/or situate the extraction processing conditions that minimize cost and maximize quality and extraction yield. Bioactive compound extraction methods are dependent on the types of the sample matrix, solvent used, and extraction method directly or indirectly alter the biomass properties, physical-chemical properties of the intended molecules and their perspective end use [114]. Hence, optimizing the extraction condition that predicts and confirms the interactive effect of the dominating factors is crucial.

4.1. Optimization Strategies Considered Processing Costs, Quality and Efficiency

Optimization strategies employing RSM coupled with central composite design, Boxe-Behnken-design and factorial designs focused on quality, cost and efficiency were mostly utilized for situating extraction conditions of bioactive compounds from seafood byproducts (Table 4). In particular, RSM coupling Boxe-Behnken-design was chosen for optimizing the improvement of extraction conditions (temperature, concentration of alkaline, time of reaction, power of microwave, Irradiation time) of chitin production from Persian Gulf shrimp waste. This optimization strategy helped to differentiate the microwave-assisted extraction method from the chemical (alkaline) technique for chitosan preparation. This method was selected due to its efficiency, less processing cost and time [58]. Extraction of carotenoid astaxanthin from shrimp (*Parapenaeus longirostris*) heads, thorax and appendix using supercritical fluid extraction (CO_2 based) was proposed as a beset method which created quality extract (attractive antioxidant activity, pro-apoptotic and anti-cancerous effects) and avoid organic solvents for extraction [76]. Similarly, an optimized extraction of fish lipids using microwave-assisted extraction was studied by Costa and Bragagnolo [115]. This optimized extraction method was fast and efficient and able to produce the fish lipids with acceptable fatty acid composition and no lipid oxidation. Employing high-energy extraction methods such as ultrasound-assisted extraction and microwave-assisted extraction is effective in recovering high-added value group bioactive compounds from the natural sample matrix. Optimized process conditions of these methods are faster, low processing cost, reproducible and repeatable. Optimization of these methods for the extraction of carotenoids from Red shrimps, *A. antennatus* head was suggested as economical and efficient [48].

Green solvent extraction methods are more cost-effective which improves quality and enhances the recovery of oil. Moreover, ionic liquids and deep eutectic solvents have attractive biocompatibility with particular selectivity on individual bioactive compound during extraction. This property demands optimization interactively with other physical parameters. A wet rendering oil recovery from catfish heads was optimized using a two-factor Taguchi orthogonal array design considering extraction temperature and time for a better oil recovery rate. This optimization strategy was proposed as both enhancing the oil extraction process and improving the cost-effective fish byproduct management [36].

Most of the reactor scales for the production of bioactive compounds using enzymatic hydrolysis and fermentation methods are performed at lower volumes, thereby the optimization of the process makes it economical and easy way. These optimized processes are validated at the enlarged portion. For example, Vázquez, *et al.* [43] studied the optimization of the protein hydrolysates production from salmonids (rainbow trout and salmon) heads, trimmings, and frames at a 100-mL-reactor, then they validated the process at a 5L-reactor scale. Consuming enzyme concentration during extraction

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is one economic case which needs optimized utilization. For instance, during the production of salmon oil from Atlantic salmon by-products increasing the 50% the enzyme concentration could facilitate the rate of oil recovery only by 5% which is not economically feasible. Hence, optimizing the enzyme concentration is critical [56]. Similarly, Iñarra, *et al.* [38] optimized protein hydrolysates extraction conditions (enzyme/substrate (protein) ratio, %solids, time) from undersized hakes (fish by-catch) using RSM coupling BBD that focused on developing a scaled-up model. They reported the most favourable conditions to confirm the laboratory scale at a 0.5 L and proposed a scaled-up model of 150 L concerning the protein extraction yield. One-variable-at-a-time optimization was employed to select the best bacterial isolates from seventy bacterial varieties which produce proteolytic enzymes. Then, the optimal chitin extraction conditions (best bacterial isolate, carbon source, shrimp waste concentration, inoculum size and fermentation time) were conducted using BBD-coupled RSM optimization. This optimization method increased extraction efficiency by 1.3-fold [31].

4.2. Best Optimization Strategies that Favour the Production of Potential Bioactive Molecules

Extraction variables intended to be studied and when extraction designs/instruments are to be investigated for the first time, the preliminary work is optimizing the process condition considering different parameters before employing it for production. This optimization stage saves the processing cost and time, as well as helps to predict quality production when applied at a larger scale.

Statistical experimental designs are very critical to establishing optimized extraction processes, hydrolysis and fermentation media conditions for the desired bioactive compound production from seafood byproducts. Multivariate statistical optimization methods such as RSM, artificial neural network and non-linear least squares (quasi-Newton) coupled with different experimental designs are applicable for evaluating multiple variables efficiently which have been applied to seafood byproduct valorization. Applications of these methods in extracting bioactive compounds in different seafood byproducts are summarized in Table 4.

4.3. Statistical Optimizations on Emerging Green Extraction Technologies

The chemical treatment-based extraction (using non-polar solvents) of bioactive compounds is less acceptable due to their side effects like toxicity, environmental problems, as well as consumption of high energy. Modern extraction methods which involve membrane breaking or cell disruption technologies such as ultrasound- and microwave-assisted extraction, freezing/thawing, pulsed electric field, sub- and supercritical fluid extraction, and high-pressure homogenization are more applicable to extract bioactive compounds from different sample matrix [116]. Other green extraction technologies such as probiotic-based fermentation, enzymatic hydrolysis, and proteolytic digestion have been recently acceptable for the extraction of bioactive compounds from seafood byproducts which can solve the above-mentioned effects of the organic solvent-based treatments [31, 38, 41].

4.3.1. Green Solvent Extraction Parameters Optimization

Green solvents are considered as solvents which avoid said effects on the final product as well as prevent wastage. These are classified into five core groups: 1) solvents with aqueous systems, 2) ionic liquids, 3) deep eutectic solvents, 4) bio-based solvents, and 5) switchable solvent systems [117]. Applying greener solvents for the extraction of bioactive substances is acceptable since they are low energy cost of synthesis, biodegradable, non-toxic, and recyclable. These are grouped into neoteric solvents (Ionic liquids, Deep Eutectic Solvent), supercritical fluids (supercritical water, supercritical carbon dioxide), bio-based solvents (Terpenes, glycerol, ethanol, ethyl lactate, D-limonene, etc.), and supramolecular solvents [118]. Commercial green solvents such as deep eutectic solvents, ethanol, synthetic ionic liquids (salt mixtures in the liquid), and carbon dioxide are considered recyclable, non-toxic, and safe for food and drug-based bioactive compounds extraction [119]. Choline chloridemalonic acid a type of deep eutectic solvent is effective green solvent utilized for chitin extraction from shrimp shells (*Marsupenaeus japonicas*) [120]. The application protocols of using these solvents and their interaction with other extraction parameters like time, sample matrix, and

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temperature should be optimized for quality and better extraction yield. Selecting and optimizing green solvents which are suitable for the ultrasonication process are also very important. In an astaxanthin extraction from shrimp (green tiger, *Penaeus semisulcatus*) shell, suitable solvents for the ultrasonic method were initially screened and the best solvent mixtures (higher polarity) were used for optimizing extraction conditions (ratio of solvents, extraction temperature, extraction time, and ultrasound amplitude) of astaxanthin [11].

Enzymatic processing and bacterial fermentations have been used for the production of bioactive metabolites (gelatinous solutions, oils, protein hydrolysates) from skins and heads from megrim, hake, boarfish, grenadier, and Atlantic horse mackerel[121]. El-Bialy and Abd El-Khalek [122] studied the extraction of astaxanthin from shrimp wastes by applying two green technologies namely lactic fermentation and edible oil extraction. In their investigation, they found that the solid-state fermentation by *Lactobacillus acidophilus* and submerged fermentation by *Streptococcus thermophilus* were the most efficient extraction yield of astaxanthin than the vegetable oil (corn, flaxseed, and sesame oils) based solvent extraction. However, the vegetable oil-based solvent extracted astaxanthin has shown improved medical properties such as extending shelf life and preventing microbial contamination. In developing the extraction model parameters such as carbon sources, type of green solvent, and fermentation time were considered. Optimizing the activity of enzymes for better extraction of bioactive compounds such as chitosanase from shrimp processing byproducts is another method to qualify the quality of the product and process [32]. Optimizing consecutive extraction processes for efficient and quality bioactive production is another strategy. For instance, Vázquez, *et al.* [41] studied a two-step proteolytic digestion for the extraction of protein hydrolysates. In the first step, they optimized the hydrolysis considering the ratio of monkfish heads to water, temperature, protease concentration, and pH as basic independent variables. Then, they validated these optimum parameters for the hydrolysis of proteins from the head and viscera of monkfish. Creating optimum enzymatic hydrolysis conditions (temperature and pH) to produce protein hydrolysates from *Scyliorhinus canicula* discards employing the non-linear least-squares (quasi-Newton) method was studied by Vázquez, *et al.* [42]. Fish skins were studied as an excellent and easily available resource for a biomolecule such as collagen extraction. This extraction processes was optimized in a two-step process by Blanco, *et al.* [49]. First, they optimized the extractability of collagen (extraction yield) from Small-Spotted Catshark (*S. canicula*) skin considering NaOH concentration, time and temperature. Then, the optimum conditions were used to design better yield and aminioacid quality optimization using acetic acid concentration, temperature and time as independent factors. Moreover, Box–Behnken coupled RSM optimization was employed for the deproteinization process of chitin extraction conditions (pH, time, temperature, agitation speed, and enzyme-to-substrate ratio) [65]. The production of protein hydrolysate from scallops (*Argopecten purpuratus*) visceral meal and defatted meal with enhanced proximal composition, amino acid composition, yield, molecular profile, protein solubility, and degree of hydrolysis were optimized using RSM coupled with BBD. Three basic independent variables (temperature, time, and enzyme concentration (enzyme/substrate level)) were optimized [60].

4.3.2. Optimizing Physical Processing (Cell Wall Breakdown) Extraction Parameters

The applications of ultrasound-assisted extraction of bioactive compounds from seafood sample matrices is due to the factors of high temperatures and pressures creates pressurized area on the bubbled solvent which then fiercely discharge the liquid part from the sample cells. Other factors such as ultrasonic frequency, intensity and processing time also interactively affect the extraction capacity [7]. Hence, statistical optimization that optimize the suitable extraction condition on better efficiency, quality, lower processing cost and time. Protein extraction optimization requires consideration of extraction parameters and technology that capacitate the cell wall breakdown. Unless suitable and optimized extraction method is developed, fish protein is highly sensitive which can be degraded by uncontrolled extraction factors like oxidation and denaturation by excessive heat.

RSM coupled BBD was employed to differentiate the efficiency of ultrasound assisted extraction and microwave-assisted extraction of carotenoids from Red shrimps (*A. antennatus)* head. In this

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study, extraction time, ultrasound, microwave power and solvent/material ratio were considered as independent variables. This ultrasound assisted extraction was efficient, had lower processing time, and a lower solvent/material ratio than the microwave-assisted extraction [48]. Microwave-assisted extraction of chitosan from Persian Gulf shrimp (species of *P. indicus*) at an optimized extraction parameters of temperature, NaOH concentration, power of irradiation and time of reaction was more effective than the chemical (alkaline) method [58]. Bioactive fish oil extraction was optimized as a sustainable valorization of fish byproduct (heads, fins) using microwave-assisted extraction. The independent variables of the extraction process considered to be optimized for obtaining high-quality and yield oil were time, microwave power, and solid/liquid ratio. The optimum microwave-assisted extraction recovered from 60% to 100% of oil at about 19 min and with less solvent utilization compared to Soxhlet extraction [3]. A typical optimization of bioactive compounds extraction from fish and shrimp byproducts using green extraction technologies is depicted in Figure 5.

Mano-thermo-sonication is a type of ultrasonic extraction which works by combining pressure, temperature and ultrasound intensity to facilitate the extraction of water-soluble bioactive compounds from a sample matrix. This is because, the method not only facilitates cell disruption but also enhances mass transfer phenomena or effective diffusivity for better extraction yield [123]. Thus, assuring the optimum interactive effect of these extraction parameters is very important.

A study was conducted to compare conventional hexane, pressing extraction methods and supercritical carbon dioxide extraction methods of cod liver oil from cod fish visceral parts. The supercritical carbon dioxide extraction method was optimized considering temperature, pressure, and CO_2 flow rate. This RSM-optimized SC-CO_2 extraction method was chosen as the most efficient, and high quality liver oil (best antioxidant and anticancer activities, highest squalene, vitamin D_3, and vitamin K content) than the other methods [81]. The combined effects of subcritical dimethyl ether extraction parameters of oil from high-moisture tuna liver was optimized using the ratio of temperature to pressure, time, and stirring speed employing RSM. At optimum extraction conditions of this method the oil extraction yield was comparable to supercritical carbon dioxide extraction of tuna liver oil [124].

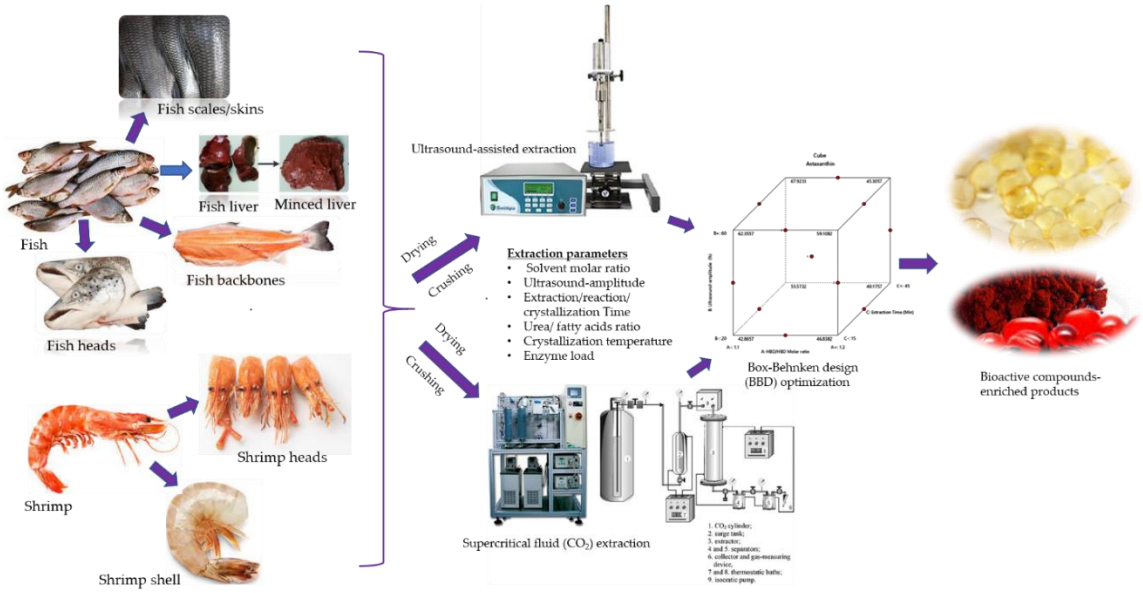

Figure 5. Typical optimization of bioactive compounds extraction from seafood byproducts using green extraction technologies [57, 125].

6. Conclusions

Selecting best statistical optimization strategies to optimize the extraction conditions of bioactive compounds from seafood byproducts using conventional and green technologies is an inevitable research activity. In this review, RSM coupling CCD and BBD have shown the most employed optimizing strategies of bioactive compound extraction parameters. The dominant extraction

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parameters considered for optimizations were enzyme/substrate ratio, pH, time, temperature and power of extraction instruments. Effects of these independent variables on extraction capacities and qualities for the bioactive compounds, chitin and chitosan, proteins and peptides, enzymes and carotenoids (Astaxanthins) were optimized using the above optimization methods. Most of the studies have shown limitations in indicating if confirmation experiment at those developed optimum points was conducted for validation of their developed optimization model.

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