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Identification and Prediction of Bioactive Peptides from Anchovy (*Engraulidae*) Based on Processing Methods: Sub-Fragment Analysis via LC-HRMS Proteomics

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Abstract

This exploratory study aimed to identify and compare peptide profiles and predicted bioactivities of anchovies (Engraulidae) in 3 forms - Raw, processed, and fortified processed - using LC HRMS based proteomics combined with BIOPEP UWM and PeptideRanker analyses. Peptides generated by tryptic digestion were analyzed by LC HRMS, matched to an Engraulidae protein database, and evaluated *in silico* to annotate potential biological activities and estimate intrinsic bioactivity scores. In this non replicated dataset, raw anchovies showed the highest proteomic complexity (254 protein IDs and 1,304 peptide sequences), whereas processed and fortified samples exhibited fewer proteins and peptides, particularly with chain lengths ≥ 4 amino acids. Across all samples, the most frequent predicted activities were Dipeptidyl Peptidase IV (DPP IV) (27% - 31%) and Angiotensin Converting Enzyme (ACE) inhibition (± 24%), supported by antioxidant, neprilysin inhibitory (3% - 6%), and other enzyme inhibitory annotations. Short peptides (2 - 3 amino acids) dominated the predicted bioactive profiles, especially in processed and fortified products, indicating a shift toward shorter chains. Although total peptide numbers decreased after processing, the proportion of peptides with multiple annotated activities and high PeptideRanker scores (≥ 0.8) was descriptively higher in fortified samples (14.58%). These findings provide a preliminary molecular map suggesting that anchovy proteins can act as precursors of peptides with putative multifunctional bioactivities and may represent candidates for anchovy based functional foods or nutraceuticals. However, all functional inferences are based solely on sequence level, in silico predictions from single run LC HRMS data; the patterns observed are hypothesis generating and require confirmation through replicated experiments and targeted *in vitro* and *in vivo* validation.

Keywords: Anchovies, Bioactive peptides, LC-HRMS, Fortification, BIOPEP, PeptideRanker

Introduction

Functional foods are defined as foods that are consumed as part of the daily diet and provide health benefits beyond their basic nutritional functions [1]. These effects are primarily mediated by bioactive components that work through specific physiological mechanisms, including the prevention and management of various chronic conditions [2,3]. In the last 2 decades, scientific attention has intensively focused on bioactive peptides, which are short fragments of amino acids stored in parent protein structures and can be released through proteolytic processes [4].

Bioactive peptides generally consist of 2 - 20 amino acid residues, and their biological activity is strongly influenced by their sequence, chain length, and chemical structure [5]. Specific interactions between peptides and molecular targets - such as enzymes or cell receptors [6] - underlie their various biological activities. In the context of animal-based foods, marine fish, particularly anchovies, represent a rich and promising source of bioactive peptides, as numerous

studies have reported the antioxidant, anti-inflammatory, antihypertensive, antimicrobial, antidiabetic, and anticancer properties of hydrolyzed fish proteins (7 - 9). The release and stability of these peptides can occur during gastrointestinal digestion, *in vitro* hydrolysis, or as a consequence of food processing [10].

In the context of protein-based foods, particularly fish and fishery products, the identification of bioactive peptides is increasingly being carried out using a mass spectrometry-based proteomics approach [11]. Techniques such as liquid chromatography-tandem mass spectrometry (LC-MS/MS) [12] and liquid chromatography-high-resolution mass spectrometry (LC-HRMS) [13] enable the identification of amino acid sequences, molecular masses, and protein origins with high precision from complex food matrices [12]. LC-HRMS specifically offers the advantages of exact mass accuracy and high resolving power, making it highly relevant for peptide mapping in real food systems [13]. This approach has been applied to various marine organisms and fish, including mesopelagic fish (14), anchovies [15], herring [16], and octopus [17], generally in combination with bioinformatics analysis to predict the potential biological activity of the identified peptides.

However, most fish proteomics studies still focus on enzymatic hydrolysates [18,19] or specially conditioned fermentation products [20]. These approaches provide important insights, but do not fully represent the conditions of ready-to-eat fish products produced through household processing, such as frying. These processes are known to modulate peptide release patterns during digestion and proteomic analysis [21]. Therefore, direct peptide mapping from processed fish products is important to understand the real impact of processing on bioactive peptide profiles. This limitation is particularly relevant for ready-to-eat fish products commonly consumed by the public.

Anchovies (Engraulidae) are small pelagic fish that are abundant in Indonesian waters and are known to be rich in macro- and micronutrients [22,23], and contain complete and easily absorbed essential and non-essential amino acids [15, 24]. Several previous studies have reported the presence of bioactive peptides in fresh anchovies [15] and in traditional fermented products [24,25]. However, these studies were generally conducted separately and focused on one form of material, without systematically comparing the peptide profiles of raw anchovies, processed anchovies, and fortified anchovies in an integrated research design. Micronutrient fortification also has the potential to modify the matrix properties of the product and the interactions between its components. Fortification with lipophilic micronutrients such as cholecalciferol (vitamin D3) is generally carried out using a carrier or encapsulation system to increase stability against heat, oxygen, and light [26].

The selection of cholecalciferol as a fortifier also has a nutritional basis, given that anchovies are known as a high source of calcium [27], while vitamin D3 plays a role in supporting calcium absorption in the body [28,29]. Fortification is primarily seen as a factor that has the potential to modify the food matrix and protein environment. The presence of carriers and accompanying changes in processing conditions may affect the stability and profile of peptides formed or detected. To date, studies that comparatively assess the effects of processing and fortification on the bioactive peptide profile of fish at the molecular level are still very limited.

Food processing methods such as heating, drying, and frying can alter the structure of fish protein through denaturation, aggregation, and oxidation [30,31,32]. These structural changes can affect the exposure of protein cleavage sites [31,32], thereby altering the release pattern and stability of peptides [30]. Variations in the sequence and length of the resulting peptides then become the basis for predicting the potential for certain biological activities, such as enzyme inhibition activity, through amino acid sequence-based in silico analysis. However, the predicted biological activity does not necessarily reflect the bioavailability and physiological effects of peptides in the body, as the processes of digestion, absorption, and metabolism have not been evaluated in this study. Therefore, this study focuses on molecular-level peptide mapping as a preliminary step prior to further biological evaluation.

Based on this, there is still a clear knowledge gap regarding the mapping of bioactive peptides in Indonesian anchovies in various forms of processing - raw, processed, and fortified - using the LC-HRMS approach. In particular, there are no reports that systematically compare the number and types of

proteins and peptides, the distribution of peptide chain lengths, the variety of predicted bioactive activities, and *in silico* bioactivity scores within a consistent analytical framework.

Therefore, this study aims to identify and compare the bioactive peptide profiles in raw anchovies, processed anchovies, and fortified anchovies using an LC-HRMS proteomics approach integrated with bioinformatics analysis (BIOPEP and PeptideRanker). This study was designed as an in silico-based exploratory mapping to provide an initial molecular overview of the impact of processing and fortification on anchovy peptide profiles. The findings are expected to provide a rational basis for further research, including *in vitro* and *in vivo* validation and process optimization, before health implications and functional food applications can be empirically confirmed.

Materials and methods

Study design

To address the research gap regarding changes in the profile of bioactive peptides caused by anchovy processing and fortification, this study used a comparative and exploratory proteomic approach based on LC-HRMS (Liquid Chromatography-High Resolution Mass Spectrometry), which enables high-precision peptide identification. Three sample groups were analyzed: raw anchovies, processed anchovy products, and processed and fortified anchovy products. This approach enabled a systematic comparison of changes in peptide complexity between sample groups, as well as an evaluation of the predicted bioactivity of peptides in silico as a result of processing and fortification at the molecular level. The study design was non-inferential and did not include biological or technical replication, thus focusing on preliminary molecular mapping - including the number of identified proteins and peptides, peptide length distribution, and predicted bioactivity diversity and scores - without intending to test for statistical differences between groups.

Samples and treatment

Anchovies (family Engraulidae) were obtained from a local market in Palu City, Central Sulawesi (Mamboro Market), which originated from the coastal fishing area of Mamboro Beach. All samples were obtained at the same time of purchase to minimize raw material variation. Three groups of samples were analyzed, namely: (1) raw anchovies, (2) Product processed anchovies, and (3) Fortified processed anchovies.

Raw samples were stored at −18 °C, while processed samples were packaged in airtight containers and stored at room temperature away from direct sunlight for one day before being sent to the laboratory for LC-HRMS analysis. Processed products were prepared by mixing anchovies with flour and spices.

The proportions of flour and spices were not quantitatively determined but were adjusted until an even coating was obtained and the anchovies did not stick together. Next, drying was carried out using a food dehydrator at 40 °C for 1 h to reduce the surface moisture content. Frying was carried out in hot cooking oil until golden brown and crispy in texture.

The fortified product follows the same procedure, but is formulated with 600 IU (15 µg) of cholecalciferol (vitamin D_3) powder per serving (± 40 g of product). This study is not intended to evaluate the effectiveness of fortified nutrition, but rather to compare the peptide profiles at the molecular level between sample groups.

LC-HRMS preparation and analysis

Proteomic analysis was performed at the Integrated Research and Testing Laboratory (LPPT), Gadjah Mada University, Yogyakarta. Peptide identification was performed using a Thermo Scientific™ Dionex Ultimate 3000 RSLCnano UHPLC system connected to a Thermo Scientific™ Q Exactive™ (Orbitrap) high-resolution mass spectrometer. Ionization was performed in positive mode using an EASY-NanoSpray ionization source. Each sample (± 15 g) was analyzed using a bottom-up proteomics approach.

Proteins were first extracted from solid samples, then underwent reduction and alkylation before enzymatic digestion using trypsin (Thermo Scientific), according to standard laboratory procedures. The digest filtrate was then filtered using a 0.22 µm membrane to remove particulate contaminants. A 5 µL aliquot of the filtrate was injected into the LC-HRMS system for analysis.

Peptide separation was performed using an EASY-Spray PepMap C18 column (15 cm×75 µm ID, particle

size 3 µm). The mobile phase consisted of MS-grade water containing 0.1% formic acid (phase A) and MS-grade acetonitrile containing 0.1% formic acid (phase B). Peptides were eluted using the following linear gradient: 5% phase B for 1 min, followed by a gradual increase from 5% to 50% phase B over 50 min, then returned to the initial conditions, with a total analysis time of 60 min. The nanoLC flow rate was set at 100 nL/min, and the column temperature was maintained at room temperature.

Mass spectra were collected using *data-dependent acquisition* (DDA) mode. Full MS scanning was performed at a resolution of 70,000 (FWHM), followed by MS/MS scanning at a resolution of 17,500 (FWHM). The mass scan range was set at m/z 150 - 2,000. Ion fragmentation was performed using higher-energy collisional dissociation (HCD) with a normalized collision energy (NCE) of 27. The spray voltage was set to approximately 3.5 kV, with a capillary temperature of 250 °C. The automatic gain control (AGC) and maximum injection time parameters were run using the Orbitrap instrument default settings.

The mass tolerance was set at ±10 ppm for precursor ions and ± 0.02 Da for fragment ions, in accordance with the Orbitrap instrument specifications. Some highly specific instrument parameters (e.g., target AGC, maximum number of ions selected per cycle, and maximum injection time) follow the standard settings of the Q Exactive instrument in the service laboratory and are not fully listed in the technical report, but are within the range commonly used for LC-HRMS-based peptide analysis.

Protein identification and reference database

Protein identification was performed using Proteome Discoverer 2.2 software (Thermo Fisher Scientific). Protein sequence matching was performed against a database downloaded from UniProt (available at the time of analysis), with trypsin set as the proteolytic enzyme and up to 2 missed cleavages allowed. Validation of protein identification results was performed using the Percolator module, with a false discovery rate (FDR) < 1% applied at the peptide spectrum match (PSM) and protein group levels. Search parameters included a mass tolerance of approximately ±10 ppm for precursor ions and ±0.02 Da for fragment ions, in accordance with Orbitrap instrument specifications and standard proteomics practices. Since the analysis was performed in an external laboratory, highly detailed instrument parameters were not fully available in the technical report, but the settings used followed software standards and MIAPE (Minimum Information About a Proteomics Experiment) guidelines.

Analysis bioinformatics

Identified peptides were analyzed *in silico* using 2 bioinformatics platforms: BIOPEP-UWM and PeptideRanker. BIOPEP-UWM was used to identify bioactive motifs and map their potential biological activities, such as antihypertensive, antioxidant, antidiabetic, and immunomodulatory effects. PeptideRanker was used to predict peptide bioactivity scores based on a machine-learning model, with values ranging from 0 to 1. Peptides with scores ≥ 0.8 were categorized as highly bioactive, those between 0.5 - 0.79 as moderately bioactive, and those < 0.5 as having low bioactivity. In this study, the BIOPEP-UWM and PeptideRanker results were used solely as exploratory *in silico* indicators of potential bioactivity and were not treated as confirmatory proof of real biological function.

Criteria peptides and classification

Peptides were classified based on 5 main parameters: (1) Number of Proteins and Peptides identified, (2) Types of biological activity (BIOPEP), (3) Peptide length (number of amino acids residues), (4) Number of biological activities per peptide and (5) Predicted bioactivity score (PeptideRanker).

The distribution of peptides according to these parameters was analyzed for each treatment (raw, processed, and fortified). The main results are presented in the primary tables of this article, while the complete peptide list is available in the supplementary data file.

Data analysis and visualization

This study represents an exploratory effort to establish an initial map of bioactive peptides in anchovies across 3 forms - raw, processed, and fortified. Data were presented in tabular form for each sample, covering the number of identified peptides, types of biological activities, peptide length distribution, number of activities per peptide, and predicted bioactivity scores.

This study was exploratory and non-inferential, as the proteomic analyses were conducted externally, and replication data for biological or technical approaches were unavailable. Such an approach is common in the initial stages of untargeted proteomics research, where the goal is to generate a preliminary mapping of bioactive peptide profiles. To ensure data reliability, protein identification parameters such as sequence coverage, number of unique peptides, and total PEP scores were applied as criteria for internal validation. The interpretation of results focused on peptide trends, distribution, and qualitative-quantitative characteristics rather than inferential statistical differences between treatments.

Research ethics

All research procedures, including sample collection, processing, and laboratory analyses, received ethical approval from the Research Ethics Committee of Gadjah Mada University, Yogyakarta, Indonesia (Ref. No: KE/FK/0618/EC/2025). The study was conducted in accordance with ethical principles for scientific research to ensure data integrity and the responsible use of biological materials.

Identification of parent peptides in raw, processed, and fortified anchovy samples

Proteomic analysis was conducted to describe the peptide profiles of raw anchovies and explore how processing and fortification might affect peptide composition, without intending to inferential test treatment effects. Three types of samples - raw anchovies, processed anchovies, and fortified processed anchovies - were analyzed using the LC-HRMS approach to identify parent peptides resulting from protein degradation. These peptides are considered as molecular components that potentially contribute to the functional characteristics and predicted bioactivity of each sample in the context of this exploratory analysis.

Identification was performed by matching peptide sequences against the *Engraulidae Coilia grayii* FASTA database, producing quantitative data in the form of protein IDs, total identified peptide sequences, and unique peptides.

These identification results form the descriptive basis for in silico bioactivity analysis in the next stage and are presented in tables and diagrams to illustrate the distribution patterns and differences observed between samples, without implying statistically validated differences.

Results and discussion

Table 1 Identification of parent peptides in raw, processed, and fortified anchovy samples.

No	Material	Raw	Processed	Fortified
1	Accession / ID protein	254	30	25
2	Sequence Peptides identified	1304	134	98
3	Peptides unique	662	51	48

Data source: LC-HRMS analysis (LPPT UGM, 2025).

Table 1 summarizes the number of protein IDs (accessions), identified peptide sequences, and unique peptides specific to each sample type. The raw sample exhibited the highest proteomic complexity, with 254 protein IDs and 1,304 identified peptide sequences. Among these, 662 were unique peptides not found in either the processed or fortified samples. In contrast, the processed and fortified samples showed a reduction in both protein and peptide counts - 30 and 25 protein IDs, and 134 and 98 peptide sequences, respectively. The number of unique peptides was also lower, with 51 in the processed and 48 in the fortified samples. This pattern is interpreted as a descriptive trend in the dataset without replication, and not to draw conclusions about the effects of processing or fortification.

The identification of protein and peptide numbers from LC-HRMS results provides essential information on the types and characteristics of proteins present in each sample, allowing for the evaluation of both the quantity and quality of identified proteins [33]. This step is crucial for improving data reliability and building a comprehensive proteomic profile, especially when comparing different samples, such as raw, processed, and fortified anchovies.

Different treatments can theoretically affect protein structure and produce variations in in the

proteomic composition of each sample [25]. The greater the number of proteins and peptides successfully identified, the more comprehensive the understanding of protein composition within a biological sample becomes. This is crucial for exploratory comparative analysis and for gain initial insights into the potential functional properties of the resulting proteins and peptides [23].

The identified proteins most likely originate from the muscle tissues of anchovies, which is widely recognized as a source of easily digestible protein and is rich in essential amino acids [15]. Previous studies have reported that enzymatic hydrolysis of fish muscle proteins can break peptide bonds and release peptides that have various functional properties [35,36], and bioactive potential such as antihypertensive (ACE inhibitor), antioxidant, antiproliferative, and antimicrobial activities [37]. Bioactive peptides can be derived from several fish parts, including muscle, collagen, gelatin from the skin, and bones [29]. However, in this study, these activities were not evaluated *in vitro* or *in vivo*. Therefore, this information is used solely as contextual literature and should not be interpreted as direct evidence of the bioactivity of the identified peptides.

Peptides identified through LC-HRMS analysis form the basis for protein identification [39]. Among these, *unique peptides* - those associated with only one protein or specific protein group without ambiguity - are particularly important [40], because they increase confidence in protein identification, reduces the risk of false positives, and strengthens automated validation. Conversely, proteins identified solely from non-unique peptides require more careful interpretation, as they may lead to less accurate identification [39].

However, not all identified proteins provide relevant information on bioactivity. Therefore, the selection of representative protein IDs was based on key criteria: A minimum of 2 unique peptides, sequence coverage, and a cumulative peptide score ≥ 10%. The presence of at least 2 unique peptides is a stronger indicator of protein identification validity, improving analytical accuracy and reliability [41]. Meanwhile, a cumulative peptide score ≥ 10% reflects high confidence in the mass spectrum matching, and sequence coverage between 20% - 30% or higher is considered sufficient for exploratory proteomics studies [32]. These thresholds help minimize reporting that proteins identified solely due to partial or coincidental matches.

Table 2 summarizes several representative proteins identified in the 3 sample types - raw, processed, and fortified anchovies - which were selected for their predicted major contributions to peptide complexity and potential bioactivity.

Table 2 Identification of Featured Protein IDs based on LC-HRMS analysis on raw, processed, and fortified anchovies.

No	Sample	Unique peptides	Coverage (%)	SUM Peptide score
1	Raw Anchovies			
	a. A0ABD1K6P1	22	32.24	94.54
	b. A0ABD1JQU7	13	59.51	93.06
	c. A0ABD1K8C4	10	35.43	107.06
	d. A0ABD1KC28	8	31.96	117.01
	e. A0ABD1K6S2	14	25.18	88.82
	f. A0ABD1JD58	7	20.97	31.99
	g. A0ABD1J185	16	28.57	51.62
	h. A0ABD1KRP3	8	21.97	51.6
	i. A0ABD1IYG6	3	26.13	56.41
	j. A0ABD1KNI6	3	29.97	47.15
2	Product processed anchovies			
	a. A0ABD1JQU7	8	33.80282	27.161

No	Sample	Unique peptides	Coverage (%)	SUM Peptide score
	b. A0ABD1JWL9	3	13.43511	61.096
	c. A0ABD1J939	2	10.30397	58.738
3	Fortified processed anchovies			
	a. A0ABD1JQU7	8	21.12676	14.701
	b. A0ABD1JWL9	2	10.2799	32.157
	c. A0ABD1J939	4	9.47965	30.765

Data source: LC-HRMS analysis (LPPT UGM, 2025).

Based on the selection criteria described above, ten key proteins were identified in raw anchovies, and 3 representative proteins each were identified in the processed and fortified products. The raw samples showed the highest proteomic complexity, with 3 - 22 unique peptides per protein. Among these, protein A0ABD1K6P1 had the highest number of unique peptides, A0ABD1JQU7 exhibited the highest sequence coverage, and A0ABD1KC28 showed the highest cumulative peptide score. In the processed and fortified samples, the number of protein IDs decreased, but A0ABD1JQU7 remained detectable, although with lower unique peptide counts and sequence coverage. Proteins A0ABD1JWL9 and A0ABD1J939 were identified with more limited representation.

The reduction in unique peptide counts, sequence coverage, and peptide scores in the processed and fortified samples may reflect protein degradation or structural modifications occurring during processing and fortification, which in turn could affect the detectable peptides. Previous studies have shown that treatments such as heating, fermentation, and fortification can lead to protein denaturation, aggregation, and fragmentation, thereby reducing proteome complexity [33]. Other studies also noted that suboptimal processing can diminish peptide stability and biological activity through degradation or modification [44]. Descriptively, the patterns observed in this study are consistent with these findings, although they cannot be used to establish a causal relationship between the treatments and changes in peptide quantity, diversity, or potential bioactivity.

Therefore, protein A0ABD1JQU7 can be tentatively considered as a candidate protein marker that remains relatively stable under the various processing and fortification conditions tested in this study. Several other proteins were also detected across all sample types but were not included in **Table 2** as they did not meet the established threshold for representation. Complete identification data are provided in Supplementary File to ensure transparency and allow further evaluation.

Distribution of biological activity types in bioactive peptides

Following the identification of primary protein IDs through LC-HRMS analysis, the resulting peptides were further examined to evaluate their potential bioactivities in silico. Peptide sequences were matched against the BIOPEP database to identify previously reported bioactive motifs, allowing each peptide to be classified into established biological activity categories. The analysis focused on the distribution of these predicted biological activity types, including antihypertensive, antioxidant, antidiabetic, immunomodulatory, and enzymatic inhibitory activities.

To streamline data presentation, the nine most abundant bioactive peptides from each sample were listed in the main table, along with one combined category labeled “other.” These bioactive peptides were selected based on their frequency of occurrence and activity type identified via BIOPEP matching. The distribution of bioactive peptide activities among raw, processed, and fortified anchovy samples is presented in **Table 3** to illustrate both quantitative and proportional differences patterns observed between sampels in this exploratory, non-replicated dataset, without statistical testing.

Table 3 Distribution of biological activity peptides bioactives identified in raw, processed, and fortified anchovy samples.

No	Activity	Sample					
		Raw		Processed		Fortified	
		Σ	%	Σ	%	Σ	%
1	Dipeptidyl Peptidase IV Inhibitor (DPP- IVi)	8,872	31.47	865	27.48	703	27.48
2	ACE Inhibitor (ACEi)	6,877	24.39	766	24.00	614	24.00
3	Antioxidant (Aox)	1,455	5.16	167	5.36	137	5.36
4	Neprilysin Inhibitor (NEPi)	1,215	4.31	216	6.53	167	6.53
5	Glutamate Carboxypeptidase II Inhibitor (GCP- IIi)	1,062	3.77	205	5.75	147	5.75
6	Stimulating (Stim)	933	3.31	130	3.48	89	3.48
7	Dipeptidyl Peptidase III Inhibitor (DPP-IIIi)	913	3.24	80	2.89	74	2.89
8	Glutamate Carboxypeptidase Inhibitor (GCP- i)	688	2.44	143	3.28	84	3.28
9	Inhibitor Of Tripeptidyl Peptidase II (TPP- IIi)	596	2.11	43	1.76	45	1.76
10	Miscellaneous (Misc)	5,582	19.80	682	19.47	498	19.47
	Total	**28,193**	**100**	**3,297**	**100**	**2,558**	**100**

Data source: LC-HRMS analysis (LPPT UGM, 2025).

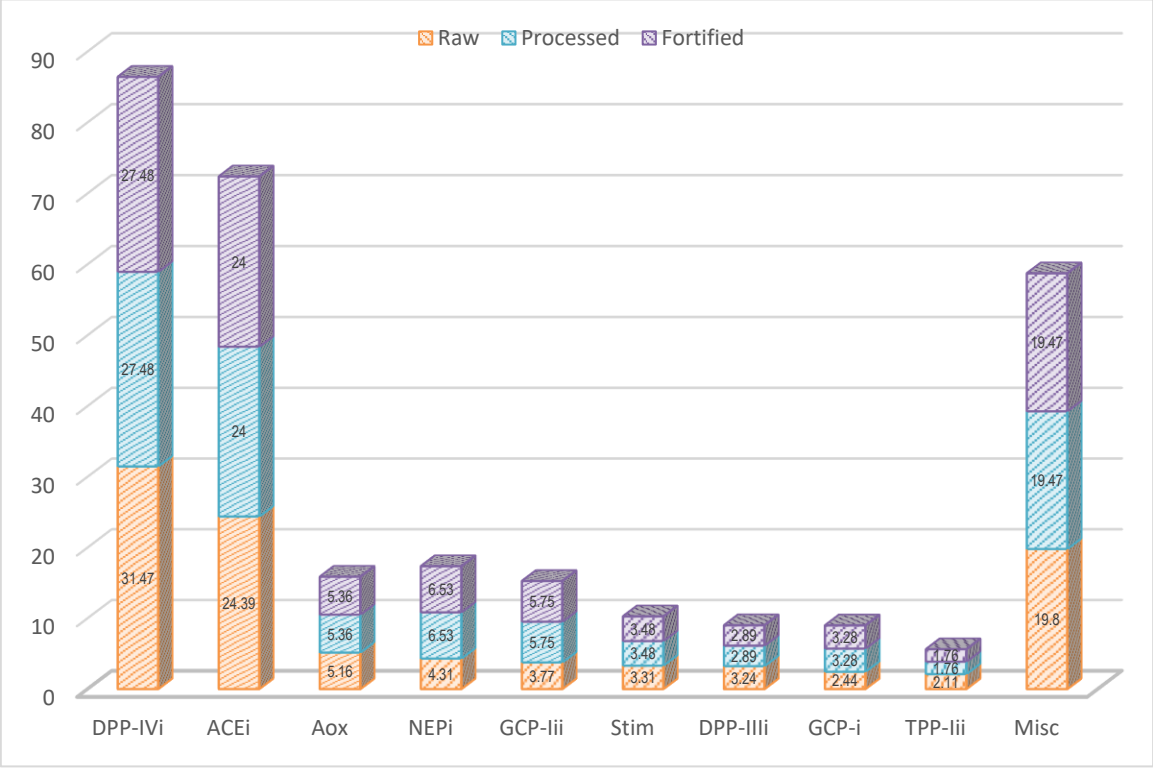

Figure 2 Distribution of biological activity peptides bioactives identified in raw, processed, and fortified anchovy samples.

Data source: LC-HRMS analysis (LPPT UGM, 2025).

The results show that although the proportional distribution of predicted bioactive activities remained broadly similar across the 3 samples, the total peptide counts decreased following processing and fortification. The dominant activity category across all groups was Dipeptidyl Peptidase IV (DPP-IV) inhibition, with the highest proportion in raw anchovies (31.47%), followed by processed and fortified anchovies (27.48%). In

previous studies, DPP-IV inhibitory peptides have been reported to regulate glucose metabolism by preventing the degradation of incretin hormones that stimulate insulin secretion, thereby improving glycaemic control and offering potential benefits for the prevention and management of type 2 diabetes [45-47].

ACE inhibitor activity was also constituted a consistently large proportion in all samples, ranging from 24.0% to 24.39%, suggesting that, at the descriptive level, processing and fortification did not markedly alter the proportion of peptides annotated as ACE inhibitors in this dataset. Anchovies, as marine organisms, have been widely reported as source of ACE inhibitory peptides with potential applications in pharmaceutical and nutraceutical development [48]. ACE (angiotensin-converting enzyme) converts angiotensin I into angiotensin II, a vasoconstrictor that raises blood pressure. By inhibiting ACE, these peptides help reduce angiotensin II formation, thereby lowering blood pressure [49,50].

Neprilysin inhibitor activity showed moderate levels, with a slightly higher proportion in fortified samples (6.53%) compared to raw samples (4.31%). Neprilysin is involved in the metabolism of natriuretic peptides, insulin, and enkephalins, and its inhibition has been linked in the literature to potential benefits for cardiovascular regulation and protection [51]. Previous studies have suggested that combined inhibition of neprilysin and the angiotensin II receptor system is associated with improved glycemic control and insulin sensitivity potentially through increased levels of beneficial peptides such as bradykinin and natriuretic peptides, which play crucial roles in glucose metabolism and cardiovascular health [52].

Other activities, including glutamate carboxypeptidase II inhibition (3.77% - 5.75%), antioxidative activity (5.16% - 5.36%), and stimulating activities (3.31% - 3.48%), were detected across all sample types. The moderate yet relatively consistent proportions of these predicted activities indicate that core categories of bioactive peptide annotations are retained despite differences in peptide composition and abundance among samples. According to previous reports, peptides annotated as Glutamate carboxypeptidase II inhibitors have been associated with potential therapeutic relevance in neurological disorders [53], while peptides annotated with antioxidant activity have been reported to contribute to cellular protection against oxidative stress through free radical scavenging and inflammation-related pathways [54]. In addition, peptides classified as stimulating have been described in the literature as being involved in regulatory processes related to gene expression, cell proliferation, differentiation, tissue repair, and the modulation of degenerative processes [55].

The lowest proportions were observed for glutamate carboxypeptidase inhibitors, dipeptidyl peptidase III inhibitors, and tripeptidyl peptidase II inhibitors, each contributing less than 3.5%. The “other” category accounted for approximately 19% - 20% of total activity, encompassing enzymatic or biological functions outside the main categories.

Overall, the findings indicate that peptides annotated as DPP-IV and ACE inhibitors represent the predominant predicted bioactive categories in anchovy samples, supported by peptides annotated with antioxidant and other enzyme-inhibitory activities. Processing and fortification were associated with only minor reductions in the relative proportions of some predicted activities, without eliminating the overall profile of annotated bioactive potential.

Previous studies have noted that food processing can influence peptide composition and activity. Certain treatments may enhance peptide release, while excessive heat or chemical fortification can mildly reduce peptide stability and function [56]. Overall, descriptive analysis based on sequence annotation shows that anchovies maintain the diversity of peptide categories predicted to have bioactive potential after processing and fortification. Although there was a decrease in the number and composition of certain peptides, the annotation patterns observed remained consistent across samples. This decrease is likely related to protein denaturation, structural changes, or peptide degradation during thermal and chemical processes, as reported in previous studies [43,44]. These findings emphasize the importance of controlling processing conditions in the context of preserving the peptide profile, although biological function stability was not experimentally evaluated in this study.

Overall, all reported bioactivities were inferred solely from sequence-based annotation and literature references. Given the exploratory and non-replicated nature of this study, these findings are presented

descriptively and are not intended to indicate direct biological efficacy.

Peptide length distribution of bioactive peptides identified in raw, processed, and fortified anchovy samples

Bioactive peptides identified from each sample were analyzed according to peptide length to characterize the distribution of predicted bioactive peptide profiles. Peptide length is an important parameter because it can influence stability, absorption, and biological relevance - particularly in food, functional, and nutraceutical contexts.

The distribution of peptide lengths was determined based on matching results from the BIOPEP database and is presented separately for raw, processed, and fortified anchovy samples. **Table 4** and **Figure 2** summarize the length distribution of predicted bioactive peptides across these samples.

Table 4 Peptide length distribution of bioactives peptides identified in raw, processed, and fortified anchovy samples.

No	Peptide length (aa)	Samples					
		Raw		Processed		Fortified	
		Σ	%	Σ	%	Σ	%
1	2 aa	309	48.97	187	75.10	181	73.88
2	3 aa	278	44.06	52	20.88	52	21.22
3	4 aa	23	3.65	8	3.21	10	4.08
4	≥ 5 aa	21	3.33	2	0.80	2	0.82
	Total	631	100	249	100	245	100

Data source: LC-HRMS analysis (LPPT UGM, 2025).

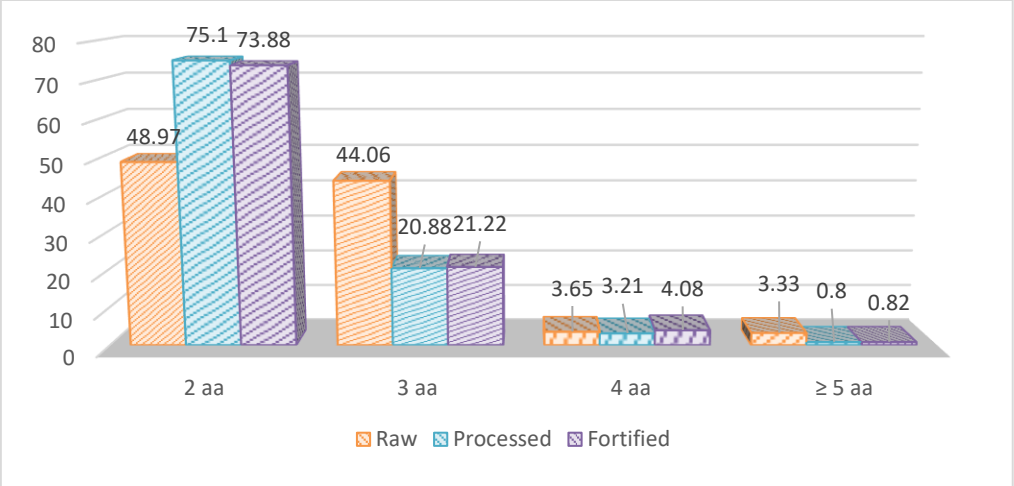

Figure 2 Peptide length distribution of bioactives peptides identified in raw, processed, and fortified anchovy samples. Data source: LC-HRMS analysis (LPPT UGM, 2025).

In the raw anchovy samples, peptide length distribution was the most diverse, with dipeptides (2 amino acids) comprising 48.97%, tripeptides (3 amino acids) 44.06%, and peptides ≥ 4 amino acids reaching a cumulative 6.98%. This distribution reflects a broader range of peptide chain lengths and greater variation in the identified peptide profiles.

In contrast, the processed and fortified products displayed a narrower peptide length distribution dominated by dipeptides, accounting for 75.10% and 73.88% respectively, while peptides ≥ 4 amino acids constituted ≤ 4%. This pattern suggests that processing and fortification may be associated with more extensive protein fragmentation, leading to shorter peptide chains. According to previous reports, short peptides, despite

their smaller size, can still retain or even exhibit enhanced biological functionality [32]. The bar chart visualization highlights differences in peptide length distributions among samples and supports a descriptive interpretation that processing and fortification are associated with shifts in peptide length profiles, rather than direct alterations of peptide structure.

The short-chain peptides identified in this study can be understood as the result of a combination of the intrinsic characteristics of the raw materials and the effects of the processing. Raw anchovies that have not undergone any treatment already contain a number of short-chain peptides, but there appears to be an increase in the proportion of short peptides in samples that have undergone processing. In line with previous reports, processing - particularly thermal treatments such as drying and frying - can cause peptide chain scissions, whereby longer protein chains are fragmented into shorter peptides. Heating and processing can trigger protein denaturation and expose previously hidden enzymatic cleavage sites, making it easier for proteolytic enzymes such as trypsin to break peptide bonds. As a result, proteins tend to degrade into shorter peptides, contributing to the dominance of short-chain peptides observed in the peptide profile of this study [57].

Peptide length plays a critical role in determining potential bioactivity. Short peptides are often reported to exhibit higher activity because they can interact more efficiently with biological targets such as enzymes or cell membranes. Their small size enhances penetration and reduces enzymatic degradation during digestion or metabolism [58]. Tripeptides and tetrapeptides with high bioactivity scores are also easier to synthesize and serve as promising candidates for nutraceutical and therapeutic development [59], due to their relatively simple structures and synthetic accessibility.

Peptides with lengths of 2 - 4 amino acids are commonly associated with antihypertensive and antioxidant functions, while antimicrobial peptides typically have longer sequences (≥ 5 amino acids) [60]. Other studies report that antioxidant peptides often range from 5 to 16 residues, with length influencing both stability and interaction with biological targets [61]. Previous research has shown that short-chain peptides such as Phenylalanine-Tryptophan (FW), Phenylalanine-Phenylalanine (FF), and Leucine-Proline-Phenylalanine (LPF) possess anti-inflammatory properties by inhibiting key enzymes involved in inflammation and prostaglandin synthesis, which are associated with pain and swelling [62]. These examples are cited here to contextualize the possible functional implications of peptide length; nevertheless, in this study, the bioactivities of the identified peptides were not experimentally validated and remain predicted based on sequence homology and literature evidence.

Distribution of bioactive peptides based on the number of biological activities in raw, processed, and fortified anchovy samples

After determining peptide activity types and peptide lengths, the next step involved grouping peptides according to the number of biological activity categories with which they were annotated. **Table 5** presents a comparative distribution across the 3 sample types-raw, processed, and fortified anchovies.

Table 5 Distribution of bioactive peptides based on the number of biological activities in raw, processed, and fortified anchovy samples.

No	Number of biological activities	Samples					
		Raw		Processed		Fortified	
		Σ	%	Σ	%	Σ	%
1	1 activity	406	64.65	141	56.63	135	55.10
2	2 activities	115	18.31	57	22.89	54	22.04
3	3 activities	51	8.12	19	7.63	22	8.98
4	4 activities	31	4.94	15	6.02	15	6.12
5	≥ 5 activities	25	3.98	17	6.83	19	7.76
	Total	628	100	249	100	245	100

Data source: LC-HRMS analysis (LPPT UGM, 2025).

The analysis revealed that the majority of peptides in all samples were annotated with only one biological activity: 64.65% in raw anchovies, 56.63% in processed anchovies, and 55.10% in fortified anchovies. However, the proportion of peptides annotated with 2 or more biological activity types increased in the processed and fortified samples. Peptides annotated with ≥ 5 biological activity categories increased from 3.98% in raw samples to 6.83% in processed and 7.76% in fortified samples. These descriptive patterns suggest that, although the total number of peptides decreased as a result of processing, the functional complexity of the remaining peptides actually increased.

In previous studies, peptides annotated with multiple biological activities are often considered superior because they can influence various physiological mechanisms simultaneously, potentially producing broader and more synergistic therapeutic effects [4], than peptides with single activities [63]. For instance, the peptides Alanine-Tryptophan (AW) and Tryptophan-Tyrosine (WY) have been reported as multifunctional bioactive peptides, acting as ACE inhibitors, antioxidants, and DPP-IV inhibitors, each with a PeptideRanker bioactivity score ≥ 0.9.

This distribution supports a descriptive interpretation that although processing and fortification reduce the overall number of peptides, they do not eliminate bioactivity potential. processed and fortification samples tends appear to contain yield peptides with more complex and diverse biological functions. In other words, while the total peptide quantity decreases, the diversity of biological activity per peptide may increase [64], thereby preserving - or even concentrating - the predicted bioactivity of anchovy-derived peptides.

A reduction in peptide quantity does not necessarily imply a loss of function, as certain peptides with higher molecular stability may exhibit stronger or more specific biological effects when confirmed experimentally [65]. In the context of this exploratory, non-replicated study, the observed patterns therefore suggest that the predicted bioactivity potential of anchovy peptides can remain largely preserved - and in some cases may appear to be concentrated in a smaller number of multifunctional sequences - following controlled processing and fortification. These observations highlight the promise of anchovy-derived peptides as candidates for functional food and nutraceutical applications, while also underscoring the need for further *in vitro* and *in vivo* studies to validate their actual biological efficacy.

Distribution of peptides based on PeptideRanker bioactivity scores in raw, processed, and fortified anchovy samples

After identifying the quantity and types of bioactive peptides and their biological activities, the next step was to evaluate the intrinsic bioactivity potential of each peptide using predictive scores from PeptideRanker. This score reflects the likelihood that a peptide may exhibit biological activity, based on the sequence order and amino acid motif patterns statistically recognized by predictive machine-learning models.

PeptideRanker score distributions were analyzed comparatively among the 3 sample types - raw anchovies, processed anchovies, and fortified anchovies - to describe whether processing and fortification were associated with changes in the proportion of peptides with high bioactivity potential (score ≥ 0.8). The complete list of peptides, along with their individual scores, is provided in the supplementary file, while the following table summarizes the score distribution in each sample as the basis for functional interpretation and peptide candidate mapping.

Table 6 Distribution of peptides based on PeptideRanker bioactivity scores in raw, processed, and fortified anchovy samples.

No	Score Range	Samples Type					
		Raw		Processed		Fortified	
		Σ	%	Σ	%	Σ	%
1	High Bioactivity (≥ 0.8)	3,854	13.67	382	11.59	373	14.58
2	Moderate Bioactivity (0.5 - 0.79)	3,303	11.72	336	10.19	293	11.45
3	Low Bioactivity (< 0.5)	21,036	74.61	2,579	78.22	1,892	73.96
	Total	28,193	100	3,297	100	2,558	100

Data source: LC-HRMS analysis (LPPT UGM, 2025).

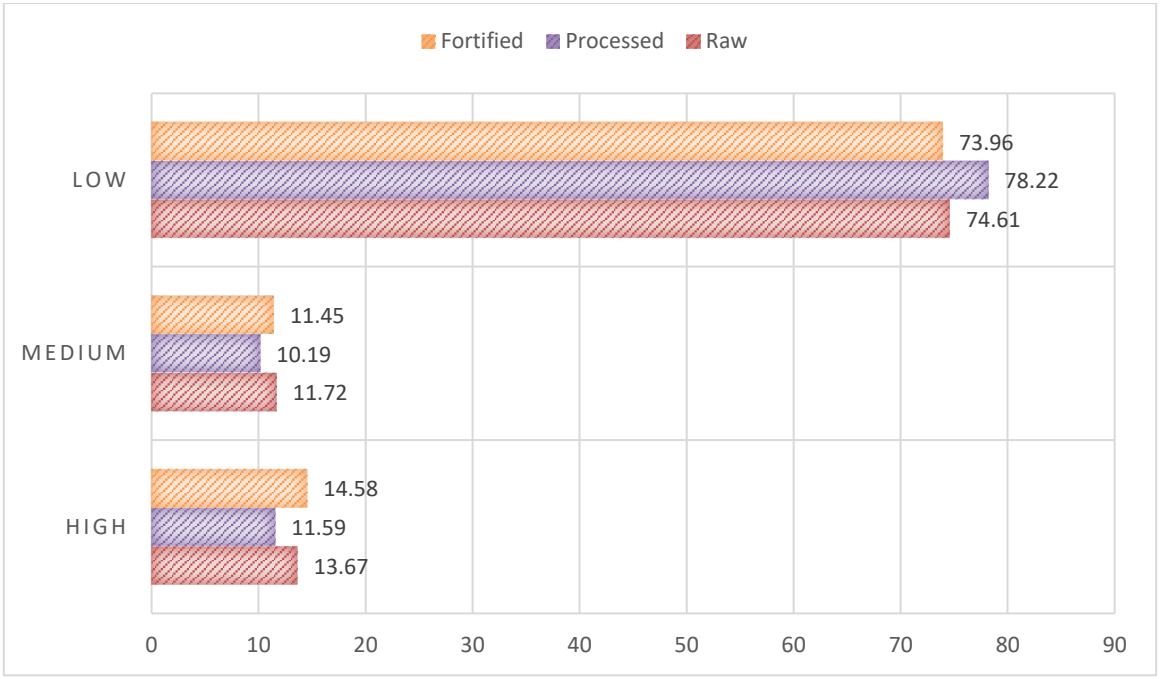

Figure 2 Distribution of peptides based on PeptideRanker bioactivity scores in raw, processed, and fortified anchovy samples.
Data source: LC-HRMS analysis (LPPT UGM, 2025).

In silico analysis using PeptideRanker revealed distinct predicted bioactivity score distributions among the 3 anchovy samples. The majority of peptides exhibited low bioactivity scores (< 0.5), accounting for 74.61% in raw anchovies, 78.22% in processed anchovies, and 73.96% in fortified anchovies - indicating that most peptides had low or suboptimal predicted intrinsic bioactivity. Nevertheless, peptides with high bioactivity scores (≥ 0.8) were still detected in meaningful proportions, particularly in fortified samples

(14.58%), followed by raw (13.67%) and processed (11.59%) samples.

These descriptive findings suggest that fortification with cholecalciferol (vitamin D_3) does not eliminate, and may be associated with a higher proportion of peptides predicted to have high bioactivity scores in this dataset. This pattern could reflect structural modifications of proteins during processing and potential interactions with fortifying agents that promote the formation of bioactive peptide sequences. The observed trend - where fortified samples exhibited the highest proportion of high-score peptides - should therefore be regarded as hypothesis generating.

Peptides with high PeptideRanker scores are often considered, in the literature, to have greater biological significance, with potential roles such as angiotensin-converting enzyme inhibition (ACE-I), dipeptidyl peptidase IV inhibition (DPP-IV), antioxidant effects, and immunomodulatory functions [63,66]. Such peptides have been reported to be more promising candidates because they are more resistant to enzymatic digestion in the gastrointestinal tract and have a greater likelihood of reaching systemic circulation to exert biological effects [59].

Not all peptides produced during proteolysis exhibit the same biological potential; therefore, evaluating peptides based on their predicted bioactivity scores provides more meaningful insight for the selection and prioritization of candidates for health-oriented and functional food applications than merely counting total peptide numbers without considering their biological relevance [66]. However, such scoring-based evaluations should be regarded as a screening tool that guides hypothesis generation and candidate selection, rather than as a substitute for experimental assessment of actual bioactivity.

Conclusions

This exploratory study descriptively identified and mapped peptides and their predicted bioactivities from raw anchovies, processed anchovies, and fortified processed anchovies using LC-HRMS proteomics combined with BIOPEP-UWM and PeptideRanker bioinformatics analysis. The results showed that raw anchovies exhibited the highest proteomic complexity, while processing and fortification led to a reduction in the number of proteins and peptides, particularly those with a chain length of ≥ 4 amino acids, in this non-replicated dataset. Nevertheless, processing did not entirely reduce bioactivity potential; in fact, peptides with high bioactivity scores (≥ 0.8) and multifunctional properties increased notably in fortified anchovy samples at a descriptive level. These patterns should be considered as hypotheses and not as evidence of actual increased bioactivity.

The predominant predicted bioactivities in anchovy-derived peptides were Dipeptidyl Peptidase IV (DPP-IV) inhibition and Angiotensin-Converting Enzyme (ACE) inhibition, followed by antioxidant, neprilysin inhibitory, and Glutamate Carboxypeptidase II inhibitory (GCPII) activities, which are considered relevant in the literature to glucose and blood-pressure regulation as well as oxidative stress and neurological pathways. The distribution of peptide lengths revealed a dominance of short peptides (2 - 3 amino acids) in processed and fortified anchovies, indicates greater protein fragmentation, likely as a result of heat treatment and fortification. Further research is needed.

Overall, the findings from these 3 sample types provide preliminary information suggesting that anchovy proteins may serve as promising precursors of peptides with putative multifunctional bioactivities and could be explored as candidates for anchovy-based functional foods or nutraceutical products once their effects are confirmed through replicated experiments and targeted *in vitro* and *in vivo* validation.

Limitations of the study

This study was exploratory and descriptive and did not include biological or technical replication. Consequently, the peptide profiles reported here should be interpreted with caution, as they may represent treatment-specific trends rather than reproducible biological effects. Without replicates, statistical comparisons cannot be performed, and causal interpretations - such as whether processing or cholecalciferol fortification truly influenced specific peptide patterns - cannot be confirmed. Nevertheless, these findings provide an initial foundation for future *in vitro* and *in vivo* studies to validate the predicted biological activities of the identified peptides.

The functional interpretation is based exclusively on in silico tools (BIOPEP-UWM and PeptideRanker), which generate motif-based predictions and ranking

scores but do not account for gastrointestinal digestion, peptide degradation, absorption barriers, or real physiological conditions. Therefore, all reported functions should be regarded as hypothetical potentials rather than validated biological activities. The predominance of very short peptides (2 - 3 amino acids) may also reflect intrinsic material properties, processing-induced fragmentation, or technical artefacts, so their predicted bioactivities must be interpreted with particular caution.

Future work should optimize processing and fortification conditions to enhance peptide stability and bioactivity, incorporate replicated LC-MS/MS analyses, and apply targeted validation strategies (e.g., PRM or SRM) together with functional assays to confirm the biological relevance and safety of prioritized peptides. In addition, the limited availability of annotated Engraulidae protein sequences in public databases may reduce identification completeness and increase the risk of misassignment; expanding proteomic resources for small pelagic fish will be essential to improve depth and accuracy in subsequent studies.

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Declaration of Generative AI in Scientific Writing

The author declares that artificial intelligence (AI) tools, including ChatGPT and Gemini AI, were used solely to refine the scientific language, enhance sentence structure, and check grammar to ensure the manuscript adheres to academic standards. All content, data interpretation, and scientific conclusions are entirely the author's own work, without any AI-generated content. The author assumes full responsibility for the authenticity and scientific accuracy of this manuscript.

CRediT Author Statement

Artika Dewie: Conceptualization Ideas, design of methodology, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Visualization; **Susetyowati Susetyowati:** Project administration Management and coordination, Validation, Resources, Data Curation, Writing - Review & Editing, Visualization, Supervision, Funding acquisition; **Diah Rumekti Hadiati:** Validation, Resources, Data Curation, Writing - Review & Editing, Visualization, Supervision.

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Supplementary Materials

Table S1 Identification of Accession/Protein IDs in raw, processed, and fortified anchovy samples.

a. Accession / Protein ID identified in raw anchovy samples.

No	Accession	Gene Names	Organisme	Status	Sum PEP Score	Coverage	Peptides	Unique peptides
1	A0ABD1JWM5	ACEWY4_013529	Coilia grayii	Uncharacterized	268.697	28.64556	65	1
2	A0ABD1KNW3	ACEWY4_004882	Coilia grayii	Uncharacterized	223.616	21.05263	51	1
3	A0ABD1KN14	ACEWY4_004883	Coilia grayii	Uncharacterized	222.224	24.70877	49	0
4	A0ABD1KN00	ACEWY4_004881	Coilia grayii	Uncharacterized	215.651	21.41383	49	1
5	A0ABD1JWL2	ACEWY4_013531	Coilia grayii	Uncharacterized	204.713	34.93031	47	0
6	A0ABD1JWK2	ACEWY4_013510	Coilia grayii	Uncharacterized	197.104	17.66529	45	2
7	A0ABD1JWK8	ACEWY4_013513	Coilia grayii	Uncharacterized	130.929	15.08264	35	1
8	A0ABD1KC28	ACEWY4_008586	Coilia grayii	Uncharacterized	117.005	31.96126	16	8
9	A0ABD1JH21	ACEWY4_018736	Coilia grayii	Uncharacterized	113.557	10.48843	26	1
10	A0ABD1JE25	ACEWY4_018741	Coilia grayii	Uncharacterized	107.8	10.21145	25	1
11	A0ABD1K8C4	ACEWY4_010117	Coilia grayii	Uncharacterized	107.058	35.43307	17	10
12	A0ABD1JE35	ACEWY4_018738	Coilia grayii	Uncharacterized	103.915	17.44186	22	1
13	A0ABD1IWR9	ACEWY4_026421	Coilia grayii	Uncharacterized	103.561	13.4068	19	19
14	A0ABD1K6P1	ACEWY4_009534	Coilia grayii	Uncharacterized	94.54	32.23844	22	22
15	A0ABD1JQU7	ACEWY4_016147	Coilia grayii	Uncharacterized	93.064	59.50704	23	13
16	A0ABD1JIK6	ACEWY4_018051	Coilia grayii	Uncharacterized	89.716	48.80637	16	7
17	A0ABD1K6S2	ACEWY4_009561	Coilia grayii	Uncharacterized	88.818	25.17815	22	14
18	A0ABD1J5R8	ACEWY4_022257	Coilia grayii	Uncharacterized	63.069	4.462086	13	13
19	A0ABD1JIG3	ACEWY4_018021	Coilia grayii	Uncharacterized	61.449	36.49123	14	1
20	A0ABD1IYG6	ACEWY4_023775	Coilia grayii	Uncharacterized	56.414	26.12613	7	3
21	A0ABD1KEJ2	ACEWY4_006819	Coilia grayii	Uncharacterized	56.196	7.489669	18	1
22	A0ABD1JFZ5	ACEWY4_017131	Coilia grayii	Uncharacterized	54.352	42.62295	14	1
23	A0ABD1J185	ACEWY4_022807	Coilia grayii	Uncharacterized	51.618	28.57143	18	16
24	A0ABD1KRP3	ACEWY4_003568	Coilia grayii	Uncharacterized	51.601	21.97802	12	8
25	A0ABD1KX80	ACEWY4_000479	Coilia grayii	Uncharacterized	51.585	27.08333	12	8
26	A0ABD1K2R0	ACEWY4_010709	Coilia grayii	Uncharacterized	47.923	14.44867	8	5
27	A0ABD1KNI6	ACEWY4_002500	Coilia grayii	Uncharacterized	47.147	29.97416	12	3
28	A0ABD1IPY3	ACEWY4_027510	Coilia grayii	Uncharacterized	44.831	33.97683	12	12
29	A0ABD1IVY3	ACEWY4_024783	Coilia grayii	Uncharacterized	42.782	18.30791	13	5
30	A0ABD1KJR0	ACEWY4_005844	Coilia grayii	Uncharacterized	32.811	16.27297	6	1
31	A0ABD1JD58	ACEWY4_019524	Coilia grayii	Uncharacterized	31.993	20.96774	7	7
32	A0ABD1IVY4	ACEWY4_026474	Coilia grayii	Uncharacterized	30.232	17.07989	6	2
33	A0ABD1JPX6	ACEWY4_015744	Coilia grayii	Uncharacterized	29.231	15.55154	6	6
34	A0ABD1KM45	ACEWY4_004653	Coilia grayii	Uncharacterized	29.097	27.8169	11	1
35	A0ABD1JS49	ACEWY4_014388	Coilia grayii	Uncharacterized	28.903	13.72549	6	6
36	A0ABD1JAD0	ACEWY4_019610	Coilia grayii	Uncharacterized	27.206	5.197133	5	1
37	A0ABD1KMJ6	ACEWY4_004432	Coilia grayii	Uncharacterized	26.69	19.56522	7	7
38	A0ABD1KXB1	ACEWY4_000667	Coilia grayii	Uncharacterized	26.365	18.35206	9	6

No	Accession	Gene Names	Organisme	Status	Sum PEP Score	Coverage	Peptides	Unique peptides
39	A0ABD1JPW8	ACEWY4_015735	Coilia grayii	Uncharacterized	26.358	4.675325	4	1
40	A0ABD1JBC5	ACEWY4_019985	Coilia grayii	Uncharacterized	25.335	5.460751	6	3
41	A0ABD1JE28	ACEWY4_018637	Coilia grayii	Uncharacterized	24.584	6.226415	9	9
42	A0ABD1KAB2	ACEWY4_008234	Coilia grayii	Uncharacterized	24.059	21.17647	5	5
43	Q9IB20	mlc2	Engraulis japonicus	Myosin light chain 2	24.029	39.53488	6	4
44	A0ABD1K6Z6	ACEWY4_009639	Coilia grayii	Uncharacterized	23.862	13.99549	4	2
45	A0ABD1JHT7	ACEWY4_017757	Coilia grayii	Uncharacterized	23.709	18.18182	4	3
46	A0ABD1KV70	ACEWY4_002231	Coilia grayii	Uncharacterized	21.712	16.41026	6	5
47	A0ABD1JVZ2	ACEWY4_013311	Coilia grayii	Uncharacterized	21.554	11.58238	7	2
48	A0ABD1JMX2	ACEWY4_016503	Coilia grayii	Uncharacterized	21.32	11.7506	5	3
49	A0ABD1K2A6	ACEWY4_010575	Coilia grayii	Uncharacterized	21.082	21.75066	7	7
50	A0ABD1K5M1	ACEWY4_009127	Coilia grayii	Uncharacterized	20.559	13.18945	5	5
51	A0ABD1KKX3	ACEWY4_004161	Coilia grayii	Uncharacterized	19.064	12.99213	4	4
52	A0ABD1KY90	ACEWY4_000991	Coilia grayii	Uncharacterized	18.369	48.125	6	5
53	A0ABD1ITD0	ACEWY4_026715	Coilia grayii	Uncharacterized	18.244	31.66667	10	1
54	A0ABD1JVU6	ACEWY4_013274	Coilia grayii	Uncharacterized	18.142	6.976744	3	3
55	A0ABD1KPN4	ACEWY4_002881	Coilia grayii	Uncharacterized	18.06	26.81159	6	6
56	A0ABD1KFY0	ACEWY4_007284	Coilia grayii	Uncharacterized	17.977	31.87919	10	1
57	A0ABD1KUF6	ACEWY4_001976	Coilia grayii	Uncharacterized	17.966	6.353861	4	3
58	A0ABD1K5F2	ACEWY4_009052	Coilia grayii	Uncharacterized	17.96	13.07692	7	3
59	A0ABD1JCU9	ACEWY4_019386	Coilia grayii	Uncharacterized	17.795	10.71975	7	3
60	A0ABD1K317	ACEWY4_010827	Coilia grayii	Uncharacterized	17.473	17.77108	6	5
61	A0ABD1J254	ACEWY4_023136	Coilia grayii	Uncharacterized	16.956	0.327668	7	5
62	A0ABD1JH61	ACEWY4_017557	Coilia grayii	Uncharacterized	16.779	14.41441	5	5
63	A0ABD1JC37	ACEWY4_020258	Coilia grayii	Uncharacterized	15.396	19.68085	3	3
64	A0ABD1KSV3	ACEWY4_001398	Coilia grayii	Uncharacterized	15.054	13.62799	6	6
65	A0ABD1J274	ACEWY4_023137	Coilia grayii	Uncharacterized	15.034	0.388258	6	4
66	A0ABD1J232	ACEWY4_023110	Coilia grayii	Uncharacterized	14.861	5.695308	5	5
67	A0ABD1KL84	ACEWY4_004269	Coilia grayii	Uncharacterized	14.675	19.70803	6	6
68	A0ABD1KS74	ACEWY4_003748	Coilia grayii	Uncharacterized	14.582	18.5	7	7
69	A0ABD1KRS6	ACEWY4_003633	Coilia grayii	Uncharacterized	14.334	23.31288	4	4
70	A0ABD1KS86	ACEWY4_003632	Coilia grayii	Uncharacterized	14.002	19.2053	2	2
71	A0ABD1J4P8	ACEWY4_023138	Coilia grayii	Uncharacterized	13.726	1.159008	4	4
72	A0ABD1JY44	ACEWY4_011561	Coilia grayii	Uncharacterized	13.573	7.274827	5	5
73	A0ABD1KP85	ACEWY4_002716	Coilia grayii	Uncharacterized	13.156	23.66864	3	1
74	A0ABD1JWK4	ACEWY4_013500	Coilia grayii	Uncharacterized	12.899	10.41667	4	1
75	A0ABD1KXC9	ACEWY4_000668	Coilia grayii	Uncharacterized	12.364	10.87344	8	1
76	A0ABD1K1M4	ACEWY4_010312	Coilia grayii	Uncharacterized	12.198	7.777778	3	3
77	A0ABD1K893	ACEWY4_010088	Coilia grayii	Uncharacterized	12.129	10	7	1
78	A0ABD1JMG4	ACEWY4_018197	Coilia grayii	Uncharacterized	11.976	13.80671	7	7
79	A0ABD1JUX2	ACEWY4_012912	Coilia grayii	Uncharacterized	11.846	18.44262	4	3
80	A0ABD1JBW2	ACEWY4_020183	Coilia grayii	Uncharacterized	11.84	11.88119	3	1

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81	A0ABD1KY68	ACEWY4_000968	Coilia grayii	Uncharacterized	11.751	8.354756	6	6
82	A0ABD1JYY6	ACEWY4_011882	Coilia grayii	Uncharacterized	11.695	10.57692	4	2
83	A0ABD1K3K3	ACEWY4_011026	Coilia grayii	Uncharacterized	10.272	5.830904	2	2
84	A0ABD1KWH5	ACEWY4_000391	Coilia grayii	Uncharacterized	10.122	48.19277	3	3
85	A0ABD1KYP3	ACEWY4_001161	Coilia grayii	Uncharacterized	10.063	9.31677	1	1
86	A0ABD1JQI0	ACEWY4_016022	Coilia grayii	Uncharacterized	10.054	19.67213	6	6
87	A0ABD1JZY7	ACEWY4_012221	Coilia grayii	Uncharacterized	10.048	12.10762	4	4
88	A0ABD1KLX4	ACEWY4_004515	Coilia grayii	Uncharacterized	9.981	16.54135	4	4
89	A0ABD1JPW7	ACEWY4_015699	Coilia grayii	Uncharacterized	9.818	17.88079	4	4
90	A0ABD1JVX5	ACEWY4_013296	Coilia grayii	Uncharacterized	9.52	5.324074	2	2
91	A0ABD1JWX3	ACEWY4_013620	Coilia grayii	Uncharacterized	9.317	7.878788	5	2
92	A0ABD1IXH7	ACEWY4_025401	Coilia grayii	Uncharacterized	9.267	17.95918	6	6
93	A0ABD1IYX6	ACEWY4_023943	Coilia grayii	Uncharacterized	9.173	5.069124	2	1
94	A0ABD1KPS6	ACEWY4_002932	Coilia grayii	Uncharacterized	9.028	19.84127	4	3
95	A0ABD1IV88	ACEWY4_027360	Coilia grayii	Uncharacterized	9	7.570978	1	1
96	A0ABD1KW84	ACEWY4_000275	Coilia grayii	Uncharacterized	8.684	5.110497	3	3
97	A0ABD1IWW4	ACEWY4_026430	Coilia grayii	Uncharacterized	8.34	17.01031	3	3
98	A0ABD1K9R6	ACEWY4_008057	Coilia grayii	Uncharacterized	8.221	24.08759	4	4
99	A0ABD1KLT4	ACEWY4_004500	Coilia grayii	Uncharacterized	7.98	2.195609	4	4
100	A0ABD1J5C4	ACEWY4_022202	Coilia grayii	Uncharacterized	7.957	15.46392	2	2
101	A0ABD1JIT1	ACEWY4_018117	Coilia grayii	Uncharacterized	7.84	2.092415	2	2
102	A0ABD1KKZ1	ACEWY4_004258	Coilia grayii	Uncharacterized	7.795	7.255521	2	2
103	A0ABD1JC51	ACEWY4_020276	Coilia grayii	Uncharacterized	7.515	26.98413	3	3
104	A0ABD1ITP6	ACEWY4_027100	Coilia grayii	Uncharacterized	7.455	10.71429	4	4
105	A0ABD1KNC7	ACEWY4_002448	Coilia grayii	Uncharacterized	7.406	10.70615	4	3
106	A0ABD1KMG0	ACEWY4_004725	Coilia grayii	Uncharacterized	7.334	5.42522	4	3
107	A0ABD1KKB0	ACEWY4_004008	Coilia grayii	Uncharacterized	7.249	11.34021	4	4
108	Q9IB21	mlc1	Engraulis japonicus	Myosin light chain 1	7.227	23.07692	4	4
109	A0ABD1JL29	ACEWY4_016687	Coilia grayii	Uncharacterized	7.223	7.816092	2	2
110	A0ABD1KM00	ACEWY4_004554	Coilia grayii	Uncharacterized	6.975	6.666667	2	2
111	A0ABD1K080	ACEWY4_012344	Coilia grayii	Uncharacterized	6.853	11.30742	2	2
112	A0ABD1JPX9	ACEWY4_015447	Coilia grayii	Uncharacterized	6.831	18.48341	4	4
113	A0ABD1JE14	ACEWY4_018622	Coilia grayii	Uncharacterized	6.614	19.32773	2	2
114	A0A2H4PU29	365059	Coilia nasus	60 kDa heat shock protein, mitochondrial	6.606	8.333333	3	3
115	A0ABD1KE79	ACEWY4_006662	Coilia grayii	Uncharacterized	6.562	3.91198	1	1
116	A0ABD1JI26	ACEWY4_017866	Coilia grayii	Uncharacterized	6.533	8.130081	3	2
117	A0ABD1KM97	ACEWY4_004708	Coilia grayii	Uncharacterized	6.465	12.8866	2	2
118	A0ABD1J9R3	ACEWY4_019450	Coilia grayii	Uncharacterized	6.452	9.803922	3	1
119	A0ABD1K7T6	ACEWY4_009938	Coilia grayii	Uncharacterized	6.389	10.81081	2	2
120	A0ABD1JMJ5	ACEWY4_015284	Coilia grayii	Uncharacterized	6.384	19.35484	3	3

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121	A0ABD1KIG1	ACEWY4_005138	Coilia grayii	Uncharacterized	6.365	4.835165	2	2
122	A0ABD1KGT8	ACEWY4_007495	Coilia grayii	Uncharacterized	6.227	12.31343	4	4
123	A0ABD1J7T3	ACEWY4_021030	Coilia grayii	Uncharacterized	6.114	21.79487	3	3
124	A0ABD1J8B0	ACEWY4_021196	Coilia grayii	Uncharacterized	5.99	2.631579	3	3
125	A0ABD1JPV8	ACEWY4_015654	Coilia grayii	Uncharacterized	5.923	26.86567	3	3
126	A0ABD1JQ28	ACEWY4_015862	Coilia grayii	Uncharacterized	5.589	21.55172	2	2
127	A0ABD1K0E4	ACEWY4_012348	Coilia grayii	Uncharacterized	5.586	10.85271	3	3
128	A0ABD1J0N5	ACEWY4_024217	Coilia grayii	Uncharacterized	5.561	8.695652	1	1
129	A0ABD1JB17	ACEWY4_019865	Coilia grayii	Uncharacterized	5.523	9.090909	2	2
130	A0ABD1J279	ACEWY4_024383	Coilia grayii	Uncharacterized	5.181	14.28571	2	2
131	A0ABD1J0I8	ACEWY4_024172	Coilia grayii	Uncharacterized	5.09	7.526882	1	1
132	A0ABD1K733	ACEWY4_009666	Coilia grayii	Uncharacterized	5.019	6.624606	2	1
133	A0ABD1KPW2	ACEWY4_002949	Coilia grayii	Uncharacterized	4.875	11.18012	2	2
134	A0ABD1KGK0	ACEWY4_007431	Coilia grayii	Uncharacterized	4.872	12.5	2	2
135	A0ABD1J2R6	ACEWY4_023337	Coilia grayii	Uncharacterized	4.835	5.445545	1	1
136	A0ABD1J1Z8	ACEWY4_024103	Coilia grayii	Uncharacterized	4.825	17.10526	3	3
137	A0ABD1JT49	ACEWY4_014705	Coilia grayii	Uncharacterized	4.805	4.72973	1	1
138	A0ABD1IZK3	ACEWY4_024170	Coilia grayii	Uncharacterized	4.688	6.926407	2	2
139	A0ABD1IWG7	ACEWY4_025056	Coilia grayii	Uncharacterized	4.67	7.727273	1	1
140	A0ABD1JTZ7	ACEWY4_015005	Coilia grayii	Uncharacterized	4.626	16.66667	2	2
141	A0ABD1K865	ACEWY4_010057	Coilia grayii	Uncharacterized	4.564	2.895323	1	1
142	A0ABD1K596	ACEWY4_009031	Coilia grayii	Uncharacterized	4.454	3.560831	1	1
143	A0ABD1KUL2	ACEWY4_002013	Coilia grayii	Uncharacterized	4.365	15.06849	2	2
144	A0ABD1J0X1	ACEWY4_024044	Coilia grayii	Uncharacterized	4.305	14.15929	1	1
145	A0ABD1JYW0	ACEWY4_011843	Coilia grayii	Uncharacterized	4.223	4.451039	1	1
146	A0ABD1KQE5	ACEWY4_003133	Coilia grayii	Uncharacterized	4.177	12.5	2	2
147	A0ABD1INT1	ACEWY4_027916	Coilia grayii	Uncharacterized	4.14	18.27411	4	4
148	A0ABD1J8Z1	ACEWY4_021365	Coilia grayii	Uncharacterized	4.104	15.48387	2	2
149	A0ABD1K3L4	ACEWY4_011034	Coilia grayii	Uncharacterized	4.095	5.46875	1	1
150	A0ABD1KHW5	ACEWY4_005296	Coilia grayii	Uncharacterized	4.081	16	3	3
151	A0ABD1IW75	ACEWY4_025871	Coilia grayii	Uncharacterized	3.973	11.84211	2	1
152	A0ABD1JYU5	ACEWY4_011844	Coilia grayii	Uncharacterized	3.952	3.254438	1	1
153	A0ABD1JDX1	ACEWY4_018594	Coilia grayii	Uncharacterized	3.872	11.04972	2	1
154	A0ABD1JIC3	ACEWY4_017558	Coilia grayii	Uncharacterized	3.829	3.550296	1	1
155	A0ABD1K654	ACEWY4_009313	Coilia grayii	Uncharacterized	3.824	4.938272	1	1
156	A0ABD1JL32	ACEWY4_017717	Coilia grayii	Uncharacterized	3.78	0.862441	2	2
157	A0ABD1KIR4	ACEWY4_005492	Coilia grayii	Uncharacterized	3.73	7.017544	2	1
158	A0ABD1IVA2	ACEWY4_024642	Coilia grayii	Uncharacterized	3.729	14.84375	2	1
159	A0ABD1J0E2	ACEWY4_025355	Coilia grayii	Uncharacterized	3.689	12.90323	2	1
160	A0ABD1KUR1	ACEWY4_002053	Coilia grayii	Uncharacterized	3.685	14.77273	3	3
161	A0ABD1KH44	ACEWY4_004992	Coilia grayii	Uncharacterized	3.672	9.160305	1	1
162	A0ABD1KWK7	ACEWY4_000412	Coilia grayii	Uncharacterized	3.646	2.539063	1	1
163	A0ABD1JCL0	ACEWY4_020420	Coilia grayii	Uncharacterized	3.604	12.8866	3	3

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164	A0ABD1JMV2	ACEWY4_016374	Coilia grayii	Uncharacterized	3.532	6.796117	1	1
165	A0ABD1J8Q8	ACEWY4_022234	Coilia grayii	Uncharacterized	3.516	12.32877	2	2
166	A0ABD1KKT0	ACEWY4_004198	Coilia grayii	Uncharacterized	3.374	4.545455	1	1
167	A0ABD1J4V6	ACEWY4_023198	Coilia grayii	Uncharacterized	3.335	6.225681	2	2
168	A0ABD1KDX7	ACEWY4_006612	Coilia grayii	Uncharacterized	3.272	4.587156	1	1
169	A0ABD1KBN8	ACEWY4_008715	Coilia grayii	Uncharacterized	3.226	15.15152	2	2
170	A0ABD1JMA5	ACEWY4_017027	Coilia grayii	Uncharacterized	3.21	1.794072	3	3
171	A0ABD1JEV4	ACEWY4_020109	Coilia grayii	Uncharacterized	3.122	5.761317	1	1
172	A0ABD1ITF3	ACEWY4_025936	Coilia grayii	Uncharacterized	3.058	1.528777	2	2
173	A0ABD1ISA0	ACEWY4_026988	Coilia grayii	Uncharacterized	2.876	5.045872	1	1
174	Q90W53	mlc3	Engraulis japonicus	Myosin light chain 3	2.832	6.395349	1	1
175	A0ABD1KN69	ACEWY4_002388	Coilia grayii	Uncharacterized	2.76	2.40481	1	1
176	A0ABD1K689	ACEWY4_009362	Coilia grayii	Uncharacterized	2.742	8.87574	1	1
177	A0ABD1KGB9	ACEWY4_007379	Coilia grayii	Uncharacterized	2.643	8.333333	1	1
178	A0ABD1ISK0	ACEWY4_025621	Coilia grayii	Uncharacterized	2.633	1.142857	1	1
179	A0ABD1KEE4	ACEWY4_006722	Coilia grayii	Uncharacterized	2.542	7.692308	2	2
180	A0ABD1J3Q6	ACEWY4_023602	Coilia grayii	Uncharacterized	2.533	2.844639	1	1
181	A0ABD1K4C1	ACEWY4_011298	Coilia grayii	Uncharacterized	2.511	0.515844	1	1
182	A0ABD1J242	ACEWY4_023116	Coilia grayii	Uncharacterized	2.493	0.6238	1	1
183	A0ABD1KNZ7	ACEWY4_002427	Coilia grayii	Uncharacterized	2.489	7.296137	2	2
184	A0ABD1K799	ACEWY4_009722	Coilia grayii	Uncharacterized	2.459	8.304498	1	1
185	A0ABD1IXK2	ACEWY4_025426	Coilia grayii	Uncharacterized	2.446	2.352941	2	1
186	A0ABD1JER4	ACEWY4_018989	Coilia grayii	Uncharacterized	2.325	4.126984	1	1
187	A0ABD1J4N7	ACEWY4_021962	Coilia grayii	Uncharacterized	2.311	7.518797	1	1
188	A0ABD1KTV1	ACEWY4_001735	Coilia grayii	Uncharacterized	2.292	8.270677	1	1
189	A0ABD1JM53	ACEWY4_016639	Coilia grayii	Uncharacterized	2.235	7.258065	1	1
190	A0ABD1K353	ACEWY4_010878	Coilia grayii	Uncharacterized	2.196	2.028398	1	1
191	A0ABD1KM24	ACEWY4_004435	Coilia grayii	Uncharacterized	2.173	7.971014	1	1
192	A0ABD1J064	ACEWY4_024342	Coilia grayii	Uncharacterized	2.109	6.716418	1	1
193	A0ABD1JVH6	ACEWY4_013093	Coilia grayii	Uncharacterized	2.036	5.882353	1	1
194	A0ABD1K580	ACEWY4_011380	Coilia grayii	Uncharacterized	2.017	3.342618	1	1
195	A0ABD1JST9	ACEWY4_014637	Coilia grayii	Uncharacterized	2.016	9.947644	1	1
196	A0ABD1JLH2	ACEWY4_017867	Coilia grayii	Uncharacterized	1.946	2.356021	1	1
197	A0ABD1K5W5	ACEWY4_009012	Coilia grayii	Uncharacterized	1.936	13.63636	1	1
198	A0ABD1IYL9	ACEWY4_023740	Coilia grayii	Uncharacterized	1.928	13.09524	1	1
199	A0ABD1K086	ACEWY4_012331	Coilia grayii	Uncharacterized	1.854	1.000834	1	1
200	A0ABD1KRF4	ACEWY4_003297	Coilia grayii	Uncharacterized	1.842	6.122449	1	1
201	A0ABD1KCI2	ACEWY4_005992	Coilia grayii	Uncharacterized	1.833	10.55276	1	1
202	A0ABD1JSG7	ACEWY4_014485	Coilia grayii	Uncharacterized	1.688	7.086614	1	1
203	A0ABD1JGN3	ACEWY4_017376	Coilia grayii	Uncharacterized	1.667	1.467269	1	1
204	A0ABD1J075	ACEWY4_024371	Coilia grayii	Uncharacterized	1.665	4.411765	1	1
205	A0ABD1KGM9	ACEWY4_007368	Coilia grayii	Uncharacterized	1.625	3.149606	1	1

No	Accession	Gene Names	Organisme	Status	Sum PEP Score	Coverage	Peptides	Unique peptides
206	A0ABD1KPN1	ACEWY4_002887	Coilia grayii	Uncharacterized	1.547	7.344633	1	1
207	A0ABD1KNX5	ACEWY4_002412	Coilia grayii	Uncharacterized	1.506	3.761062	1	1
208	A0ABD1JY61	ACEWY4_013916	Coilia grayii	Uncharacterized	1.492	5.594406	1	1
209	A0ABD1JB29	ACEWY4_019906	Coilia grayii	Uncharacterized	1.471	1.591896	1	1
210	A0ABD1K075	ACEWY4_012316	Coilia grayii	Uncharacterized	1.459	4.278075	1	1
211	A0ABD1KXX2	ACEWY4_000876	Coilia grayii	Uncharacterized	1.433	2.739726	1	1
212	A0ABD1KPX4	ACEWY4_002991	Coilia grayii	Uncharacterized	1.401	1.033592	1	1
213	A0ABD1KVZ3	ACEWY4_000197	Coilia grayii	Uncharacterized	1.345	3.76569	1	1
214	A0ABD1KQT1	ACEWY4_003057	Coilia grayii	Uncharacterized	1.338	7.142857	1	1
215	A0ABD1JWH4	ACEWY4_013346	Coilia grayii	Uncharacterized	1.33	6.923077	1	1
216	A0ABD1KCV7	ACEWY4_006238	Coilia grayii	Uncharacterized	1.327	2.254098	1	1
217	A0ABD1KND0	ACEWY4_002370	Coilia grayii	Uncharacterized	1.318	4.878049	1	1
218	A0ABD1K4J0	ACEWY4_011352	Coilia grayii	Uncharacterized	1.309	3.703704	1	1
219	A0ABD1K3J6	ACEWY4_011031	Coilia grayii	Uncharacterized	1.308	4.733728	1	1
220	A0ABD1KQW0	ACEWY4_003298	Coilia grayii	Uncharacterized	1.245	4.895105	1	1
221	A0ABD1JQ43	ACEWY4_015868	Coilia grayii	Uncharacterized	1.197	3.645833	1	1
222	A0ABD1J596	ACEWY4_022077	Coilia grayii	Uncharacterized	1.191	1.878914	1	1
223	A0ABD1K1G9	ACEWY4_010284	Coilia grayii	Uncharacterized	1.15	2.222222	1	1
224	A0ABD1KR41	ACEWY4_003400	Coilia grayii	Uncharacterized	1.123	6.21118	1	1
225	A0ABD1J3C2	ACEWY4_023222	Coilia grayii	Uncharacterized	1.112	1.465798	1	1
226	A0ABD1K770	ACEWY4_009679	Coilia grayii	Uncharacterized	1.043	2.409639	1	1
227	A0ABD1KBH7	ACEWY4_008524	Coilia grayii	Uncharacterized	1.023	4.464286	1	1
228	A0ABD1JAS6	ACEWY4_019792	Coilia grayii	Uncharacterized	1.011	8.130081	1	1
229	A0ABD1J6Z1	ACEWY4_020727	Coilia grayii	Uncharacterized	1.004	1.17096	1	1
230	A0ABD1JBH9	ACEWY4_020050	Coilia grayii	Uncharacterized	1	0.656045	1	1
231	A0ABD1IZD2	ACEWY4_024072	Coilia grayii	Uncharacterized	1	4.530744	1	1
232	A0ABD1IUQ5	ACEWY4_026054	Coilia grayii	Uncharacterized	0.997	12.7451	1	1
233	A0ABD1K9V5	ACEWY4_008066	Coilia grayii	Uncharacterized	0.983	2.723735	1	1
234	A0ABD1JTV4	ACEWY4_015009	Coilia grayii	Uncharacterized	0.958	13.04348	1	1
235	A0ABD1JDD8	ACEWY4_018412	Coilia grayii	Uncharacterized	0.957	4.137931	1	1
236	A0ABD1JQC6	ACEWY4_015940	Coilia grayii	Uncharacterized	0.945	6.756757	1	1
237	A0ABD1K6Y7	ACEWY4_009604	Coilia grayii	Uncharacterized	0.94	2.39521	1	1
238	A0ABD1KYV2	ACEWY4_001197	Coilia grayii	Uncharacterized	0.914	1.672241	1	1
239	A0ABD1JEE7	ACEWY4_018759	Coilia grayii	Uncharacterized	0.914	4.188482	1	1
240	A0ABD1J3N7	ACEWY4_023079	Coilia grayii	Uncharacterized	0.896	3.954802	1	1
241	A0ABD1J0Q6	ACEWY4_022636	Coilia grayii	Uncharacterized	0.877	4.661487	1	1
242	A0ABD1KCC0	ACEWY4_006042	Coilia grayii	Uncharacterized	0.867	0.95511	1	1
243	A0ABD1J206	ACEWY4_023083	Coilia grayii	Uncharacterized	0.858	4.040404	1	1
244	A0ABD1IY42	ACEWY4_023658	Coilia grayii	Uncharacterized	0.857	1.034208	1	1
245	A0ABD1KSM1	ACEWY4_001315	Coilia grayii	Uncharacterized	0.829	1.620029	1	1
246	A0A2H4PU25	365059	Coilia grayii	Uncharacterized	0.818	7.070707	1	1
247	A0ABD1KR66	ACEWY4_003423	Coilia grayii	Uncharacterized	0.811	3.478261	1	1
248	A0ABD1KRI9	ACEWY4_003134	Coilia grayii	Uncharacterized	0.805	0.995025	1	1

No	Accession	Gene Names	Organisme	Status	Sum PEP Score	Coverage	Peptides	Unique peptides
249	A0ABD1ISX4	ACEWY4_025780	Coilia grayii	Uncharacterized	0.794	0.196415	1	1
250	A0ABD1IQ00	ACEWY4_026576	Coilia grayii	Uncharacterized	0.78	0.804388	1	1
251	A0ABD1JNT2	ACEWY4_016689	Coilia grayii	Uncharacterized	0.77	1.687764	1	1
252	A0ABD1KDD8	ACEWY4_006349	Coilia grayii	Uncharacterized	0.769	4.554455	1	1
253	A0ABD1KM53	ACEWY4_004596	Coilia grayii	Uncharacterized	0.766	1.296296	1	1
254	A0ABD1J7W4	ACEWY4_021057	Coilia grayii	Uncharacterized	0.764	4.268293	1	1

b. Accession / Protein ID identified in processed anchovy samples.

No	Accession	Gene Names	Organisme	Status	Sum PEP Score	Coverage	Peptides	Unique Peptides
1	A0ABD1JWL9	ACEWY4_013515	Coilia grayii	Uncharacterized	61.096	13.43511	21	3
2	A0ABD1JWK7	ACEWY4_013535	Coilia grayii	Uncharacterized	60.874	11.66495	19	1
3	A0ABD1KN14	ACEWY4_004883	Coilia grayii	Uncharacterized	59.142	12.32373	18	0
4	A0ABD1J939	ACEWY4_022354	Coilia grayii	Uncharacterized	58.738	10.30397	19	2
5	A0ABD1JQU7	ACEWY4_016147	Coilia grayii	Uncharacterized	27.161	33.80282	12	8
6	A0ABD1JFA2	ACEWY4_018739	Coilia grayii	Uncharacterized	15.491	3.343621	5	1
7	A0ABD1KM45	ACEWY4_004653	Coilia grayii	Uncharacterized	8.396	13.38028	5	1
8	A0ABD1IYJ9	ACEWY4_023816	Coilia grayii	Uncharacterized	5.59	2.075612	3	3
9	A0ABD1IUL2	ACEWY4_026353	Coilia grayii	Uncharacterized	4.74	3.742204	3	3
10	A0ABD1JAD0	ACEWY4_019610	Coilia grayii	Uncharacterized	4.218	1.25448	1	1
11	A0ABD1JXM3	ACEWY4_013721	Coilia grayii	Uncharacterized	4.088	2.008032	3	3
12	A0ABD1KPS6	ACEWY4_002932	Coilia grayii	Uncharacterized	3.84	19.84127	3	3
13	A0ABD1ITZ7	ACEWY4_026034	Coilia grayii	Uncharacterized	3.371	9.549072	3	3
14	A0ABD1JIG3	ACEWY4_018021	Coilia grayii	Uncharacterized	3.265	7.719298	2	2
15	A0ABD1KC28	ACEWY4_008586	Coilia grayii	Uncharacterized	2.766	5.084746	1	1
16	A0ABD1JLG9	ACEWY4_016482	Coilia grayii	Uncharacterized	2.652	17.64706	2	2
17	A0ABD1KY90	ACEWY4_000991	Coilia grayii	Uncharacterized	2.508	10.625	1	1
18	A0ABD1KRP3	ACEWY4_003568	Coilia grayii	Uncharacterized	1.982	3.846154	1	1
19	A0ABD1JWX3	ACEWY4_013620	Coilia grayii	Uncharacterized	1.754	1.818182	1	1
20	A0ABD1KRB8	ACEWY4_003461	Coilia grayii	Uncharacterized	1.334	0.791855	1	1
21	A0ABD1J5R8	ACEWY4_022257	Coilia grayii	Uncharacterized	1.205	0.301122	1	1
22	A0ABD1K3K3	ACEWY4_011026	Coilia grayii	Uncharacterized	1.15	3.206997	1	1
23	A0ABD1JTV4	ACEWY4_015009	Coilia grayii	Uncharacterized	1.115	13.04348	1	1
24	A0ABD1KP75	ACEWY4_002693	Coilia grayii	Uncharacterized	1.109	0.561224	1	1
25	A0ABD1J7T3	ACEWY4_021030	Coilia grayii	Uncharacterized	0.991	5.769231	1	1
26	A0ABD1JHG8	ACEWY4_018886	Coilia grayii	Uncharacterized	0.961	0.481026	1	1
27	A0ABD1K6Z6	ACEWY4_009639	Coilia grayii	Uncharacterized	0.829	3.160271	1	1
28	A0ABD1J219	ACEWY4_023944	Coilia grayii	Uncharacterized	0.799	6.048387	1	1
29	A0ABD1KP80	ACEWY4_002722	Coilia grayii	Uncharacterized	0.753	2.137405	1	1
30	A0ABD1JVZ2	ACEWY4_013311	Coilia grayii	Uncharacterized	0.736	1.305057	1	1

c. Accession / Protein ID identified in fortified anchovy samples

No	Accession	Gene Names	Organisme	Status	Sum PEP Score	Coverage	Peptides	Unique Peptides
1	A0ABD1JWL9	ACEWY4_013515	Coilia grayii	Uncharacterized	32.157	10.2799	17	2
2	A0ABD1JWK7	ACEWY4_013535	Coilia grayii	Uncharacterized	31.461	9.455293	16	1
3	A0ABD1J939	ACEWY4_02235	Coilia grayii	Uncharacterized	30.765	9.47965	18	4
4	A0ABD1JQU7	ACEWY4_016147	Coilia grayii	Uncharacterized	14.701	21.12676	8	8
5	A0ABD1ITZ7	ACEWY4_026034	Coilia grayii	Uncharacterized	7.351	20.4244	6	3
6	A0ABD1K8C4	ACEWY4_010117	Coilia grayii	Uncharacterized	7.262	17.84777	5	5
7	A0ABD1IYJ9	ACEWY4_023816	Coilia grayii	Uncharacterized	5.141	1.408451	2	2
8	A0ABD1KNI6	ACEWY4_002500	Coilia grayii	Uncharacterized	3.336	12.4031	4	1
9	A0ABD1JAD0	ACEWY4_019610	Coilia grayii	Uncharacterized	3.239	1.88172	2	2
10	A0ABD1KRB8	ACEWY4_003461	Coilia grayii	Uncharacterized	3.066	0.791855	1	1
11	A0ABD1KRP3	ACEWY4_003568	Coilia grayii	Uncharacterized	2.536	3.846154	1	1
12	A0ABD1IUL2	ACEWY4_026353	Coilia grayii	Uncharacterized	2.398	3.950104	3	3
13	A0ABD1JXM3	ACEWY4_013721	Coilia grayii	Uncharacterized	2.348	1.405622	2	2
14	A0ABD1IT58	ACEWY4_025870	Coilia grayii	Uncharacterized	1.923	12.75168	2	2
15	A0ABD1J219	ACEWY4_023944	Coilia grayii	Uncharacterized	1.663	6.048387	1	1
16	A0ABD1K6Z6	ACEWY4_009639	Coilia grayii	Uncharacterized	1.635	3.160271	1	1
17	A0ABD1KN37	ACEWY4_002332	Coilia grayii	Uncharacterized	1.62	0.624133	1	1
18	A0ABD1JLG9	ACEWY4_016482	Coilia grayii	Uncharacterized	1.426	10.45752	1	1
19	Q9IB20	mlc2	Engraulis japonicus	Myosin light chain 2	1.425	5.232558	1	1
20	A0ABD1JTV4	ACEWY4_015009	Coilia grayii	Uncharacterized	1.172	13.04348	1	1
21	A0ABD1KAB2	ACEWY4_008234	Coilia grayii	Uncharacterized	1.149	4.313725	1	1
22	A0ABD1JQ28	ACEWY4_015862	Coilia grayii	Uncharacterized	1.058	10.34483	1	1
23	A0ABD1K3K3	ACEWY4_011026	Coilia grayii	Uncharacterized	0.938	3.206997	1	1
24	A0ABD1JHG8	ACEWY4_018886	Coilia grayii	Uncharacterized	0.666	0.481026	1	1
25	A0ABD1J7T3	ACEWY4_021030	Coilia grayii	Uncharacterized	0.64	5.769231	1	1

Table S2 Distribution of bioactive activities and peptide sequences identified in raw, processed, and fortified anchovy samples.

a. Distribution of Bioactive Activities and Peptide Sequences Identified in raw anchovy samples.

No	Activity	Number	Sequence peptides
1	dipeptidyl peptidase IV inhibitor	8872	GP, PP, MP, VA, MA, KA, LA, FA, AP, PA, LP, VP, LL, VV, HA, IPA, VPL, APG, IP, TP, WP, SP, FP, RP, KP, HP, YP, GPA, GA, IA, RA, WA, EP, NP, TA, QP, FL, WV, HL, EK, AL, SL, GL, VR, LPL, AA, PL, AIAV, IIAP, PPL, PPG, GPGA, WR, WK, WL, WI, WN, WM, WY, WT, WS, LW, MW, WE, WF, IQP, VGL, AW, YT, WG, AD, AE, AF, AG, AH, AS, AT, AV, AY, DN, DP, DQ, DR, EG, EH, EI, ES, ET, EV, EW, EY, FN, FQ, FR, GE, GF, GG, GH, GI, GV, GW, GY, HD, HE, HF, HH, HI, HR, HS, HT, HV, HW, HY, IH, II, IL, IM, IN, IQ, IR, IW, KE, KF, KG, KI, KK, KR, KS, KT, KV, KY, LH, LI, LM, LN, LT, LV, ME, MF, MG, MH, MI, MK, ML, MM, MN, MQ, MR, MV, MY, NA, ND, NE, NF, NG, NH, NL, NM, NN, NQ, NR, NT, NV, NW, NY,

No	Activity	Number	Sequence peptides
			PF, PG, PH, PI, PK, PM, PN, PQ, PS, PT, PV. PW, PY, QA, QD, QE, QF, QG, QH, QI, QL, QN, QQ, QS, QT, QV, QW, QY, RG, RH, RI, RK, RL, RM, RR, SF, SH, SI, SK, SV, SW, SY, TD, TE, TF, TG, TH, TI, TK, TL, TM, TN, TQ, TR, TS, TT, TV, TW, TY, VD, VE, VF, VG, VH, VI, VK, VL, VM, VN, VQ, VS, VT, VW, VY, WD, WH, YA, YD, YE, YF, YG, YH, YI, YK, YL, YM, YN, YQ, YR, YS, YV, YY, GPV, GPM, LPQ, VAPEEHPT, FF, IGL, VPTP, GPIN, GPVG, LPF, PGR, GPR, MAM, EYF, VC.
2	ACE inhibitor	6877	EA, DL, AA, EG, ER, LA, EI, EK, TE, GG, IE, AV, KK, EV, LEE, DA, VE, GS, AI, AK, KL, LQ, IA, TG, GK, GE, GE, AG, NK, YE, GA, VV, GI, LN, VG, GT, ST, GV, VK, GL, FL, LG, LR, GD, IL, QK, IP, IG, DG, SG, AR, KE, HL, DR, LP, AF, VP PA, AP, GQ, SY, FK, LF, PG, ME, PL, MM, LY, AH, EF, VF, QG, YA, IR, KR, VR, KA, GR, SF, TF, EY, PT, DY, TP LAA, YK, YQ, FG, DF, YV, RL, VY, GY, NF, IAE, TQ GP, GF, HP, RP, FR, DM, FQ, AEL, IF, MG, KP, IY, MF, FP, GM, PH, LEK, PR, YL, KG, YP, KY, PP, YG, YN, YS, IW, PQ, GTG, FF, YI, FY, GH, AY, HG, NY, VW, IPA, IWH, VM, GGY, VAA, NG, KF, RR, LVE, ASL, WL, LVL, GW, RA, LFR, DW, LW, LQQ, DLP, DGL, AVL, YH, QP, PM, WP, EW, ALP, IFL, AFL, FW, AVP, FAP, HY, LPG, AW, LAP, WG, HK, IAQ, EAP, GIL, GEF, ILP, MY, RF, VAP, PAP, IAP, LNF, LEF, VVF, VLY, RG, AGS, VVR, IIAPPER, IAF, VLP, DMIPAQK, GRP, IPP, LNP, FNQ, GPL, GPV, IRP, VFPS, YVP, NKL, FAL, VIPEL, LVY, GLY, VQV, AQL, LGI, YPG, VTR, VGP, WA, FGF, WT, LVS, PLP, IWHHT, IKP, LKL, FGK, VRP, GKP, LAY, AIP, LLP, VFK, PGL, IVY, LKP, LYP, YQY, LTF, TNP, ITT, GYK, YNK, LIY, VIY, VVRP, LLF, VAF, IQY, MYPGIA, IVF, IVGRPR, HQG, HHT, LPF, AGP, LVQ, FDK, RPP, FYN, GVY, WM, SGP, TGP, VVGP, TLS, AVV, DFY, GVR, VVL, NLR, PPL, SLR, YY, LPL, LQL, RPA, LPK, IWHHTFYNELR, VMP, IYP, GPA, ALPHA, GYALPHA, YALPHA, AAP, AKK, RY, LPP, TAP, ALPP, HIR, VPP, LVR, VAY, YGL, VPK, RPK, GTW, LGP, GLP, GEP, FFL, NPP, YGG, IYK, IEP, IQP, AFLL, VNP, VKP, FVP, FTTQ, IYEGY, IITNW, GSH, YVA, GKV, GPM, MW, MGP, DLGP, PPP, LDY, LGV, VHW, GHS, PFP, NPR, EQR, KLP, VAPEEHPV, LEEAF, LVF, YLR, YLL, ILKP, SRP, YRP, LGF, GPR, PGR, KKK, MAM, ILR, VC, WY.
3	antioxidative	1455	EL, LK, LT, VE, GA, HL, KD, MM, LY, AH, YA, IR, TY, YQ, VY, GP, LH, LEQQVDDLEGSLEQEKK, KP, IY, PH, KAI, YL, PK, FY, AY, VW, DIDDLELTLAK, HPH, PHF, EAK, GAA, GW, PY, LW, PEL, YF, TDY, AW, WG, DLEE, YNI, YVY, LHD, MY, YVGD, IIAPPER, ADF, LHF, LHL, YVL, PW, FGF, VFL, LAN, TIL, HH, IKK, WHH, YGY, LHM, LWG, PHK, IQY, LKP, TW, LLR, LPM, SVL, LPL, LQL, LFV, LAF, GPE, SGP, IVF, LLF, HLH, SDF, WY, DYY, YDY, YYE, HYY, YYF, YLY, YSY, LHI, LHK, LHN, LHQ, LHS, LWE, LWK, LWT, PHA, PHL, PHN, PHQ, PHR, PHS, PWI, PWL, PWV, RHV, GAH, KVI, TFE, VKP, WDDMEK, LDY, FIGP,

No	Activity	Number	Sequence peptides
			ELLI, QYP, GTW, YLL, YVE, NEN, DYK, VAPEEHPV, RDY, GSH, LEEAF, RY, AYI, LGF, YSQ, CW, VC.
4	neprilysin inhibitor	1215	EA, AE, ER, GG, KK, VE, AK, GE, VV, GD, AR, FG, GP, RP, FW, RG, GPA
5	glutamate carboxypeptidase II inhibitor	1062	EE, AE, DD, DA, DE, GE, YE, EEE, DF
6	stimulating	933	EE, SE, LL, VL, LV, IL, LI, IV, EEE, II, LLL, VPL
7	dipeptidyl peptidase III inhibitor	913	YY, LR, MR, YF, YH, YL, YK, YR, RR, TF, GE, GF, PR, RF, RV, DA, HL, HK, HF, HP, IH, LW, LA, FA, FR, FL, FM, PE, PF, WM, SM, YG, VY, YI, KA, GFL
8	glutamate carboxypeptidase inhibitor	688	EE, IE, GE, YE, FE, HE
9	inhibitor of tripeptidyl peptidase II	596	AA, VA, VV, AF, AP, MM, VF, VY, GY, GF, VM, GW, AAA, GVF, MY, GGF, APA
10	alpha-glucosidase inhibitor	553	EA, VE, AD, LR, PE, YP, PP, FY, VW, WH, EAK, IIAPPER, YPG, IPP, WS.
11	hypouricemic	474	LT, TL, LR, FK, TT, PT, TP, FR, VW, FVR, LW, YH, FW, GTL, ATL, FH, IAT, SITA, VGT
12	neuropeptide	404	EG, FL, IL, GQ, RP, LH, YL, YI, YR, RR, WL, PY, MH, KPT, KPS
13	hypotensive	391	AA, ER, TF, VY, GP, FY, GVR, IWHHTFYNELR, IVF, LLF, GHS, LGF
14	citrate lyase deacetylase inhibitor	366	EE
15	thymidylate synthase inhibitor	366	EE
16	renin inhibitor	353	LR, LY, EF, YA, IR, NR, SF, TF, QF, FT, KF, LW, WG, LPL, LQL, LEEAF, LGF
17	inhibitor of cytosol alanyl aminopeptidase	285	AA, LL, GGA, AAA, LLL, GGF
18	regulating	212	SL, PG, DY, GP, FW, GFL, GLY, PGP, GLF, LLF
19	antibacterial	175	AA, RR, YVL, IQY, EIPT, VALTGLTVAEYFR
20	lactocepin inhibitor	167	LL, LP, PL, LGG
21	tubulin-tyrosine ligase inhibitor	161	YE, YA, EY, YG, AY, YY
22	alanine carboxypeptidase inhibitor	159	GA, YA, HA, FA
23	D-Ala-D-Ala dipeptidase inhibitor	158	AA
24	binding	147	EG, TAT
25	activating ubiquitin-mediated proteolysis	143	LA, RA, WA

No	Activity	Number	Sequence peptides
26	bacterial permease ligand	94	KK, KKK
27	CaMPDE inhibitor	79	IR, AGA, KF, EF
28	antithrombotic	78	PG, GP, DEE, PGP, GPR
29	anticancer	68	LKK, YK, VVV, VWV, RPK
30	antiamnestic	66	PG, GP, VPL, PGP
31	immunostimulating	56	EAE, YG, GFL, GVM, KEEAE, YGG
32	neuroprotective	56	LR, LPI
33	toxic	55	LKK, YK, RPK
34	pancreatic lipase inhibitor	45	TF, EW, IIAPPER, SW, VAPEEHPV, ASF, AGY
35	peptidylprolyl isomerase inhibitor	40	AP
36	xaa-pro inhibitor	40	PL, VPL
37	PAM inhibitor	38	PG
38	hypolipidemic	35	EF
39	calpain 1 inhibitor	34	VF
40	neprilysin 2 inhibitor	34	VF
41	anti inflammatory	30	NLQ, PY, HY, IPP, ANP, LPF, VPP
42	HMG-CoA reductase inhibitor	29	GGV, IVG, IAF, QGF, QDF
43	acylaminoacyl peptidase inhibitor	22	GF
44	neurolysin inhibitor	21	RP
45	Leucyltransferase inhibitor	17	RR, RF, RG
46	ACE2 inhibitor	15	PF, IPP, LPP, VPP
47	alpha-amylase inhibitor	15	FY, IPP
48	immunomodulating	13	WL, GLF, DNIQGITKPAIR
49	pseudolysin inhibitor	13	FF
50	osteoanabolic	11	IPA, KSA
51	ACE 2 activator	10	IRP, IQY, ARF, LKP, IRY
52	antiartherosclerotic	10	WH
53	antiviral	10	RR, FFK
54	haemolytic	9	RR
55	phospholipase A2 inhibitor	8	PY
56	inhibitor	7	PGP, PPAP, GGYR, GAW
57	antidiabetic	6	VPV, LPVP, VPF, YPI
58	chemotactic	3	PGP

No	Activity	Number	Sequence peptides
59	alcohol dehydrogenase activator	1	PEF
	Total	**28193**	

b. Distribution of Bioactive Activities and Peptide Sequences Identified in processed anchovies samples.

No	Activity	Number	Sequence peptides
1	dipeptidyl peptidase IV inhibitor	865	AE, QL, VE, GP, LA, GA, AL, EI, GE, AG, EG, TL, VA, TA, AT, QG, ET, AA, NL, TG, ES, EV, LT, LV, QE, GG, YE, AS, PA, GPA, GL, NA, QA, SY, TE, VD, GPAG, DQ, LM, ME, MI, SK, DR, LN, QV, SL, AV, NQ, VQ, IQ, KE, KK, MN, PG, QI, QQ, QS, TD, GPR, HE, KR, MK, NM, NR, SI, VK, VV, IA, HL, EK, AD, GF, GV, PT, QD, QH, SV, VG, VL, VT, LP, VR, AF, GY, II, IM, IN, KF, LI, NE, NT, PQ, PV, RI, TH, TI, TK, TR, TV, VI, VN, YA, PP, MA, FA, AP, VP, LL, TP, SP, YP, WA, EP, FL, VLGP, PPG, AY, DN, EH, FN, GI, IL, KI, LH, MF, MR, ND, NF, NV, PI, PN, PS, QN, QT, RG, RR, SF, TN, TT, TY, VF, VS, VW, VY, YD, YK, YS, YV, GPV, IGL, VPTP, GPVG.
2	ACE inhibitor	766	EA, LQ, ER, DL, VE, LEE, GP, LA, GA, GE, EI, IE, AG, EG, QK, QG, AA, TG, AK, DA, EV, GG, YE, LG, GPA, GL, SY, TE, PA, ME, AEL, GK, LN, AGP, DR, PR, GQ, AV, LAA, IG, SG, PG, NK, AR, KE, GPR, KK, LQQ, VK, KR, GT, DG, LVE, AQL, ST, IA, GF, VG, HL, GS, GV, AI, GD, PT, EK, TGP, LR, VV, GY, AF, YA, GM, VR, KF, KL, PQ, ASL, QGP, GTG, EQR, EF, LP, LY, IY, VF, MF, LVL, VW, VY, VAA, LF, FY, FNQ, LAY, AY, YP, PGL, LGP, GLP, LPG, GPV, AP, VP, GI, GR, LIY, NF, SF, YK, RR, GPP, IAE, PP, VAF, YPG, VGP, DY, TP, DM, YV, IL, WA, SGP, EQGP, TLS, RG, LGV, VVL, AGDDAPR, GIL, FK, YS, FL.
3	neprilysin inhibitor	216	AE, AK, AR, EA, ER, GG, GD, GE, GP, GPA, KK, VV, RG, VE
4	glutamate carboxypeptidase II inhibitor	205	EE, AE, GE, DD, EEE, DE, DA, YE
5	antioxidative	167	EL, VE, GA, GP, LT, LK, HL, QGAR, YA, LH, AY, LY, IY, LHL, TY, VY, VW, GAA, GPP, DLEE, YVE, AGDDAPR, FY, SGP.
6	glutamate carboxypeptidase inhibitor	143	FE, YE, EE, GE, HE, IE
7	stimulating	130	EEE, VL, LV, IV, IL, LI, II, LL, EE, SE
8	thymidylate synthase inhibitor	80	EE
9	dipeptidyl peptidase III inhibitor	80	LA, GE, DA, PR, LR, GF, HL, MR, YK, RR, FA, FL, FM, SM, VY
10	citrate lyase deacetylase inhibitor	80	EE
11	alpha-glucosidase inhibitor	71	VW, YP, YPG, EA, PP, VE, AD, AGDDAPR, LR, FY

No	Activity	Number	Sequence peptides
12	hypotensive	65	ER, GP, AA, FY, VY
13	inhibitor of tripeptidyl peptidase II	43	VA, VF, VY, GF, AA, AF, AP, GVF, GY, VV
14	hypouricemic	43	TL, LT, LR, PT, FK, VW, IAT, ATL, GTL, TP, TT
15	regulating	38	DY, GP, PG, GPGG, PGP, SL
16	antithrombotic	38	GPR, GP, PGP, PG, DEE, GPGG
17	antiamnestic	31	GPGG, PGP, PG, GP
18	neuropeptide	28	GQ, RR, IL, EG, FL, LH
19	alanine carboxypeptidase inhibitor	26	YA, GA, FA
20	activating ubiquitin-mediated proteolysis	24	LA, WA
21	binding	20	EG, TAT
22	inhibitor of cytosol alanyl aminopeptidase	19	AA, GGA, LL
23	renin inhibitor	15	LR, KF, EF, NR, SF, YA, LY
24	tubulin-tyrosine ligase inhibitor	14	AY, YA, YE
25	antibacterial	14	RR, AA
26	D-Ala-D-Ala dipeptidase inhibitor	13	AA
27	immunostimulating	12	EAE
28	CaMPDE inhibitor	8	AGA, KF, EF
29	PAM inhibitor	5	PG
30	bacterial permease ligand	5	KK
31	anti inflammatory	4	NLQ
32	neuroprotective	3	LR
33	lactocepin inhibitor	3	LL, LP
34	acylaminoacyl peptidase inhibitor	3	GF
35	Leucyltransferase inhibitor	2	RG, RR
36	inhibitor	2	PGP
37	hypolipidemic	2	EF
38	HMG-CoA reductase inhibitor	2	GGV
39	chemotactic	2	PGP
40	toxic	1	YK
41	peptidylprolyl isomerase isnhibitor	1	AP
42	pancreatic lipase inhibitor	1	AGDDAPR

No	Activity	Number	Sequence peptides
43	neprilysin 2 inhibitor	1	VF
44	haemolytic	1	RR
45	calpain 1 inhibitor	1	VF
46	antiviral	1	RR
47	anticancer	1	YK
48	alpha-amylase inhibitor	1	FY
49	alcohol dehydrogenase activator	1	AGFAGDDAPR
	Total	**3297**	

c. Distribution of Bioactive Activities and Peptide Sequences Identified in fortified anchovy samples.

No	Activity	Number	Sequence peptides
1	dipeptidyl peptidase IV inhibitor	703	VG, GL, ET, KV, LM, ME, VI, VR, GG, LN, NA, NL, QA, QS, QV, SV, TE, VN, GPR, HL, DR, KK, LH, PS, PT, QD, QQ, VF, AP, LP, SL, AD, AF, AH, GF, GV, GY, KR, NM, NR, NT, PG, PV, QT, TD, TK, TT, VL, YV, MA, LL, VV, FP, RP, IA, EK, DP, II, IQ, NE, MQ, PN, PQ, SF, TI, YS, PP, VP, TP, HP, RA, WA, EP, NP, VLGP, PL, PPG, AY, DN, EH, FN, FR, GI, HE, IL, IM, IN, KE, KF, KI, LI, MK, MR, NF, NV, NY, PI, PK, QH, RG, RI, RR, TM, TV, VK, VS, VT, VW, VY, YG, YK, YL, GPV, VAPEEHPT, FF, IGL, VPTP, GPVG.
2	ACE inhibitor	614	GP, EA, ER, LQ, DL, DA, VE, GE, GA, AG, QG, LA, TG, EG, SY, EV, AV, LEE, GPA, IG, IE, YE, AK, PA, QK, EI, AGP, PR, AA, VG, GL, ME, AQL, GG, SG, VR, TE, LN, GPR, VF, LAA, HL, HL, GQ, GD, DG, PT, TGP, ST, DR, KK, GY, AF, AP, KR, GF, GM, GR, GV, GK, PG, KL, AR, AH, YV, AEL, EF, LP, GRP, FP, VFPS, IA, RP, GS, GT, LG, SF, LVE, PQ, EK, ASL, IVGRPR, QGP, GTG, LR, EQR, VV, YS, LVL, VW, VY, VAA, VAP, LNP, YL, YG, FY, FNQ, LAY, AY, LQQ, LGP, GLP, LPG, PL, GPV, VK, LAP, VP, RA, FR, GI, AI, NY, NF, KF, YK, RR, GPP, PP, KE, HP, VAF, LVY, LNF, IAQ, EAP, VGP, TP, IL, WA, SGP, EQGP, RG, LGV, VVL, FF, GIL, GEF, FK.
3	neprilysin inhibitor	167	AE, EA, ER, GP, VE. GE, AK, GPA, GG, GD, KK, AR, VV, RP, RG.
4	glutamate carboxypeptidase II inhibitor	147	EE, AE, DA, GE, DD, YE, DE, EEE
5	antioxidative	137	EL, GP, VE, GA, LK, LT, LH, HL, LHL, AH, YVGD, AY, QGAR, VY, VW, GAA, GPP, DLEE, FY, YL, PK, SGP
6	stimulating	89	EE, LV, SE, EEE, VL, IV, II, LL, IL, LI
7	glutamate carboxypeptidase inhibitor	84	EE, GE, YE, IE, FE, HE

No	Activity	Number	Sequence peptides
8	dipeptidyl peptidase III inhibitor	74	DA, GE, LA, PR, HL, GF. LR, MR, YL, YK, RR, HP, FR, FM, PE, YG, VY,
9	hypotensive	55	ER, GP, AA, FY, VY
10	alpha-glucosidase inhibitor	50	EA, VE, AD, LR, VW, PP, PE, FY
11	citrate lyase deacetylase inhibitor	45	EE
12	inhibitor of tripeptidyl peptidase II	45	VA, AA, VF, GF, AF, AP, GY, VV, VY, GVF
13	thymidylate synthase inhibitor	45	EE
14	hypouricemic	40	TL, LT, PT, TT, LR, FK, FR, VW, TP
15	antithrombotic	34	GP, GPR. PG, PGP, DEE, GPGG
16	regulating	31	GP, PG, SL, GPGG, PGP
17	antiamnestic	28	GP, PG, GPGG, PGP
18	neuropeptide	23	EG, GQ, LH, RP, YL, RR, IL
19	activating ubiquitin-mediated proteolysis	14	LA, RA, WA
20	alanine carboxypeptidase inhibitor	14	GA
21	binding	12	EG, TAT
22	renin inhibitor	12	EF, NR, LR, SF, FT, KF
23	tubulin-tyrosine ligase inhibitor	11	YE, YG, AY
24	inhibitor of cytosol alanyl aminopeptidase	9	AA, LL
25	antibacterial	8	RR, AA
26	CaMPDE inhibitor	7	EF, AGA, KF
27	D-Ala-D-Ala dipeptidase inhibitor	7	AA
28	lactocepin inhibitor	6	LP, LL, PL
29	bacterial permease ligand	4	KK
30	calpain 1 inhibitor	4	VF
31	HMG-CoA reductase inhibitor	4	IVG

No	Activity	Number	Sequence peptides
32	immunostimulating	4	EAE, YG
33	neprilysin 2 inhibitor	4	VF
34	acylaminoacyl peptidase inhibitor	3	GF
35	hypolipidemic	3	EF
36	PAM inhibitor	3	PG
37	peptidylprolyl isomerase isnhibitor	3	AP
38	Leucyltransferase inhibitor	2	RG, RR
39	neurolysin inhibitor	2	RP
40	neuroprotective	2	LR
41	alpha-amylase inhibitor	1	FY
42	anticancer	1	YK
43	antiviral	1	RR
44	chemotactic	1	PGP
45	haemolytic	1	RR
46	inhibitor	1	PGP
47	pseudolysin inhibitor	1	FF
48	toxic	1	YK
49	xaa-pro inhibitor	1	PL
	Total	**2558**	

Table S3 Distribution of Bioactive Peptides Based on Total Activity in raw, processed, and fortified anchovy samples.

a. Distribution of Bioactive Peptides Based on Total Activity in raw anchovy samples.

No	Total activities	Sequences peptide		Activity type	Occurens
		Number	Name		
1	1 aktivitas	1911	DL, AK, GS, AI, KL, LQ, GK, NK, GT, ST, LG, QK, DG, IG, SG, LF, GR, DM, IF, GM, HG, DW, LEE, LAA, IAE, AEL, LEK, GTG, IWH, ASL, GGY, LVE, VAA, LFR, LVL, AVL, DGL, DLP, LQQ, AFL, ALP, IFL, AVP, EAP, FAP, GEF, GIL, IAQ, LAP, LPG, AGS, IAP, ILP, LEF, LNF,	ACE inhibitor	165

No	Total activities	Sequences peptide		Activity type	Occurens
		Number	Name		
			PAP, VAP, VLY, VVF, VVR, AQL, FAL, FNQ, GPL, GRP, LGI, LNP, LVS, LVY, NKL, VGP, VLP, VQV, VTR, YVP, AGP, AIP, AVV, DFY, FDK, FGK, FYN, GKP, GVY, GYK, HHT, HQG, IKP, ITT, IVY, LAY, LIY, LKL, LLP, LPK, LTF, LVQ, LYP, NLR, PGL, PLP, RPA, RPP, SLR, TGP, TLS, TNP, VAF, VFK, VIY, VRP, VVL, YNK, AAP, AKK, EQR, FFL, FVP, GEP, GKV, GLP, HIR, IEP, ILR, IYK, IYP, KLP, LGP, LGV, LVF, LVR, MGP, NPP, NPR, PFP, PPP, SRP, TAP, VAY, VHW, VMP, VNP, VPK, YGL, YLR, YRP, YVA, VFPS, VVGP, VVRP, AFLL, ALPP, DLGP, FTTQ, ILKP, VIPEL, IWHHT, ALPHA, IITNW, IYEGY, IVGRPR, MYPGIA, YALPHA, DMIPAQK, GYALPHA		
	1 aktivitas	3472	AL, QE, VD, TA, NL, QV, ES, QL, AT, VT, ET, AS, TD, TV, NA, QQ, SK, VN, TK, VI, MA, QT, SV, QI, SI, DQ, NV, VS, IQ, IN, NT, TI, VQ, MN, QS, ND, DN, NE, QA, KV, LM, MK, MV, PV, DP, QN, QH, EP, TS, NQ, NN, TR, YD, NM, NP, PI, PS, QD, TM, VH, PN, MI, KT, IM, ML, EH, MQ, QY, WV, FN, HV, TH, WR, KS, HI, HT, HD, QW, RK, YT, HR, SH, HS, MP, KI, NW, SP, WI, YM, NH, RI, WK, WN, WE, HW, RH, RM, WD, WF, VGL, APG, EYF, IGL, LPQ, PPG, IIAP, GPIN, VPTP, AIAV, GPGA, GPVG, VAPEEHPT.	dipeptidyl peptidase IV inhibitor	113
	1 aktivitas	633	EL, LK, KD, CW, KAI, HPH, PHF, GAA, PEL, TDY, YNI, LHD, YVY, ADF, LAN, LHF,	antioxidative	74

No	Total activities	Sequences peptide		Activity type	Occurens
		Number	Name		
			LHL, TIL, VFL, GPE, IKK, LAF, LFV, LHM, LLR, LPM, LWG, PHK, SVL, WHH, AYI, DYK, DYY, GAH, HLH, HYY, KVI, LHI, LHK, LHN, LHQ, LHS, LWE, LWK, LWT, NEN, PHA, PHL, PHN, PHQ, PHR, PHS, PWI, PWL, PWV, QYP, RDY, RHV, SDF, TFE, YDY, YLY, YSQ, YSY, YVE, YYE, YYF, DLEE, YVGD, ELLI, FIGP, WDDMEK, DIDDLELLTLAK, LEQQVDDLEGSLEQEKK		
	1 aktivitas	29	FH, FVR, GTL, ATL, IAT, VGT, SITA	hypouricemic	7
	1 aktivitas	6	VPV, VPF, YPI, LPVP	antidiabetic	4
	1 aktivitas	25	GGV, QGF, IVG, QDF	HMG-CoA reductase inhibitor	4
	1 aktivitas	20	SM, FM, RV	dipeptidyl peptidase III inhibitor	3
	1 aktivitas	37	EAE, GVM, KEEAE	immunostimulating	3
	1 aktivitas	4	GAW, PPAP, GGYR	inhibitor	3
	1 aktivitas	3	ARF, IRY	ACE 2 activator	2
	1 aktivitas	11	NLQ, ANP	anti inflammatory	2
	1 aktivitas	3	EIPT, VALTGLTVAEYFR	antibacterial	2
	1 aktivitas	13	VVV, VWV	anticancer	2
	1 aktivitas	193	DD, DE	glutamate carboxypeptidase II inhibitor	2
	1 aktivitas	8	GVF, APA	inhibitor of tripeptidyl peptidase II	2
	1 aktivitas	3	KPT, KPS	neuropeptide	2
	1 aktivitas	2	AGY, ASF	pancreatic lipase inhibitor	2
	1 aktivitas	140	SE, IV	stimulating	2
	1 aktivitas	1	PEF	alcohol dehydrogenase activator	1
	1 aktivitas	14	DEE	antithrombotic	1
	1 aktivitas	1	FFK	antiviral	1
	1 aktivitas	7	TAT	binding	1
	1 aktivitas	4	AGA	CaMPDE inhibitor	1
	1 aktivitas	45	FE	glutamate carboxypeptidase inhibitor	1
	1 aktivitas	2	DNIQGITKPAIR	immunomodulating	1

No	Total activities	Sequences peptide		Activity type	Occurens
		Number	Name		
	1 aktivitas	28	GGA	inhibitor of cytosol alanyl aminopeptidase	1
	1 aktivitas	3	LGG	lactocepin inhibitor	1
	1 aktivitas	1	LPI	neuroprotective	1
	1 aktivitas	1	KSA	osteoanabolic	1
	1 aktivitas	21	FT	renin inhibitor	1
2	2 aktivitas	3718	EI, EK, TE, AV, EV, IA, TG, AG, GI, LN, VG, GV, GL, VK, IP, KE, DR, PA, VP, SY, ME, QG, KR, VR, YV, NF, RL, TQ, FQ, MG, FP, MF, KG, KY, YN, YS, IW, PQ, GH, NY, NG, PM, QP, WP, WT, MW, GPV, PPL, GPM, IQP, MAM, PGR.	ACE inhibitor, dipeptidyl peptidase IV inhibitor	52
	2 aktivitas	64	IY, RY, FGF, SGP, YQY, GSH, GTW, LDY, VKP, YLL	ACE inhibitor, antioxidative	10
	2 aktivitas	100	TY, PK, PW, HH, TW	antioxidative, dipeptidyl peptidase IV inhibitor	5
	2 aktivitas	474	VL, LV, LI, II	dipeptidyl peptidase IV inhibitor, stimulating	4
	2 aktivitas	10	GVR, GHS, IWHHTFYNELR	ACE inhibitor, hypotensive	3
	2 aktivitas	246	GD, AR, FG	ACE inhibitor, neprilysin inhibitor	3
	2 aktivitas	118	HF, IH, MR	dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor	3
	2 aktivitas	42	PR, HK	ACE inhibitor, dipeptidyl peptidase III inhibitor	2
	2 aktivitas	60	DY, GLY	ACE inhibitor, regulating	2
	2 aktivitas	156	AD, WS	alpha-glucosidase inhibitor, dipeptidyl peptidase IV inhibitor	2
	2 aktivitas	268	TL, TT	dipeptidyl peptidase IV inhibitor, hypouricemic	2
	2 aktivitas	116	NR, QF	dipeptidyl peptidase IV inhibitor, renin inhibitor	2
	2 aktivitas	20	AAA, GGF	inhibitor of cytosol alanyl aminopeptidase, inhibitor of tripeptidyl peptidase II	2
	2 aktivitas	6	IRP	ACE 2 activator, ACE inhibitor	1
	2 aktivitas	2	LPP	ACE inhibitor, ACE2 inhibitor	1
	2 aktivitas	6	YPG	ACE inhibitor, alpha-glucosidase inhibitor	1

No	Total activities	Sequences peptide		Activity type	Occurens
		Number	Name		
	2 aktivitas	2	KKK	ACE inhibitor, bacterial permease ligand	1
	2 aktivitas	50	DF	ACE inhibitor, glutamate carboxypeptidase II inhibitor	1
	2 aktivitas	192	IE	ACE inhibitor, glutamate carboxypeptidase inhibitor	1
	2 aktivitas	8	IAF	ACE inhibitor, HMG-CoA reductase inhibitor	1
	2 aktivitas	78	FK	ACE inhibitor, hypouricemic	1
	2 aktivitas	2	YGG	ACE inhibitor, immunostimulating	1
	2 aktivitas	80	GQ	ACE inhibitor, neuropeptide	1
	2 aktivitas	58	HA	alanine carboxypeptidase inhibitor, dipeptidyl peptidase IV inhibitor	1
	2 aktivitas	18	EAK	alpha-glucosidase inhibitor, antioxidative	1
	2 aktivitas	104	PE	alpha-glucosidase inhibitor, dipeptidyl peptidase III inhibitor	1
	2 aktivitas	6	YVL	antibacterial, antioxidative	1
	2 aktivitas	56	LKK	anticancer, toxic	1
	2 aktivitas	58	HE	dipeptidyl peptidase IV inhibitor, glutamate carboxypeptidase inhibitor	1
	2 aktivitas	208	VA	dipeptidyl peptidase IV inhibitor, inhibitor of tripeptidyl peptidase II	1
	2 aktivitas	6	MH	dipeptidyl peptidase IV inhibitor, neuropeptide	1
	2 aktivitas	6	SW	dipeptidyl peptidase IV inhibitor, pancreatic lipase inhibitor	1
	2 aktivitas	210	SL	dipeptidyl peptidase IV inhibitor, regulating	1
	2 aktivitas	92	EEE	glutamate carboxypeptidase II inhibitor, stimulating	1
	2 aktivitas	4	GLF	immunomodulating, regulating	1
	2 aktivitas	10	LLL	inhibitor of cytosol alanyl aminopeptidase, stimulating	1
	3 aktivitas	315	AW, AH, YQ, KP, PH, VC, WY	ACE inhibitor, antioxidative, dipeptidyl peptidase IV inhibitor	7
	3 aktivitas	117	LY, LQL, LEEAF	ACE inhibitor, antioxidative, renin inhibitor	3

No	Total activities	Sequences peptide		Activity type	Occurens
		Number	Name		
	3 aktivitas	162	KA, HP, WM	ACE inhibitor, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor	3
	3 aktivitas	225	AF, GY, VM	ACE inhibitor, dipeptidyl peptidase IV inhibitor, inhibitor of tripeptidyl peptidase II	3
	3 aktivitas	33	RA, WA	ACE inhibitor, activating ubiquitin-mediated proteolysis, dipeptidyl peptidase IV inhibitor	2
	3 aktivitas	90	PP, YP	ACE inhibitor, alpha-glucosidase inhibitor, dipeptidyl peptidase IV inhibitor	2
	3 aktivitas	21	HY, LPF	ACE inhibitor, anti inflammatory, dipeptidyl peptidase IV inhibitor	2
	3 aktivitas	162	PT, TP	ACE inhibitor, dipeptidyl peptidase IV inhibitor, hypouricemic	2
	3 aktivitas	303	GG, GPA	ACE inhibitor, dipeptidyl peptidase IV inhibitor, neprilysin inhibitor	2
	3 aktivitas	6	LKP	ACE 2 activator, ACE inhibitor, antioxidative	1
	3 aktivitas	3	VPP	ACE inhibitor, ACE2 inhibitor, anti inflammatory	1
	3 aktivitas	600	EA	ACE inhibitor, alpha-glucosidase inhibitor, neprilysin inhibitor	1
	3 aktivitas	3	RPK	ACE inhibitor, anticancer, toxic	1
	3 aktivitas	6	IVF	ACE inhibitor, antioxidative, hypotensive	1
	3 aktivitas	3	VAPEEHPV	ACE inhibitor, antioxidative, pancreatic lipase inhibitor	1
	3 aktivitas	3	GPR	ACE inhibitor, antithrombotic, dipeptidyl peptidase IV inhibitor	1
	3 aktivitas	267	DA	ACE inhibitor, dipeptidyl peptidase III inhibitor, glutamate carboxypeptidase II inhibitor	1
	3 aktivitas	12	RF	ACE inhibitor, dipeptidyl peptidase III inhibitor, Leucyltransferase inhibitor	1

No	Total activities	Sequences peptide		Activity type	Occurens
		Number	Name		
	3 aktivitas	129	LP	ACE inhibitor, dipeptidyl peptidase IV inhibitor, lactocepin inhibitor	1
	3 aktivitas	30	IPA	ACE inhibitor, dipeptidyl peptidase IV inhibitor, osteoanabolic	1
	3 aktivitas	18	EW	ACE inhibitor, dipeptidyl peptidase IV inhibitor, pancreatic lipase inhibitor	1
	3 aktivitas	39	FF	ACE inhibitor, dipeptidyl peptidase IV inhibitor, pseudolysin inhibitor	1
	3 aktivitas	87	SF	ACE inhibitor, dipeptidyl peptidase IV inhibitor, renin inhibitor	1
	3 aktivitas	81	EY	ACE inhibitor, dipeptidyl peptidase IV inhibitor, tubulin-tyrosine ligase inhibitor	1
	3 aktivitas	408	ER	ACE inhibitor, hypotensive, neprilysin inhibitor	1
	3 aktivitas	30	PF	ACE2 inhibitor, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor	1
	3 aktivitas	78	FA	alanine carboxypeptidase inhibitor, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor	1
	3 aktivitas	30	WH	alpha-glucosidase inhibitor, antiartherosclerotic, dipeptidyl peptidase IV inhibitor	1
	3 aktivitas	18	YF	antioxidative, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor	1
	3 aktivitas	336	LT	antioxidative, dipeptidyl peptidase IV inhibitor, hypouricemic	1
	3 aktivitas	60	LH	antioxidative, dipeptidyl peptidase IV inhibitor, neuropeptide	1
	3 aktivitas	36	YR	dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, neuropeptide	1

No	Total activities	Sequences peptide		Activity type	Occurens
		Number	Name		
	3 aktivitas	12	GFL	dipeptidyl peptidase III inhibitor, immunostimulating, regulating	1
	3 aktivitas	573	AE	dipeptidyl peptidase IV inhibitor, glutamate carboxypeptidase II inhibitor, neprilysin inhibitor	1
3	4 aktivitas	196	MY, MM, GW	ACE inhibitor, antioxidative, dipeptidyl peptidase IV inhibitor, inhibitor of tripeptidyl peptidase II	3
	4 aktivitas	28	WG, LPL	ACE inhibitor, antioxidative, dipeptidyl peptidase IV inhibitor, renin inhibitor	2
	4 aktivitas	112	YH, FR	ACE inhibitor, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, hypouricemic	2
	4 aktivitas	284	FL, YI	ACE inhibitor, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, neuropeptide	2
	4 aktivitas	8	IQY	ACE 2 activator, ACE inhibitor, antibacterial, antioxidative	1
	4 aktivitas	528	LA	ACE inhibitor, activating ubiquitin-mediated proteolysis, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor	1
	4 aktivitas	288	GA	ACE inhibitor, alanine carboxypeptidase inhibitor, antioxidative, dipeptidyl peptidase IV inhibitor	1
	4 aktivitas	16	IIAPPER	ACE inhibitor, alpha-glucosidase inhibitor, antioxidative, pancreatic lipase inhibitor	1
	4 aktivitas	176	HL	ACE inhibitor, antioxidative, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor	1
	4 aktivitas	44	AY	ACE inhibitor, antioxidative, dipeptidyl peptidase IV inhibitor, tubulin-tyrosine ligase inhibitor	1
	4 aktivitas	8	LLF	ACE inhibitor, antioxidative, hypotensive, regulating	1

No	Total activities	Sequences peptide		Activity type	Occurens
		Number	Name		
	4 aktivitas	4	LGF	ACE inhibitor, antioxidative, hypotensive, renin inhibitor	1
	4 aktivitas	372	KK	ACE inhibitor, bacterial permease ligand, dipeptidyl peptidase IV inhibitor, neprilysin inhibitor	1
	4 aktivitas	560	EG	ACE inhibitor, binding, dipeptidyl peptidase IV inhibitor, neuropeptide	1
	4 aktivitas	36	KF	ACE inhibitor, CaMPDE inhibitor, dipeptidyl peptidase IV inhibitor, renin inhibitor	1
	4 aktivitas	140	EF	ACE inhibitor, CaMPDE inhibitor, hypolipidemic, renin inhibitor	1
	4 aktivitas	8	YY	ACE inhibitor, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, tubulin-tyrosine ligase inhibitor	1
	4 aktivitas	36	WL	ACE inhibitor, dipeptidyl peptidase IV inhibitor, immunomodulating, neuropeptide	1
	4 aktivitas	288	VV	ACE inhibitor, dipeptidyl peptidase IV inhibitor, inhibitor of tripeptidyl peptidase II, neprilysin inhibitor	1
	4 aktivitas	160	AP	ACE inhibitor, dipeptidyl peptidase IV inhibitor, inhibitor of tripeptidyl peptidase II, peptidylprolyl isomerase inhibitor	1
	4 aktivitas	148	PL	ACE inhibitor, dipeptidyl peptidase IV inhibitor, lactocepin inhibitor, xaa-pro inhibitor	1
	4 aktivitas	16	RG	ACE inhibitor, dipeptidyl peptidase IV inhibitor, Leucyltransferase inhibitor, neprilysin inhibitor	1
	4 aktivitas	208	IL	ACE inhibitor, dipeptidyl peptidase IV inhibitor, neuropeptide, stimulating	1

No	Total activities	Sequences peptide		Activity type	Occurens
		Number	Name		
	4 aktivitas	24	FW	ACE inhibitor, hypouricemic, neprilysin inhibitor, regulating	1
	4 aktivitas	12	VPL	antiamnestic, dipeptidyl peptidase IV inhibitor, stimulating, xaa-pro inhibitor	1
	4 aktivitas	336	LL	dipeptidyl peptidase IV inhibitor, inhibitor of cytosol alanyl aminopeptidase, lactocepin inhibitor, stimulating	1
	≥ 5 aktivitas	15	IPP	ACE inhibitor, ACE2 inhibitor, alpha-amylase inhibitor, alpha-glucosidase inhibitor, anti inflammatory	1
	≥ 5 aktivitas	110	GF	ACE inhibitor, acylaminoacyl peptidase inhibitor, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, inhibitor of tripeptidyl peptidase II	1
	≥ 5 aktivitas	60	FY	ACE inhibitor, alpha-amylase inhibitor, alpha-glucosidase inhibitor, antioxidative, hypotensive	1
	≥ 5 aktivitas	50	VW	ACE inhibitor, alpha-glucosidase inhibitor, antioxidative, dipeptidyl peptidase IV inhibitor, hypouricemic	1
	≥ 5 aktivitas	435	VE	ACE inhibitor, alpha-glucosidase inhibitor, antioxidative, dipeptidyl peptidase IV inhibitor, neprilysin inhibitor	1
	≥ 5 aktivitas	130	YK	ACE inhibitor, anticancer, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, toxic	1
	≥ 5 aktivitas	155	IR	ACE inhibitor, antioxidative, CaMPDE inhibitor, dipeptidyl peptidase IV inhibitor, renin inhibitor	1
	≥ 5 aktivitas	80	YL	ACE inhibitor, antioxidative, dipeptidyl peptidase III inhibitor,	1

No	Total activities	Sequences peptide		Activity type	Occurens
		Number	Name		
				dipeptidyl peptidase IV inhibitor, neuropeptide	
	≥ 5 aktivitas	170	VF	ACE inhibitor, calpain 1 inhibitor, dipeptidyl peptidase IV inhibitor, inhibitor of tripeptidyl peptidase II, neprilysin 2 inhibitor	1
	≥ 5 aktivitas	70	YG	ACE inhibitor, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, immunostimulating, tubulin-tyrosine ligase inhibitor	1
	≥ 5 aktivitas	375	YE	ACE inhibitor, dipeptidyl peptidase IV inhibitor, glutamate carboxypeptidase II inhibitor, glutamate carboxypeptidase inhibitor, tubulin-tyrosine ligase inhibitor	1
	≥ 5 aktivitas	105	RP	ACE inhibitor, dipeptidyl peptidase IV inhibitor, neprilysin inhibitor, neurolysin inhibitor, neuropeptide	1
	≥ 5 aktivitas	40	PY	anti inflammatory, antioxidative, dipeptidyl peptidase IV inhibitor, neuropeptide, phospholipase A2 inhibitor	1
	≥ 5 aktivitas	15	PGP	antiamnestic, antithrombotic, chemotactic, inhibitor, regulating	1
	≥ 5 aktivitas	1830	EE	citrate lyase deacetylase inhibitor, glutamate carboxypeptidase II inhibitor, glutamate carboxypeptidase inhibitor, stimulating, thymidylate synthase inhibitor	1
	≥ 5 aktivitas	192	YA	ACE inhibitor, alanine carboxypeptidase inhibitor, antioxidative, dipeptidyl peptidase IV inhibitor, renin inhibitor, tubulin-tyrosine ligase inhibitor	1
	≥ 5 aktivitas	330	LR	ACE inhibitor, alpha-glucosidase inhibitor, dipeptidyl peptidase III inhibitor,	1

No	Total activities	Sequences peptide		Activity type	Occurens
		Number	Name		
				hypouricemic, neuroprotective, renin inhibitor	
	≥ 5 aktivitas	228	PG	ACE inhibitor, antiamnestic, antithrombotic, dipeptidyl peptidase IV inhibitor, PAM inhibitor, regulating	1
	≥ 5 aktivitas	1106	AA	ACE inhibitor, antibacterial, D-Ala-D-Ala dipeptidase inhibitor, dipeptidyl peptidase IV inhibitor, hypotensive, inhibitor of cytosol alanyl aminopeptidase, inhibitor of tripeptidyl peptidase II	1
	≥ 5 aktivitas	144	VY	ACE inhibitor, antioxidative, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, hypotensive, inhibitor of tripeptidyl peptidase II	1
	≥ 5 aktivitas	42	LW	ACE inhibitor, antioxidative, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, hypouricemic, renin inhibitor	1
	≥ 5 aktivitas	462	GE	ACE inhibitor, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, glutamate carboxypeptidase II inhibitor, glutamate carboxypeptidase inhibitor, neprilysin inhibitor	1
	≥ 5 aktivitas	174	TF	ACE inhibitor, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, hypotensive, pancreatic lipase inhibitor, renin inhibitor	1
	≥ 5 aktivitas	176	GP	ACE inhibitor, antiamnestic, antioxidative, antithrombotic, dipeptidyl peptidase IV inhibitor, hypotensive, neprilysin inhibitor, regulating	1
	≥ 5 aktivitas	72	RR	ACE inhibitor, antibacterial, antiviral, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, haemolytic, Leucyltransferase inhibitor, neuropeptide	1

b. Distribution of Bioactive Peptides Based on Total Activity in processed anchovy samples.

No	Total activities	Sequences peptide		Activity type	Occurens
		jml	Nama		
1	1 aktivitas	331	AL, AS, AT, DN, DQ, EH, EP, ES, ET, FN, IM, IN, IQ, KI, LM, MA, MI, MK, MN, NA, ND, NE, NL, NM, NQ, NT, NV, PI, PN, PS, PV, QA, QD, QE, QH, QI, QL, QN, QQ, QS, QT, QV, RI, SI, SK, SP, SV, TA, TD, TH, TI, TK, TN, TR, TV, VD, VI, VN, VQ, VS, VT, YD, IGL, PPG, GPAG, GPVG, VLPG, VPTP	dipeptidyl peptidase IV inhibitor	68
	1 aktivitas	212	AI, DG, DL, DM, GK, GM, GR, GS, GT, IG, KL, LF, LG, LQ, NK, QK, SG, ST, AEL, AGP, AQL, ASL, EQR, FNQ, GIL, GLP, GTG, IAE, LAA, LAY, LEE, LGP, LGV, LIY, LPG, LQQ, LVE, LVL, QGP, TGP, TLS, VAA, VAF, VGP, VVL, EQGP, PGL	ACE inhibitor	47
	1 aktivitas	69	EL, LK, GAA, LHL, YVE, DLEE, QGAR	antioxidative	7
	1 aktivitas	3	ATL, GTL, IAT	hypouricemic	3
	1 aktivitas	2	FM, SM	dipeptidyl peptidase III inhibitor	2
	1 aktivitas	30	DD, DE	glutamate carboxypeptidase II inhibitor	2
	1 aktivitas	14	IV, SE	stimulating	2
	1 aktivitas	1	AGFAGDDAPR	alcohol dehydrogenase activator	1
	1 aktivitas	4	NLQ	anti inflammatory	1
	1 aktivitas	2	DEE	antithrombotic	1
	1 aktivitas	2	TAT	binding	1
	1 aktivitas	4	AGA	CaMPDE inhibitor	1
	1 aktivitas	4	FE	glutamate carboxypeptidase inhibitor	1
	1 aktivitas	2	GGV	HMG-CoA reductase inhibitor	1
	1 aktivitas	12	EAE	immunostimulating	1
	1 aktivitas	5	GGA	inhibitor of cytosol alanyl aminopeptidase	1
	1 aktivitas	1	GVF	inhibitor of tripeptidyl peptidase II	1
2	2 aktivitas	364	AG, AV, DR, EI, EK, EV, GI, GL, GV, IA, KE, KR, LN, ME, MF, NF, PA, PQ, QG, SY, TE,	ACE inhibitor, dipeptidyl peptidase IV inhibitor	29

No	Total activities	Sequences peptide		Activity type	Occurens
		jml	Nama		
			TG, VG, VK, VP, VR, YS, YV, GPV		
	2 aktivitas	38	II, LI, LV, VL	dipeptidyl peptidase IV inhibitor, stimulating	4
	2 aktivitas	6	IY, GPP, SGP	ACE inhibitor, antioxidative	3
	2 aktivitas	42	AR, GD, AK	ACE inhibitor, neprilysin inhibitor	3
	2 aktivitas	38	TL. TT	dipeptidyl peptidase IV inhibitor, hypouricemic	2
	2 aktivitas	2	YPG	ACE inhibitor, alpha-glucosidase inhibitor	1
	2 aktivitas	3	LY	ACE inhibitor, antioxidative, renin inhibitor	1
	2 aktivitas	12	PR	ACE inhibitor, dipeptidyl peptidase III inhibitor	1
	2 aktivitas	44	IE	ACE inhibitor, glutamate carboxypeptidase inhibitor	1
	2 aktivitas	2	FK	ACE inhibitor, hypouricemic	1
	2 aktivitas	12	GQ	ACE inhibitor, neuropeptide	1
	2 aktivitas	2	DY	ACE inhibitor, regulating	1
	2 aktivitas	6	AD	alpha-glucosidase inhibitor, dipeptidyl peptidase IV inhibitor	1
	2 aktivitas	3	GPGG	antiamnestic, antithrombotic, regulating	1
	2 aktivitas	2	TY	antioxidative, dipeptidyl peptidase IV inhibitor	1
	2 aktivitas	2	MR	dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor	1
	2 aktivitas	8	HE	dipeptidyl peptidase IV inhibitor, glutamate carboxypeptidase inhibitor	1
	2 aktivitas	32	VA	dipeptidyl peptidase IV inhibitor, inhibitor of tripeptidyl peptidase II	1
	2 aktivitas	12	SL	dipeptidyl peptidase IV inhibitor, regulating	1
	2 aktivitas	8	NR	dipeptidyl peptidase IV inhibitor, renin inhibitor	1
	2 aktivitas	30	EEE	glutamate carboxypeptidase II inhibitor, stimulating	1
	3 aktivitas	6	PP, YP	ACE inhibitor, alpha-glucosidase inhibitor, dipeptidyl peptidase IV inhibitor	2
	3 aktivitas	12	PT, TP	ACE inhibitor, dipeptidyl peptidase IV inhibitor, hypouricemic	2
	3 aktivitas	12	AF, GY	ACE inhibitor, dipeptidyl peptidase IV inhibitor, inhibitor of tripeptidyl peptidase II	2
	3 aktivitas	60	GG, GPA	ACE inhibitor, dipeptidyl peptidase IV inhibitor, neprilysin inhibitor	2

No	Total activities	Sequences peptide		Activity type	Occurens
		jml	Nama		
3	3 aktivitas	3	WA	ACE inhibitor, activating ubiquitin-mediated proteolysis, dipeptidyl peptidase IV inhibitor	1
	3 aktivitas	105	EA	ACE inhibitor, alpha-glucosidase inhibitor, neprilysin inhibitor	1
	3 aktivitas	15	GPR	ACE inhibitor, antithrombotic, dipeptidyl peptidase IV inhibitor	1
	3 aktivitas	36	DA	ACE inhibitor, dipeptidyl peptidase III inhibitor, glutamate carboxypeptidase II inhibitor	1
	3 aktivitas	6	LP	ACE inhibitor, dipeptidyl peptidase IV inhibitor, lactocepin inhibitor	1
	3 aktivitas	3	SF	ACE inhibitor, dipeptidyl peptidase IV inhibitor, renin inhibitor	1
	3 aktivitas	81	ER	ACE inhibitor, hypotensive, neprilysin inhibitor	1
	3 aktivitas	3	FA	alanine carboxypeptidase inhibitor, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor	1
	3 aktivitas	36	LT	antioxidative, dipeptidyl peptidase IV inhibitor, hypouricemic	1
	3 aktivitas	3	LH	antioxidative, dipeptidyl peptidase IV inhibitor, neuropeptide	1
	3 aktivitas	105	AE	dipeptidyl peptidase IV inhibitor, glutamate carboxypeptidase II inhibitor, neprilysin inhibitor	1
	4 aktivitas	92	LA	ACE inhibitor, activating ubiquitin-mediated proteolysis, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor	1
	4 aktivitas	92	GA	ACE inhibitor, alanine carboxypeptidase inhibitor, antioxidative, dipeptidyl peptidase IV inhibitor	1
	4 aktivitas	4	AGDDAPR	ACE inhibitor, alpha-glucosidase inhibitor, antioxidative, pancreatic lipase inhibitor	1
	4 aktivitas	12	HL	ACE inhibitor, antioxidative, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor	1
	4 aktivitas	4	AY	ACE inhibitor, antioxidative, dipeptidyl peptidase IV inhibitor, tubulin-tyrosine ligase inhibitor	1

No	Total activities	Sequences peptide		Activity type	Occurens
		jml	Nama		
	4 aktivitas	20	KK	ACE inhibitor, bacterial permease ligand, dipeptidyl peptidase IV inhibitor, neprilysin inhibitor	1
	4 aktivitas	72	EG	ACE inhibitor, binding, dipeptidyl peptidase IV inhibitor, neuropeptide	1
	4 aktivitas	8	KF	ACE inhibitor, CaMPDE inhibitor, dipeptidyl peptidase IV inhibitor, renin inhibitor	1
	4 aktivitas	8	EF	ACE inhibitor, CaMPDE inhibitor, hypolipidemic, renin inhibitor	1
	4 aktivitas	4	FL	ACE inhibitor, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, neuropeptide	1
	4 aktivitas	12	VV	ACE inhibitor, dipeptidyl peptidase IV inhibitor, inhibitor of tripeptidyl peptidase II, neprilysin inhibitor	1
	4 aktivitas	4	AP	ACE inhibitor, dipeptidyl peptidase IV inhibitor, inhibitor of tripeptidyl peptidase II, peptidylprolyl isomerase isnhibitor	1
	4 aktivitas	4	RG	ACE inhibitor, dipeptidyl peptidase IV inhibitor, Leucyltransferase inhibitor, neprilysin inhibitor	1
	4 aktivitas	4	IL	ACE inhibitor, dipeptidyl peptidase IV inhibitor, neuropeptide, stimulating	1
	4 aktivitas	4	LL	dipeptidyl peptidase IV inhibitor, inhibitor of cytosol alanyl aminopeptidase, lactocepin inhibitor, stimulating	1
	≥ 5 aktivitas	15	GF	ACE inhibitor, acylaminoacyl peptidase inhibitor, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, inhibitor of tripeptidyl peptidase II.	1
	≥ 5 aktivitas	12	YA	ACE inhibitor, alanine carboxypeptidase inhibitor, antioxidative, dipeptidyl peptidase IV inhibitor, renin inhibitor, tubulin-tyrosine ligase inhibitor	1
	≥ 5 aktivitas	5	FY	ACE inhibitor, alpha-amylase inhibitor, alpha-glucosidase inhibitor, antioxidative, hypotensive	1
	≥ 5 aktivitas	5	VW	ACE inhibitor, alpha-glucosidase inhibitor, antioxidative, dipeptidyl peptidase IV inhibitor, hypouricemic	1
	≥ 5 aktivitas	120	VE	ACE inhibitor, alpha-glucosidase inhibitor, antioxidative, dipeptidyl peptidase IV inhibitor, neprilysin inhibitor	1

No	Total activities	Sequences peptide		Activity type	Occurens
		jml	Nama		
	≥ 5 aktivitas	18	LR	ACE inhibitor, alpha-glucosidase inhibitor, dipeptidyl peptidase III inhibitor, hypouricemic, neuroprotective, renin inhibitor	1
	≥ 5 aktivitas	184	GP	ACE inhibitor, antiamnestic, antioxidative, antithrombotic, dipeptidyl peptidase IV inhibitor, hypotensive, neprilysin inhibitor, regulating	1
	≥ 5 aktivitas	30	PG	ACE inhibitor, antiamnestic, antithrombotic, dipeptidyl peptidase IV inhibitor, PAM inhibitor, regulating	1
	≥ 5 aktivitas	8	RR	ACE inhibitor, antibacterial, antiviral, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, haemolytic, Leucyltransferase inhibitor, neuropeptide	1
	≥ 5 aktivitas	91	AA	ACE inhibitor, antibacterial, D-Ala-D-Ala dipeptidase inhibitor, dipeptidyl peptidase IV inhibitor, hypotensive, inhibitor of cytosol alanyl aminopeptidase, inhibitor of tripeptidyl peptidase II.	1
	≥ 5 aktivitas	5	YK	ACE inhibitor, anticancer, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, toxic.	1
	≥ 5 aktivitas	6	VY	ACE inhibitor, antioxidative, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, hypotensive, inhibitor of tripeptidyl peptidase II	1
	≥ 5 aktivitas	5	VF	ACE inhibitor, calpain 1 inhibitor, dipeptidyl peptidase IV inhibitor, inhibitor of tripeptidyl peptidase II, neprilysin 2 inhibitor	1
	≥ 5 aktivitas	132	GE	ACE inhibitor, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, glutamate carboxypeptidase II inhibitor, glutamate carboxypeptidase inhibitor, neprilysin inhibitor	1
	≥ 5 aktivitas	55	YE	ACE inhibitor, dipeptidyl peptidase IV inhibitor, glutamate carboxypeptidase II inhibitor, glutamate carboxypeptidase inhibitor, tubulin-tyrosine ligase inhibitor	1
	≥ 5 aktivitas	10	PGP	antiamnestic, antithrombotic, chemotactic, inhibitor, regulating	1
	≥ 5 aktivitas	400	EE	citrate lyase deacetylase inhibitor, glutamate carboxypeptidase II inhibitor, glutamate	1

No	Total activities	Sequences peptide		Activity type	Occurens
		jml	Nama		
				carboxypeptidase inhibitor, stimulating, thymidylate synthase inhibitor	

c. Distribution of Bioactive Peptides Based on Total Activity in fortified anchovy samples.

No	Total activities	Sequences peptide		Activity type	Occurens
		Number	Name		
1	1 aktivitas	256	AL, AS, AT, DN, DP, DQ, EH, EP, ES, ET, FN, IM, IN, IQ, KI, KV, LM, MA, MI, MK, NA, NE, NL, NM, NP, NQ, NT, NV, PI, PN, PS, PV, QA, QD, QE, QH, QL, QQ, QS, QT, QV, RI, SI, SK, SV, TA, TD, TI, TK, TM, TV, VD, VI, VN, VS, VT, IGL, PPG, GPAG, GPVG, VLGP, VPTP, VAPEEHPT	dipeptidyl peptidase IV inhibitor	63
	1 aktivitas	166	AI, DG, DL, GK, GM, GR, GS, GT, IG, KL, LG, LQ, QK, SG, ST, AEL, AGP, AQL, ASL, EAP, EQR, FNQ, GEF, GIL, GLP, GRP, GTG, IAQ, LAA, LAP, LAY, LEE, LGP, LGV, LNF, LNP, LPG, LQQ, LVE, LVL, LVY, QGP, TGP, VAA, VAF, VAP, VGP, VVL, EQGP, VFPS, IVGRPR	ACE inhibitor	51
	1 aktivitas	54	EL, LK, GAA, LHL, DLEE, QGAR, YVGD	antioxidative	7
	1 aktivitas	25	DD, DE	glutamate carboxypeptidase II inhibitor	2
	1 aktivitas	4	GGV, IVG	HMG-CoA reductase inhibitor	2
	1 aktivitas	14	IV, SE	stimulating	2
	1 aktivitas	1	DEE	antithrombotic	1
	1 aktivitas	2	TAT	binding	1
	1 aktivitas	3	AGA	CaMPDE inhibitor	1
	1 aktivitas	1	FM	dipeptidyl peptidase III inhibitor	1
	1 aktivitas	3	FE	glutamate carboxypeptidase inhibitor	1
	1 aktivitas	3	EAE	immunostimulating	1
	1 aktivitas	1	GVF	inhibitor of tripeptidyl peptidase II	1
	1 aktivitas	1	FT	renin inhibitor	1

No	Total activities	Sequences peptide		Activity type	Occurens
		Number	Name		
2	2 aktivitas	296	EV, FP, GI, GL, GV, IA, KE, KR, LN, ME, NF, NY, PA, PQ, QG, SY, TE, TG, VG, VK, VP, VR, YS, YV, GPV, AG, AV, DR, EI, EK	ACE inhibitor, dipeptidyl peptidase IV inhibitor	30
	2 aktivitas	38	II, LI, LV, VL	dipeptidyl peptidase IV inhibitor, stimulating	4
	2 aktivitas	32	AK, AR, GD	ACE inhibitor, neprilysin inhibitor	3
	2 aktivitas	4	GPP, SGP	ACE inhibitor, antioxidative	2
	2 aktivitas	42	TL, TT	dipeptidyl peptidase IV inhibitor, hypouricemic	2
	2 aktivitas	14	PR	ACE inhibitor, dipeptidyl peptidase III inhibitor	1
	2 aktivitas	18	IE	ACE inhibitor, glutamate carboxypeptidase inhibitor	1
	2 aktivitas	2	FK	ACE inhibitor, hypouricemic	1
	2 aktivitas	8	GQ	ACE inhibitor, neuropeptide	1
	2 aktivitas	2	PE	alpha-glucosidase inhibitor, dipeptidyl peptidase III inhibitor	1
	2 aktivitas	6	AD	alpha-glucosidase inhibitor, dipeptidyl peptidase IV inhibitor	1
	2 aktivitas	2	PK	antioxidative, dipeptidyl peptidase IV inhibitor	1
	2 aktivitas	2	MR	dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor	1
	2 aktivitas	2	HE	dipeptidyl peptidase IV inhibitor, glutamate carboxypeptidase inhibitor	1
	2 aktivitas	36	VA	dipeptidyl peptidase IV inhibitor, inhibitor of tripeptidyl peptidase II	1
	2 aktivitas	6	SL	dipeptidyl peptidase IV inhibitor, regulating	1
	2 aktivitas	6	NR	dipeptidyl peptidase IV inhibitor, renin inhibitor	1
	2 aktivitas	16	EEE	glutamate carboxypeptidase II inhibitor, stimulating.	1
	3 aktivitas	6	RA, WA	ACE inhibitor, activating ubiquitin-mediated proteolysis, dipeptidyl peptidase IV inhibitor	2
	3 aktivitas	3	PT, TP	ACE inhibitor, dipeptidyl peptidase IV inhibitor, hypouricemic	2
	3 aktivitas	69	GY, AF	ACE inhibitor, dipeptidyl peptidase IV inhibitor, inhibitor of tripeptidyl peptidase II	2
	3 aktivitas	9	GPA, GG	ACE inhibitor, dipeptidyl peptidase IV inhibitor, neprilysin inhibitor	2
	3 aktivitas	15	PP	ACE inhibitor, alpha-glucosidase inhibitor, dipeptidyl peptidase IV inhibitor	1
	3 aktivitas	3	EA	ACE inhibitor, alpha-glucosidase inhibitor, neprilysin inhibitor	1

No	Total activities	Sequences peptide		Activity type	Occurens
		Number	Name		
	3 aktivitas	57	AHA	ACE inhibitor, antioxidative, dipeptidyl peptidase IV inhibitor.	1
	3 aktivitas	15	GPR	ACE inhibitor, antithrombotic, dipeptidyl peptidase IV inhibitor	1
	3 aktivitas	18	HP	ACE inhibitor, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor	1
	3 aktivitas	9	DA	ACE inhibitor, dipeptidyl peptidase III inhibitor, glutamate carboxypeptidase II inhibitor	1
	3 aktivitas	42	LP	ACE inhibitor, dipeptidyl peptidase IV inhibitor, lactocepin inhibitor	1
	3 aktivitas	3	FF	ACE inhibitor, dipeptidyl peptidase IV inhibitor, pseudolysin inhibitor.	1
	3 aktivitas	6	SF	ACE inhibitor, dipeptidyl peptidase IV inhibitor, renin inhibitor	1
	3 aktivitas	69	ER	ACE inhibitor, hypotensive, neprilysin inhibitor.	1
	3 aktivitas	3	GPGG	antiamnestic, antithrombotic, regulating	1
	3 aktivitas	27	LT	antioxidative, dipeptidyl peptidase IV inhibitor, hypouricemic	1
	3 aktivitas	12	LH	antioxidative, dipeptidyl peptidase IV inhibitor, neuropeptide	1
	3 aktivitas	72	AE	dipeptidyl peptidase IV inhibitor, glutamate carboxypeptidase II inhibitor, neprilysin inhibitor.	1
3	4 aktivitas	48	LA	ACE inhibitor, activating ubiquitin-mediated proteolysis, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor	1
	4 aktivitas	56	GA	ACE inhibitor, alanine carboxypeptidase inhibitor, antioxidative, dipeptidyl peptidase IV inhibitor.	1
	4 aktivitas	16	HL	ACE inhibitor, antioxidative, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor	1
	4 aktivitas	4	AY	ACE inhibitor, antioxidative, dipeptidyl peptidase IV inhibitor, tubulin-tyrosine ligase inhibitor.	1
	4 aktivitas	16	KK	ACE inhibitor, bacterial permease ligand, dipeptidyl peptidase IV inhibitor, neprilysin inhibitor	1
	4 aktivitas	40	EG	ACE inhibitor, binding, dipeptidyl peptidase IV inhibitor, neuropeptide.	1
	4 aktivitas	4	KF	ACE inhibitor, CaMPDE inhibitor, dipeptidyl peptidase IV inhibitor, renin inhibitor.	1

No	Total activities	Sequences peptide		Activity type	Occurens
		Number	Name		
	4 aktivitas	12	EF	ACE inhibitor, CaMPDE inhibitor, hypolipidemic, renin inhibitor.	1
	4 aktivitas	4	FR	ACE inhibitor, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, hypouricemic	1
	4 aktivitas	8	VV	ACE inhibitor, dipeptidyl peptidase IV inhibitor, inhibitor of tripeptidyl peptidase II, neprilysin inhibitor	1
	4 aktivitas	12	AP	ACE inhibitor, dipeptidyl peptidase IV inhibitor, inhibitor of tripeptidyl peptidase II, peptidylprolyl isomerase inhibitor.	1
	4 aktivitas	4	PL	ACE inhibitor, dipeptidyl peptidase IV inhibitor, lactocepin inhibitor, xaa-pro inhibitor	1
	4 aktivitas	4	RG	ACE inhibitor, dipeptidyl peptidase IV inhibitor, Leucyltransferase inhibitor, neprilysin inhibitor	1
	4 aktivitas	4	IL	ACE inhibitor, dipeptidyl peptidase IV inhibitor, neuropeptide, stimulating	1
	4 aktivitas	8	LL	dipeptidyl peptidase IV inhibitor, inhibitor of cytosol alanyl aminopeptidase, lactocepin inhibitor, stimulating	1
4	≥ 5 aktivitas	15	GF	ACE inhibitor, acylaminoacyl peptidase inhibitor, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, inhibitor of tripeptidyl peptidase II.	1
	≥ 5 aktivitas	5	FY	ACE inhibitor, alpha-amylase inhibitor, alpha-glucosidase inhibitor, antioxidative, hypotensive	1
	≥ 5 aktivitas	5	VW	ACE inhibitor, alpha-glucosidase inhibitor, antioxidative, dipeptidyl peptidase IV inhibitor, hypouricemic	1
	≥ 5 aktivitas	90	VE	ACE inhibitor, alpha-glucosidase inhibitor, antioxidative, dipeptidyl peptidase IV inhibitor, neprilysin inhibitor	1
	≥ 5 aktivitas	12	LR	ACE inhibitor, alpha-glucosidase inhibitor, dipeptidyl peptidase III inhibitor, hypouricemic, neuroprotective, renin inhibitor	1
	≥ 5 aktivitas	184	GP	ACE inhibitor, antiamnestic, antioxidative, antithrombotic, dipeptidyl peptidase IV inhibitor, hypotensive, neprilysin inhibitor, regulating.	1
	≥ 5 aktivitas	18	PG	ACE inhibitor, antiamnestic, antithrombotic, dipeptidyl peptidase IV inhibitor, PAM inhibitor, regulating	1
	≥ 5 aktivitas	8	RR	ACE inhibitor, antibacterial, antiviral, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV	1

No	Total activities	Sequences peptide		Activity type	Occurens
		Number	Name		
				inhibitor, haemolytic, Leucyltransferase inhibitor, neuropeptide	
	≥ 5 aktivitas	49	AA	ACE inhibitor, antibacterial, D-Ala-D-Ala dipeptidase inhibitor, dipeptidyl peptidase IV inhibitor, hypotensive, inhibitor of cytosol alanyl aminopeptidase, inhibitor of tripeptidyl peptidase II.	1
	≥ 5 aktivitas	5	YK	ACE inhibitor, anticancer, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, toxic	1
	≥ 5 aktivitas	6	VY	ACE inhibitor, antioxidative, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, hypotensive, inhibitor of tripeptidyl peptidase II	1
	≥ 5 aktivitas	5	YL	ACE inhibitor, antioxidative, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, neuropeptide	1
	≥ 5 aktivitas	20	VF	ACE inhibitor, calpain 1 inhibitor, dipeptidyl peptidase IV inhibitor, inhibitor of tripeptidyl peptidase II, neprilysin 2 inhibitor.	1
	≥ 5 aktivitas	102	GE	ACE inhibitor, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, glutamate carboxypeptidase II inhibitor, glutamate carboxypeptidase inhibitor, neprilysin inhibitor.	1
	≥ 5 aktivitas	5	YG	ACE inhibitor, dipeptidyl peptidase III inhibitor, dipeptidyl peptidase IV inhibitor, immunostimulating, tubulin-tyrosine ligase inhibitor	1
	≥ 5 aktivitas	45	YE	ACE inhibitor, dipeptidyl peptidase IV inhibitor, glutamate carboxypeptidase II inhibitor, glutamate carboxypeptidase inhibitor, tubulin-tyrosine ligase inhibitor	1
	≥ 5 aktivitas	10	RP	ACE inhibitor, dipeptidyl peptidase IV inhibitor, neprilysin inhibitor, neurolysin inhibitor, neuropeptide	1
	≥ 5 aktivitas	5	PGP	antiamnestic, antithrombotic, chemotactic, inhibitor, regulating	1
	≥ 5 aktivitas	225	EE	citrate lyase deacetylase inhibitor, glutamate carboxypeptidase II inhibitor, glutamate carboxypeptidase inhibitor, stimulating, thymidylate synthase inhibitor.	1

Table S4 Distribution of Bioactive Peptide Lengths in raw, processed, and fortified anchovy samples.

	Length peptide (aa)	Raw		Processed		Fortification	
		Jml	**%**	**Jml**	**%**	**Jml**	**%**
1	2 aa	307	49.0	187	75.1	181	73.9
2	3 aa	278	44.1	52	20.9	52	21.2
3	4 aa	23	3.6	8	3.2	10	4.1
4	5 aa	7	1.1	-	0.0	-	0.0
5	6 aa	4	0.6	-	0.0	1	0.4
6	7 aa	3	0.5	1	0.4	-	0.0
7	8 aa	2	0.3	-	0.0	1	0.4
8	10 aa	-	0.0	1	0.4	-	0.0
9	11 aa	2	0.3	-	0.0	-	0.0
10	12 aa	1	0.2	-	0.0	-	0.0
11	13 aa	1	0.2	-	0.0	-	0.0
12	17 aa	1	0.2	-	0.0	-	0.0
	Total	**628**	**100.0**	**249**	**100.0**	**245**	**100.0**

a. Bioactive peptides according to peptide length and bioactivity score in raw anchovy samples.

No	**Length peptide**	**Bioactivity score**		
		> 0.8	**0.5 - 0.79**	**< 0.5**
1	2 aa	FF, WF, FW, MF, FM, CW, MW, WM, GF, FP, PF, WP, GW, FG, PW, WG, FL, WL, LF, RF, FR, LW, FY, YF, WR, WY, AF, MM, AW, MP, WA, FA, WH, GM, HW, FH, PM, FN, HF, IF, SF, QF, IW, MG, DF, NF, WI, WN, NW, SW, QW, WD, FQ, KF, GP, ML, WS, GG, PP, PG, WK, LM, FK, MR, RM, MY, YM, TF, RP, FT, VF, TW, WT, PL, GL, VW	LP, PR, WV, GR, GY, YP, RG, PY, LG, MH, MI, IM, MA, YG, MN, SM, AP, RL, LL, DM, NM, EF, FE, EW, IP, WE, YL, LR, HP, RR, MQ, AG, PI, RY, PH, PA, GH, YR, GA, GI, LY, SP, IG,	NP, DP, HG, PN, YY, QP, MK, AL, SG, AR, PS, PQ, IL, DG, KP, NG, QG, GD, GQ, HL, TM, RH, RA, GS, LI, VC, RI, LH, IR, HR, SL, VM, PK, DL, MV, IY, KG, LA, YI, HY, GK, YH, QL, NL, DR, TP, YA, SY, NR, LN, PT, YN, DY, VP, KL, QY, GT, NY, II, DW, HH, IH, KR, HI, AH, PV, AI, ME, LQ, AA, TG, RK, YD, YQ, HA, YS, IA, GV, KY, LK, VG, SH, SI, EP, IN, AY, PE, YK, TL, NH, QH, HS, AD, DA, QI, HD, TR, VL, AS, IQ, QA, RV, NA, LT, VR, TY, YT, GE, HK, QN, DN, NN, EG, KI, LV, VY, DD, KA, AK, YV, QD, QQ, DQ, QS, ND, NQ, HT, TH, SK, AE, EL, TI, AT, AV, ER, VH, HV, EY, YE, KS, KD, NK, VI, QK, IV, ST, KK, TA, VA, HE, EH, SV, TN, QT, IE, EI, NT, VN, TS, SE, TD, VS, QV, VD, TQ, EA, NV, VQ, ES, DE, KT, QE, KV, NE, VK, TK, TT, VT, KE, VV, TV, EK, ET, TE, EE, VE, EV
2	3 aa	FGF, FFL, GGF, PFP, PWL, GFL, FFK, GLF, LPF, LWG, LGF, GAW, GPM, FAP, MGP, QGF,	LPG, WHH, LGP, ASF, SDF, YRP, IPP, YGG, PAP, VFL, APG, LWK, LWT, QDF, NPP, LPL,	AIP, LNP, GPV, TGP, IRY, KLP, HPH, APA, AAP, LGI, IAP, IPA, LPQ, VGP, ILR, LPK, SLR, GGV, GAH, ANP, RPK, DYY, PHA, VFK, GTG, HYY, LHL, VPL,

No	Length peptide	Bioactivity score		
		> 0.8	**0.5 - 0.79**	**< 0.5**
		PWI, LFR, YYF, LLF, PHF, PPP, ARF, AFL, PPG, PGP, IFL, PPL, LPM, FAL, GPL, GRP, VPF, GTW, LAF, DFY, PWV, GPR, RPP, PLP, GLP, PGL, MAM, FGK, LHF, GVF, FYN, PGR, LNF, LPP, FVP, YPG, IAF, GGY, IWH, ADF	AGP, GPA, YGL, PEF, GEF, GLY, LGG, LLP, SGP, LYP, FVR, LTF, IRP, LVF, ALP, GGA, LFV, SRP, FNQ, LHM, GIL, PHL, YLL, ILP, VHW, FDK, YPI, VMP, IYP, IVF, PHR, GKP, LLL, IGL, GVM, EYF, VAF, NPR, YLR, QYP, DLP, AGY, YLY, LWE, DGL, LPI, RPA, LLR, VPP, LAP, LEF	PHN, GYK, VRP, LKP, GPE, YDY, AGA, GAA, IQP, GTL, LIY, NLR, VLP, GEP, GSH, VWV, YQY, PHQ, YSY, IKP, RDY, VVF, YVP, LAY, PHS, PHK, HLH, VGL, AYI, GVR, LQL, GVY, LDY, HIR, GHS, HQG, ASL, LKL, AGS, LHI, PEL, AQL, TFE, AVP, YNI, LGV, LAA, IQY, VAP, KPS, YVL, TNP, LHN, YVY, VLY, IYK, LVL, LAN, LHD, VNP, LHS, IEP, LHQ, AAA, LHK, IVG, KPT, EAP, TIL, NKL, ATL, LVR, NLQ, YYE, DYK, YSQ, AVL, YNK, LVY, VPK, SVL, VKP, IAQ, VIY, IVY, GKV, RHV, LQQ, HHT, VAY, TAP, TDY, KAI, LKK, VPV, YVA, IAT, AEL, VGT, TLS, KSA, AKK, IKK, LVS, VAA, LVQ, EQR, IAE, VVL, KKK, VVR, VTR, KVI, ITT, LEK, TAT, YVE, AVV, NEN, EAK, LVE, LEE, VQV, EAE, DEE, VVV, EEE
3	4 aa	FIGP, AFLL, PPAP	GPGA, ALPP, GGYR, DLGP, GPVG, VFPS, GPIN, LPVP	IIAP, ILKP, VPTP, FTTQ, VVGP, VVRP, YVGD, ELLI, EIPT, SITA, AIAV, DLEE
4	≥ 5 aa	-	MYPGIA, IWHHTFYNELR	IWHHT, WDDMEK, GYALPHA, IITNW, ALPHA, IVGRPR, IIAPPER, YALPHA, DMIPAQK, IYEGY, DNIQGITKPAIR, DIDDLELTLAK, LEEAF, VIPEL, VAPEEHPT, VAPEEHPV, LEQQVDDLEGSLEQEKK, VALTGLTVAEYFR, KEEAE

b. Bioactive peptides according to peptide length and bioactivity score in processed anchovy samples.

No	Length peptide	Bioactivity score		
		> 0.8	**0.5 - 0.79**	**< 0.5**
1	2 aa	MF, FM, GF, FL, LF, FY, AF, WA, FA, GM, FN, SF, NF, KF, GP, GG, PP, PG, LM, FK, MR, VF, GL, VW	LP, PR, GR, GY, YP, RG, LG, MI, IM, MA, MN, SM, AP, LL, DM, NM, EF, FE, LR, RR, AG, PI, PA, GA, GI, LY, SP, SP, IG	PN, MK, AL, SG, AR, PS, PQ, IL, DG, QG, GD, GQ, HL, GS, LI, RI, LH, SL, DL, IY, LA, GK, QL, NL, DR, TP, YA, SY, NR, LN, PT, DY, VP, KL, GT, II, KR, PV, AI, ME, LQ, AA, TG, YD, YS, IA, GV, LK, VG, SI, EP, IN, AY, YK, TL, QH, AD, DA, QI, TR, VL, AS, IQ, QA, NA, LT, VR, TY, GE, QN, DN, EG, KI, LV, VY, DD, AK, YV, QD,

				QQ, DQ, QS, ND, NQ, TH, SK, AE, EL, TI, AT, AV, ER, YE, NK, VI, QK, IV, ST, KK, TA, VA, HE, EH, SV, TN, QT, IE, EI, NT, VN, SE, TD, VS, QV, VD, EA, NV, VQ, ES, DE, QE, NE, VK, TK, TT, VT, KE, VV, TV, EK, ET, TE, EE, VE, EV
2	3 aa	GPP, PPG, PGP, GPR, GLP, PGL, GVF, YPG	LPG, LGP, AGP, GPA, SGP, QGP, GGA, FNQ, GIL, IGL, VAF	GPV, TGP, VGP, GGV, GTG, LHL, AGA, GAA, GTL, LIY, LAY, ASL, AQL, LGV, LAA, LVL, ATL, NLQ, LQQ, IAT, AEL, TLS, VAA, EQR, IAE, VVL, TAT, YVE, LVE, LEE, EAE, DEE, EEE
3	4 aa	-	GPGG, GPAG, GPVG	VLGP, QGAR, VPTP, EQGP, DLEE
4	≥ 5 aa		AGFAGDDAPR, AGDDAPR	-

c. Bioactive peptides according to peptide length and bioactivity score in fortified processed samples.

No	**Length peptide**	**Bioactivity score**		
		> 0.8	**0.5 - 0.79**	**< 0.5**
1	2 aa	FF, FM, GF, FP, FR, FY, AF, WA, GM, FN, SF, NF, KF, GP, GG, PP, PG, LM, FK, MR, RP, FT, VF, PL, GL, VW	LP, PR, GR, GY, RG, LG, MI, IM, MA, YG, AP, LL, NM, EF, FE, YL, LR, HP, RR, AG, PI, PA, GA, GI, IG	NP, DP, PN, MK, AL, SG, AR, PS, PQ, IL, DG, QG, GD, GQ, HL, TM, RA, GS, LI, RI, LH, SL, PK, DL, LA, GK, QL, NL, DR, TP, SY, NR, LN, PT, VP, KL, GT, NY, II, KR, AH, PV, AI, ME, LQ, AA, TG, YS, IA, GV, LK, VG, SI, EP, IN, AY, PE, YK, TL, QH, AD, DA, VL, AS, IQ, QA, NA, LT, VR, GE, DN, EG, KI, LV, VY, DD, AK, YV, QD, QQ, DQ, QS, NQ, SK, AE, EL, TI, AT, AV, ER, YE, VI, QK, IV, ST, KK, TA, VA, HE, EH, SV, QT, IE, EI, NT, VN, SE, TD, VS, QV, VD, EA, NV, ES, DE, QE, KV, NE, VK, TK, TT, VT, KE, VV, TV, EK, ET, TE, EE, VE, EV,
2	3 aa	GPP, PPG, PGP, GRP, GPR, GLP, GVF, LNF	LPG, LGP, AGP, GPA, GEF, SGP, QGP, FNQ, GIL, IGL, VAF, LAP	LNP, GPV, TGP, VGP, GGV, GTG, LHL, AGA, GAA, LAY, ASL, AQL, LGV, LAA, VAP, LVL, IVG, EAP, LVY, IAQ, LQQ, AEL, VAA, EQR, VVL, TAT, LVE, LEE, EAE, DEE, EEE
3	4 aa	GPGG, GPAG	GPVG, VFPS	VLGP, QGAR, VPTP, EQGP, YVGD, DLEE
4	≥ 5 aa	-	-	IVGRPR, VAPEEHPT