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Investigating the addition of rainbow trout visceral hydrolyzed protein on the quality properties of cooked hamburger

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ARTICLE INFO	ABSTRACT
	Fat oxidation during the storage period is one of the important factors in the deterioration of food quality. Hydrolyzed fish waste is one of the most important sources of bioactive peptides as a natural antioxidant. The purpose of this research is adding bioactive peptide that obtained from the enzymatic hydrolysis of rainbow trout waste (viscera) to the cooked hamburger formulation and measure the characteristics of the hamburger. Fish wastes were hydrolyzed in optimum conditions (temperature 59°C, time 118 minutes and concentration of 2% alcalase enzyme and then, 0.5, 1, 1.5 and 2% by weight, were added to the hamburger samples. A hamburger is also as a blank. The tests were performed in 3 repetitions and the averages were compared with Duncan's test to check the significance of the variables at P < 0.05 and the data were reported as mean ± standard deviation. The results showed that the cooked hamburger formulation contains 2% viscera by weight, has the highest percentage of cooked yield, fat and moisture retention, and the lowest amount of hardness, cooking loss and shrinkage. Using of a waste source, in order to turn it into a valuable product with antioxidant properties, lead to reduce the amount of fish waste and helps the environment. With this method, natural antioxidants can be used instead of synthetic sources.

1- Introduction

Today, various meat products are widely used around the world. Hamburgers are among the popular meat products consumed by millions of consumers globally. Formulating meat products without altering their flavor, mouthfeel, and other organoleptic characteristics is a very precise and specialized process [1]. Lipid oxidation during storage is a significant factor in the decline of the quality of this food product and is a concern for hamburger producers. The oxidation of lipids during food storage and processing not only leads to a loss of nutritional quality but also generates products such as free radicals. The free radicals produced in food systems cause auto-oxidation and the formation of undesirable chemical compounds, resulting in bitterness and off-flavors in the food. Additionally, free radicals in biological and living systems contribute to the onset of many diseases, particularly cancer. Antioxidants are compounds that effectively prevent the oxidation of fats [2]. While the use of chemically sourced antioxidants is beneficial for maintaining quality, extending shelf life, and preventing economic losses, it can reduce health benefits. Today, there is extensive research aimed at eliminating or reducing synthetic and chemical compounds in food products, focusing on replacing these chemicals with natural alternatives. Significant efforts have been made to find natural antioxidants from marine sources. Currently, bioactive compounds are recognized as natural ingredients or supplements in foods with the potential to enhance health and provide nutritional value beyond that of the original product [3]. Bioactive peptides are explored as protein components that are inactive in the primary protein structure but exhibit various physicochemical functions once released through enzymatic hydrolysis. These peptides range from 2 to 20 amino acids in length and have a molecular weight of less than 6000 Daltons. Their amino acid composition and sequences impact their bioactive properties [4]. Based on their structural characteristics and amino acid composition, they demonstrate various roles, such as inhibiting trace elements, enhancing the immune system, exhibiting antimicrobial activity, providing antioxidant effects, reducing cholesterol, and displaying antihypertensive activity [5]. One of the most important sources of bioactive peptides is aquatic organisms. Typically, low-value fish and marine by-products are utilized in the form of fish oil, fertilizers, compost, fish meal, and silage products. However, many of these recovered products have low economic value, whereas producing bioactive compounds can lead to higher added value [6]. Enzymatic hydrolysis is the most common method used for producing bioactive peptides from aquatic sources. Among the advantages of this method are the production of bioactive peptides with low molecular weight and desirable biological functions and properties, a reduction in the allergenic potential of proteins, and the absence of toxic chemical compounds [7]. Today, significant research is being conducted to eliminate or reduce chemical and synthetic compounds in food products by replacing them with natural alternatives. Given that the use of preservatives in hamburgers is prohibited, numerous studies have focused on finding natural antioxidants from various sources. One such study was conducted by Shahrokh-Shabani et al., in 2014, who used garlic aqueous extract as a natural antioxidant at concentrations of 1, 2, and 3 cc in the formulation of 100-gram hamburger samples. The prepared hamburger samples were stored under refrigerated conditions at 0-4 degrees Celsius for 1 and 2 weeks, while some samples were kept frozen at -18 degrees Celsius for 1, 2, and 3 months. The level of oxidation in all samples was then evaluated using the thiobarbituric acid (TBA) test to determine the most effective concentration of garlic aqueous extract in reducing oxidation. Statistical analysis showed that hamburger samples containing 2 and 3 cc of garlic aqueous extract had the greatest effect in preventing oxidation and inhibiting spoilage. The results of this study demonstrated that garlic aqueous extract acts as an effective natural antioxidant, significantly preventing fat oxidation in hamburgers and meat products, thereby ensuring the health and safety of the product [8]. Saghari et al., (2015) conducted research on the effect of thyme on the properties of hamburgers during storage. According to the results obtained, thyme extract significantly delayed lipid oxidation in treated beef burgers. The number of psychrotrophic bacteria during the storage period remained below the acceptable limit in the hamburgers treated with thyme extract, and microbial spoilage in these samples was significantly reduced compared to the control samples. Sensory evaluation results indicated that the

treated samples were generally acceptable in terms of overall acceptance. Overall, the findings demonstrate the antioxidant and antimicrobial effects of thyme extract on beef burgers during storage, contributing to an increased shelf life [9]. Monica Bergamaschi et al., (2023) investigated the antioxidant properties and sensory characteristics of pork hamburgers containing green coffee bean extract. The samples included a control and those with 0.15%, 0.3%, and 0.6% extract, stored at 4 degrees Celsius for 14 days. The results indicated that at higher concentrations, phenolic compounds and free radical scavenging activity significantly increased. The aim of this research is to add bioactive peptides derived from the enzymatic hydrolysis of rainbow trout (viscera) waste as a natural antioxidant to hamburger formulations, measuring the characteristics of the cooked hamburgers and determining the optimal percentage of added hydrolyzed waste containing bioactive peptides [10]. The significance of this study lies in examining the effects of natural antioxidants (bioactive peptides) on the physicochemical properties of hamburgers during the cooking process. Additionally, it highlights the importance of using natural-source antioxidants as substitutes for synthetic antioxidants, optimizing the use of fish waste to transform it into a source with antioxidant properties, reducing fish waste, and contributing to environmental sustainability.

2-Materials and methods
2-1- Materials
In this study, the raw materials used for producing hamburger samples were sourced from the local market, as outlined in Table 1. The entrails and offal of rainbow trout were purchased from the fish market. The enzyme used for hydrolysis was Alcalase, which was obtained from Merck and has an enzymatic activity of 0.75 IU/Kg and a density of 1.25 g/ml. Alcalase is an alkaline protease of microbial origin, extracted from the bacterium *Bacillus licheniformis*.

Table 1. Proportion of raw materials in hamburger production

Materials	Weight ratio (g/100g)
The calf minced meat	91
Onion	5
Garlic	0.7
Salt	1.2
Pepper	0.3
Spice	0.8
Flour	2

2-2- Production method
To produce hamburger samples, the raw materials were weighed according to the formulation using a laboratory scale, model AND-GE120, and mixed together to achieve a uniform dough. The mixture was then ground using a meat grinder, model National MK-G28NR, with a 10 mm mesh to ensure homogeneity. The prepared dough was shaped separately on each surface using a manual hamburger press and packaged in polyethylene wraps. The samples were stored in a freezer at -18 degrees Celsius until testing. To hydrolyze fish waste (viscera), the fish waste was first hydrolyzed under optimal conditions (temperature of 59 degrees Celsius, duration of 118 minutes, and enzyme concentration of 2 percent) and then added to the hamburger sample in amounts of 0.5%, 1%, 1.5%, and 2% [11]. One hamburger treatment served as a control sample, lacking hydrolyzed fish waste (and thus lacking bioactive peptides). Finally, the hamburger samples were cooked in frying oil at 80 °C for 5 min.

2-3- Measurement of texture properties
To measure the textural properties of the hamburger, including gumminess, hardness, cohesiveness, springiness, and chewiness, the samples were cooked at a temperature of 170 °C for 5 min and then stored at a temperature of

4 °C for 12 h to allow the center temperature of the samples to decrease to 4 degrees. The samples were then cut into cubes measuring 1×1 cm and subjected to a compressive test using a Texture Analyzer. The force required to compress the samples to 70% of their original height was measured at a constant speed [12].

2-4- Cooking loss assessment

For this purpose, the hamburger samples were first weighed and then fried for 5 minutes in oil at a temperature of 170°C. After frying, the samples were placed on a strainer for 10 minutes to remove excess oil and then weighed again. The loss during frying was calculated using the following equation [13].

Cooking loss (%) = [(Raw hamburger weight – Cooked hamburger weight) – Raw hamburger weight] × 100

2-5- Cooking yield assessment

To measure the cooking yield, the weights of the burger samples and the control sample were measured before and after cooking using a digital scale with an accuracy of 0.01. The cooking yield was determined using the following equation [13].

Cooking yield (%) = (weight of raw sample / weight of cooked sample) × 100

2-6- Measurement of diameter reduction (Shrinkage)

In order to conduct this test, samples weighing 15 grams were taken from raw hamburger patties and shaped into circular disks. The diameter of the samples was measured before and after frying (in vegetable oil specifically for frying at a temperature of 155 degrees Celsius for 5 minutes) using calipers. The percentage reduction in the diameter of the samples (DR %) was calculated using the following equation [14].

Diameter reduction (%) = (Raw burger diameter - Cooked burger diameter) / (Raw burger diameter) × 100

2-7- Fat retention assessment

In order to measure the fat retention capacity of hamburger samples and the control sample, they were randomly selected, and their total fat content was measured using the Soxhlet method before and after cooking. The fat retention capacity was then determined using the following equation [15].

Fat retention (%) = (Cooked sample fat / Raw sample fat) × Cooking yield

2-8- Moisture retention assessment

To measure the moisture retention capacity of hamburger samples and the control hamburger sample, they were randomly selected, and their moisture content was measured using a Memmert UE500 oven before and after cooking. The moisture retention was then calculated using the following equation [15].

Moisture retention (%) = (Cooked sample moisture / Raw sample moisture) × Cooking yield =

2-9- Sensory evaluation assay

To conduct the sensory evaluation test, 50 g were taken from each treatment and placed in a hamburger press. The resulting hamburger was then uniformly fried using a deep fryer and specific frying oil at a temperature of 170 degrees Celsius for 5 minutes, and was provided to a panel group. The sensory evaluation test was conducted with a trained panel group consisting of 15 individuals. After assessing the taste, aroma, texture, color, and overall acceptability of each treatment, these individuals recorded their opinions on questionnaires that were prepared in advance based on the hedonic scale (ATSM-1960) with slight modifications [16].

2-10- Statistical analysis

In this study, to analyze the data obtained from the addition of hydrolyzed fish by-products to the cooked hamburger formulation and to examine the interaction effects of treatment-time, SPSS software version 2018 was used. All tests were conducted in 3 replicates, and the means were compared using Duncan's multiple range test to assess the significance of the variables at $P < 0.05$. The data were reported as mean ± standard deviation.

3- Results and discussion

3-1- Textural properties

Hardness: The interaction effect of time-treatment in all treatments shows that the highest hardness factor was in the control treatment on day 90, equal to 79.33, while the lowest hardness factor was in the treatment containing 2% viscera on day 0, equal to 41.46.

Table 2. Interaction effects of treatment-time (Treatment-day) on Hardness, Cohesiveness, Springiness, Gumminess and Chewiness of cooked hamburger

Treatment-day	Hardness (N)	Cohesiveness	Springiness (cm)	Gumminess (kg)	Chewiness (kg.cm)
Blank-Day0	53.52 ± 0.02^m	0.81 ± 0.02^g	1.14 ± 0.02^a	43.08 ± 0.79^{jk}	48.88 ± 0.25^{gh}
Blank-Day30	62.12 ± 0.01^g	1.14 ± 0.02^e	1.12 ± 0.02^{ab}	70.50 ± 0.94^e	78.97 ± 2.46^d
Blank-Day60	71.14 ± 0.02^c	1.92 ± 0.01^a	0.97 ± 0.02^c	136.59 ± 0.68^a	131.81 ± 2.70^a
Blank-Day90	79.33 ± 0.02^a	1.65 ± 0.03^b	0.97 ± 0.01^c	130.50 ± 2.02^b	126.60 ± 3.26^b
0.5%VH-Day0	49.83 ± 0.00^q	0.72 ± 0.01^h	1.12 ± 0.02^{ab}	35.87 ± 0.50^l	40.00 ± 1.10^j
0.5%VH-Day30	57.32 ± 0.01^k	0.93 ± 0.01^f	1.07 ± 0.02^b	53.30 ± 0.58^h	57.02 ± 0.45^f
0.5%VH-Day60	65.65 ± 0.02^e	1.24 ± 0.02^d	0.96 ± 0.01^{cd}	81.07 ± 1.01^d	77.41 ± 0.56^{de}
0.5%VH-Day90	72.32 ± 0.01^b	1.44 ± 0.02^c	0.92 ± 0.01^{cd}	104.14 ± 1.44^c	95.79 ± 0.28^c
1%VH-Day0	46.57 ± 0.02^r	0.63 ± 0.02^{ij}	0.97 ± 0.01^c	29.10 ± 0.69^m	28.08 ± 0.81^m
1%VH-Day30	53.23 ± 0.02^n	0.84 ± 0.02^g	0.97 ± 0.02^c	44.44 ± 0.81^j	42.89 ± 1.45^{ij}
1%VH-Day60	61.44 ± 0.01^h	0.95 ± 0.03^f	0.94 ± 0.02^{cd}	56.83 ± 0.30^g	53.41 ± 0.86^{fg}
1%VH-Day90	66.59 ± 0.02^d	1.20 ± 0.02^d	0.93 ± 0.03^{cd}	79.57 ± 1.02^d	73.63 ± 2.94^e
1.5%VH-Day0	44.80 ± 0.02^s	0.52 ± 0.01^k	0.95 ± 0.02^{cd}	23.29 ± 0.44^n	22.02 ± 0.77^n
1.5%VH-Day30	51.13 ± 0.02^o	0.66 ± 0.03^i	0.91 ± 0.02^{cde}	33.49 ± 1.30^l	30.49 ± 1.85^{lm}
1.5%VH-Day60	57.46 ± 0.01^j	0.84 ± 0.02^g	0.95 ± 0.03^{cd}	47.97 ± 0.87^i	45.31 ± 0.38^{hi}
1.5%VH-Day90	63.77 ± 0.02^f	0.96 ± 0.02^f	0.90 ± 0.03^{de}	61.21 ± 1.29^f	54.82 ± 2.69^f
2%VH-Day0	41.46 ± 0.01^t	0.47 ± 0.01^l	0.94 ± 0.02^{cd}	19.28 ± 0.21^o	18.02 ± 0.10^n
2%VH-Day30	50.97 ± 0.02^p	0.59 ± 0.01^j	0.92 ± 0.02^{cd}	29.81 ± 0.26^m	27.43 ± 0.84^m
2%VH-Day60	56.19 ± 0.01^l	0.73 ± 0.02^h	0.86 ± 0.02^{ef}	40.74 ± 0.84^k	34.81 ± 0.10^{kl}
2%VH-Day90	59.39 ± 0.02^i	0.83 ± 0.01^g	0.81 ± 0.03^f	48.99 ± 0.31^i	39.44 ± 1.47^{jk}

Means ± SE (n = 10) with different letters in each column indicate significant difference (P<0.05)

Cohesiveness: The interaction effect of time-treatment in all treatments shows that the highest cohesiveness factor was in the control treatment on day 60, equal to 1.92, while the lowest cohesiveness factor was in the treatment containing 2% viscera on day 0, equal to 0.47.

Springiness: The interaction effect of time-treatment in all treatments shows that the highest elasticity factor was in the control treatment on day 0, equal to 1.14, while the lowest elasticity factor was in the treatment containing 2% viscera on day ninety, equal to 0.81.

Gumminess: The interaction effect of time-treatment in all treatments shows that the highest gumminess factor was in the control treatment on day 60 of storage, equal to 136.59, while the lowest gumminess factor was in the treatment containing 2% viscera on day 0, equal to 19.28.

Chewiness: It is derived from the product of the hardness and cohesiveness factors. The trend of its changes in all treatments aligns with the two dependent factors [16].

As the percentage of viscera increases in the cooked hamburger samples, the water-holding capacity, as well as water and fat retention, increases. The results also indicate that the lowest hardness factor and the highest connectivity property were measured in the samples containing 2% viscera.

3-2 Cooking loss, cooking yield, and diameter reduction

The interaction effect of time-treatment in all treatments shows that the highest cooking loss factor was in the control treatment on day 90, equal to 25.75, while the lowest cooking loss was in the treatment containing 2% viscera on day 0, equal to 12.87. The factors affecting protein denaturation are heat, pH changes, and freezing. According to the results, the cooking loss over 90 days of storage in all treatments significantly increased. The cause of protein denaturation is the cooking process followed by the freezing of the treatments. During denaturation, proteins unfold from their coiled state and spread out. In this state, the hydrophobic regions that were previously trapped within the structure come to the surface, leading to reduced solubility of the protein. Additionally, one of the other effects of denaturation is the decrease in water absorption capacity. Amino acids can exist in anionic form (in alkaline conditions) and cationic form (in acidic conditions) or as zwitterions (having both positive and negative charges) depending

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on the pH of the environment. The pH at which amino acids are electrically neutral and exist in zwitterionic form is called the isoelectric point. At the isoelectric pH, solubility, emulsifying capacity, and water absorption capacity are minimized, causing proteins to precipitate at this pH. The isoelectric pH of proteins is approximately 5.5 to 6, and under these conditions, denaturation occurs easily [17]. Enzymatic hydrolysis of fish by-products was conducted at a pH of around 8, which is above the isoelectric point, resulting in proteins being in anionic form. This allows for a high ability to absorb H^+ ions, subsequently increasing the water-holding capacity. Additionally, the presence of mild alkaline conditions causes carboxyl groups of proteins to become ionized, and the resulting negative groups repel each other, leading to increased protein solubility. All these factors can contribute to enhancing water-holding capacity and fat retention, as well as reducing cooking loss, due to the increased percentage of viscera in the hamburger formulation. The interaction effect of time-treatment in all treatments shows that the highest cooking yield factor was in the treatment containing 2% viscera on day 0, equal to 87.42, while the lowest cohesiveness factor was in the control treatment on day 90, equal to 71.14. In fact, in these treatments, we observe the highest and lowest water-holding capacity and water retention, respectively. This phenomenon is apparently due to water and fat retention as well as the water-holding capacity in the treatments. The interaction effect of time-treatment in all treatments also indicates that the highest shrinkage factor was in the control treatment on day 90, equal to 40.24, while the lowest connectivity factor was in the treatment containing 2% viscera on day 0, equal to 30.44. The loss of fat, water, and protein denaturation are the main reasons for diameter reduction during cooking. Since bioactive peptides have the ability to form gels and retain moisture, it can be concluded that the high retention of moisture and fat in samples containing the maximum amount of viscera prevented diameter reduction and shrinkage. The results of measuring water and fat retention also support this assertion.

Table 3. Interaction effects of treatment (Treatment-Day) on Cooking loss, Shrinkage and Cooking yield of cooked hamburger

Treatment-day	Cooking loss (%)	Shrinkage (%)	Cooking yield (%)
Blank-Day0	18.17±0.02[i]	37.22±0.01[f]	79.66±0.01[k]
Blank-Day30	20.66±0.02[f]	37.96±0.01[d]	75.83±0.02[p]
Blank-Day60	22.52±0.01[c]	39.19±0.02[b]	73.98±0.02[r]
Blank-Day90	25.75±0.02[a]	40.24±0.02[a]	71.14±0.02[t]
0.5%VH-Day0	17.24±0.01[j]	35.62±0.01[j]	81.54±0.01[h]
0.5%VH-Day30	19.23±0.01[h]	36.28±0.01[h]	78.90±0.02[m]
0.5%VH-Day60	21.65±0.00[d]	37.45±0.03[e]	76.24±0.01[o]
0.5%VH-Day90	23.55±0.01[b]	38.63±0.01[c]	73.70±0.02[s]
1%VH-Day0	15.79±0.01[l]	33.61±0.02[m]	82.49±0.02[f]
1%VH-Day30	17.59±0.02[j]	34.73±0.02[k]	80.15±0.02[j]
1%VH-Day60	19.13±0.02[h]	35.66±0.01[i]	77.71±0.02[n]
1%VH-Day90	21.18±0.01[e]	36.53±0.02[g]	75.47±0.02[q]
1.5%VH-Day0	14.65±0.02[m]	31.87±0.02[q]	85.29±0.02[c]
1.5%VH-Day30	16.25±0.03[k]	32.70±0.01[o]	83.19±0.01[e]
1.5%VH-Day60	17.50±0.48[j]	33.65±0.02[m]	81.27±0.02[i]
1.5%VH-Day90	19.62±0.01[g]	34.54±0.01[l]	79.15±0.03[l]
2%VH-Day0	12.87±0.01[n]	30.44±0.01[s]	87.42±0.01[a]
2%VH-Day30	14.37±0.02[m]	31.19±0.01[r]	85.52±0.01[b]
2%VH-Day60	15.90±0.02[l]	31.96±0.02[p]	83.66±0.02[d]
2%VH-Day90	17.30±0.02[j]	32.75±0.01[n]	81.70±0.02[g]

Means ± SE (n = 10) with different letters in each column indicate significant difference (P<0.05)

3-4- Fat and moisture retention

The interaction effect of time-treatment in all treatments shows that the highest fat retention factor was in the treatment containing 2% viscera on day 0, equal to 76.67, while the lowest connectivity factor was in the control treatment on day 90, equal to 63.23. The interaction effect of time-treatment in all treatments also indicates that the highest water retention factor was in the treatment containing 2% viscera on day 0, equal to 48.5, while the lowest water retention was in the control treatment on day 90, equal to 35.93. Water-holding capacity, cooking yield, and diameter reduction of the samples are among the most important tests for determining the quality of meat products. The release of fat and water is the main factor leading to a reduction in these indices, which depends on the gel-forming ability of proteins and the changes in the nature of proteins during the heating process. The fat retention levels of the treatments are also related to the cooking yield and emulsifying capacity [19]. The results of Lopez et al., (2011) on the effects of cooking on the properties of low-fat burgers indicate that the increase in cooking yield can be attributed to better retention of fat and moisture in the texture during cooking, which aligns with the findings of this study [20].

Table 4. Interaction effects of treatment-time (Treatment-day) on fat retention and moisture retention of cooked hamburger

Treatment-day	Fat retention (%)	Moisture retention (%)
Blank-Day0	64.87±0.02[q]	37.46±0.01[p]
Blank-Day30	64.50±0.01[r]	36.94±0.02[q]
Blank-Day60	64.15±0.03[s]	36.44±0.01[r]
Blank-Day90	63.23±0.02[t]	35.93±0.02[s]
0.5%VH-Day0	66.84±0.02[m]	38.69±0.02[m]
0.5%VH-Day30	66.15±0.01[n]	38.24±0.01[n]
0.5%VH-Day60	65.38±0.01[o]	37.78±0.02[o]
0.5%VH-Day90	65.81±0.02[p]	37.42±0.01[p]
1%VH-Day0	69.27±0.02[i]	41.17±0.02[i]
1%VH-Day30	68.92±0.02[j]	40.81±0.02[j]
1%VH-Day60	68.57±0.03[k]	40.43±0.02[k]
1%VH-Day90	68.32±0.01[l]	40.11±0.02[l]
1.5%VH-Day0	73.43±0.03[e]	45.37±0.01[e]
1.5%VH-Day30	73.11±0.02[f]	45.09±0.02[f]
1.5%VH-Day60	72.86±0.02[g]	44.72±0.01[g]
1.5%VH-Day90	72.52±0.01[h]	44.42±0.03[h]
2%VH-Day0	76.67±0.02[a]	48.55±0.01[a]
2%VH-Day30	76.42±0.02[b]	48.27±0.02[b]
2%VH-Day60	76.14±0.03[c]	47.94±0.03[c]
2%VH-Day90	75.88±0.02[d]	47.64±0.02[d]

Means ± SE (n = 10) with different letters in each column indicate significant difference ($P<0.05$)

3-5 Sensory evaluation

In all treatments, undesirable odors arise from oxidative fat spoilage, the formation of low molecular weight compounds, protein degradation, and changes in the composition of trimethylamine oxide. Flavor and color are two important quality factors of meat products that affect consumer acceptance and shelf life. In this study, the scores given for the six evaluated indices (taste, odor, color, texture, appearance, and overall acceptability) decreased during the 90 days of storage. The treatments had a fresh odor and appearance in the initial days, which gradually diminished. Additionally, the indices of odor, appearance, and overall acceptability received higher scores

compared to the indices of taste, color, and texture. The decreasing trend for all measured factors aimed at sensory

evaluation of the quality of the treatments was significant on most days.

Table 5. Interaction effects of treatment-time (Treatment-Day) on sensory evaluation of cooked hamburger

Treatment-day	Odor	Taste	Color	Texture	Appearance	Overall acceptability
Blank-Day0	6.66 ± 0.01^a	5.82 ± 0.02^{ab}	4.71 ± 0.01^a	5.81 ± 0.01^a	7.42 ± 0.02^a	7.57 ± 0.02^a
Blank-Day30	6.54 ± 0.01^b	5.71 ± 0.01^{abc}	4.45 ± 0.01^c	5.61 ± 0.02^c	7.24 ± 0.01^b	7.31 ± 0.02^b
Blank-Day60	6.23 ± 0.02^d	$5.34\pm0.02^{a-e}$	3.86 ± 0.01^g	5.38 ± 0.01^f	7.13 ± 0.02^c	7.23 ± 0.02^c
Blank-Day90	6.17 ± 0.02^e	$4.86\pm0.01^{b-f}$	3.51 ± 0.01^l	4.82 ± 0.02^l	6.85 ± 0.01^d	7.16 ± 0.03^d
0.5%VH-Day0	6.56 ± 0.01^b	5.73 ± 0.02^{abc}	4.53 ± 0.02^b	5.67 ± 0.02^b	7.27 ± 0.02^b	7.21 ± 0.01^c
0.5%VH-Day30	6.42 ± 0.02^c	$5.32\pm0.02^{a-e}$	4.20 ± 0.02^e	5.39 ± 0.01^{ef}	7.16 ± 0.01^c	6.86 ± 0.01^f
0.5%VH-Day60	6.21 ± 0.02^{de}	$4.67\pm0.02^{b-f}$	3.73 ± 0.02^i	5.33 ± 0.01^{gh}	6.65 ± 0.01^f	6.71 ± 0.02^{gh}
0.5%VH-Day90	5.83 ± 0.02^f	4.27 ± 0.02^{ef}	3.51 ± 0.01^l	5.25 ± 0.00^i	6.53 ± 0.01^g	6.66 ± 0.03^h
1%VH-Day0	6.24 ± 0.02^d	$5.58\pm0.02^{a-d}$	4.24 ± 0.01^d	5.48 ± 0.01^d	7.14 ± 0.02^c	6.91 ± 0.02^e
1%VH-Day30	5.71 ± 0.02^g	$5.42\pm0.01^{a-e}$	4.14 ± 0.02^f	5.31 ± 0.01^h	6.77 ± 0.02^e	6.75 ± 0.03^g
1%VH-Day60	5.43 ± 0.02^i	$5.22\pm0.01^{a-f}$	3.72 ± 0.02^i	5.23 ± 0.01^i	6.66 ± 0.02^f	6.52 ± 0.02^j
1%VH-Day90	4.86 ± 0.01^m	$4.73\pm0.00^{b-f}$	3.51 ± 0.01^l	5.13 ± 0.02^j	6.55 ± 0.03^g	6.33 ± 0.01^l
1.5%VH-Day0	5.69 ± 0.02^{gh}	$5.33\pm0.01^{a-e}$	3.80 ± 0.01^h	5.43 ± 0.03^e	6.88 ± 0.01^d	6.61 ± 0.02^i
1.5%VH-Day30	5.66 ± 0.01^h	$5.12\pm0.02^{a-f}$	3.64 ± 0.02^j	5.30 ± 0.02^h	6.76 ± 0.01^e	6.42 ± 0.02^k
1.5%VH-Day60	5.38 ± 0.01^j	$4.84\pm0.02^{b-f}$	3.54 ± 0.02^{kl}	5.13 ± 0.01^j	6.39 ± 0.02^h	6.30 ± 0.01^{lm}
1.5%VH-Day90	5.20 ± 0.02^l	6.19 ± 1.49^a	3.25 ± 0.01^o	4.90 ± 0.02^k	6.25 ± 0.03^j	6.22 ± 0.02^{no}
2%VH-Day0	5.42 ± 0.02^i	$4.78\pm0.01^{b-f}$	3.56 ± 0.01^k	5.36 ± 0.03^{fg}	6.52 ± 0.02^g	6.44 ± 0.02^k
2%VH-Day30	5.24 ± 0.01^k	$4.65\pm0.02^{c-f}$	3.43 ± 0.02^m	5.22 ± 0.01^i	6.31 ± 0.01^i	6.26 ± 0.01^{mn}
2%VH-Day60	4.89 ± 0.01^m	4.43 ± 0.03^{def}	3.38 ± 0.01^n	4.83 ± 0.02^l	6.26 ± 0.01^j	6.18 ± 0.01^o
2%VH-Day90	4.65 ± 0.01^n	4.16 ± 0.01^f	3.21 ± 0.01^o	4.76 ± 0.01^m	6.13 ± 0.02^k	5.85 ± 0.02^p

Means $\pm$ SE (n = 10) with different letters in each column indicate significant difference (P<0.05)

4- Conclusion

Optimal use of a low-value waste resource to transform it into a valuable product with antioxidant properties reduces the volume of fish waste and contributes to environmental sustainability. Moreover, this method allows for the use of natural antioxidants instead of synthetic antioxidant sources. The results of this

study showed that using hydrolyzed by-products from rainbow trout, which contain bioactive peptides, as a natural antioxidant in the formulation of cooked hamburgers improves cooking loss, cooking yield, and diameter reduction. Additionally, the water retention and fat retention in the hamburger samples increased with the percentage of viscera. Furthermore, the examination of textural properties revealed that the highest

connectivity factor and the lowest hardness factor were in the sample containing 2% viscera. According to sensory evaluation results, the control treatment received a higher score compared to the other treatments.

5- References

[1] Kumar, N. S., Nazeer, R. A., & Ganesh, R. J. (2012). Functional properties of tein hydrolysates from different body parts of horse mackerel (Magalaspis cordyla) and croaker (Otolithes ruber). editerranean Journal of Nutrition and Metabolism, 5(2), 105–110.

[2] Ovissipour, M., Abedian Kenari, A., Nazari, R., Motamedzadegan, A., & Rasco, B. (2014). Tuna viscera protein hydrolysate: nutritive and disease resistance properties for Persian sturgeon (Acipenser persicus L.) larvae. Aquaculture Research, 45(4), 591–601.

[3] Kumar, N. S., Nazeer, R. A., & Jaiganesh, R. (2011). Purification and biochemical characterization of antioxidant peptide from horse mackerel (Magalaspis cordyla) viscera protein. Peptides, 32(7), 1496–1501.

[4] Soumya, N. P., Mini, S., Sivan, K. Sh., & Mondal, S. (2021). Bioactive compounds in functional food and their role as therapeutics. Bioactive compounds in health and disease. 4(3), 24-39.

[5] Boutin Y, Paradis ME, Couture P, Immunological effect of fish protein supplementation on healthy adults. J Nat Prod 5 (2012) 37-44.

[6] El-Nassage, D. E., Refaat, W. A. (2017). Antioxidant and antibacterial activites of beef burger by using kiwi fruits peel powder. Journal of home economics, 27(1), 31-53.

[7] Wang, Sh., Zhao, M., Fan, H., & Wu, J. (2023). Peptidomics study of plant-based meat analogs as a source of bioactive peptides. Foods journal MDPI. 12(5), 1-15.

[8] Shaabani, Sh., Hoseini, E., Mahasti, P & Goodarzi, L. (2015). An investigation on antioxidant effect of garlic aqueous extract on uncooked hamburger. Journal of food science and Technology, 2(3), 31-37.

[9] Gorran, A., Shivazad, M & Rezaeian, M. (2017). Efficacy of Aqueous Extract of *Thymus Daenensis* to Ameliorate the Adverse Effects of Aflatoxin in

Japanese Quail. Research on Animal Production. 8(15), 25-32.

[10] Bergamaschi, M., Simoncini, N., Spezzano, V. M., Ferri, M., & Tassoni, A. (2023). Antioxidant and sensory properties of raw and cooked pork meat burgers formulated with extract from non-compliant green coffee beans. Foods journal MDPI, 12(6), 1-16.

[11] Shafiee, S., Goli, M., Khoshkhoo, Zh & Hosseini, E. (2021). Optimization of hydrolysis conditions (temperature, time, and concentration of alkalase) of rainbow trout viscera using the response surface methodology. Journal of Food Processing and Preservation, 45(5), e15456.

[12] Thiansilakul, Y., Benjakul, S., & Shahidi, F. (2007). Compositions, functional properties and anti-oxidative activity of protein hydrolysates prepared from round scad (Decapterus maruadsi). Food Chemistry, 103(4), 1385–1394.

[13] Rodrigues de souza, M. L., Gasparino, E., Coradini, M. F., Vieira, V. I., Siemer, S., & Feihrmann, A. C. (2022). Fish carcass flours from different species and their incorporation in tapioca cookies. Futures food. Doi: 10.1016/j. fufo. 2022. 100132.

[14] Liu, Y., Li, X., Chen, Z., Yu, J., Wang, F., & Wang, J. (2014). Characterization of structural and functional properties of fish protein hydrolysates from surimi processing by-products. Food Chemistry, 151, 459–465.

[15] Niu, J., Zhang, Y. Q., Liu, Y. J., Tian, L. X., Lin, H. Z., Chen, X., et al. (2014). Effects of graded replacement of fish meal by fish protein hydrolysate on growth performance of early post-larval Pacific white shrimp (Litopenaeus vannamei, Boone). Journal of Applied Animal Research, 42(1), 6–15.

[16] Nikoo M, Benjakul S, Potential application of seafood-derived peptides as bio functional ingredients, antioxidant–cryoprotectant: A review. JFunct Foods 10 (2015) 753-764.

[17] Nchienzia, H. A., Morawicki, R. O., & Gadang, V. P. (2010). Enzymatic hydrolysis of poultry meal with endo- and exopeptidases. Poultry Science, 89(10), 2273–2280.

[18] Wang, W., Li, Z., Liu, J., Wang, Y., Liu, S., & Sun, M. (2013). Comparison between thermal hydrolysis and enzymatic proteolysis process for the preparation of tilapia skin collagen hydrolysates. Czech Journal of Food Science, 31(1), 1–4.

[19] Zakey, A. A., Gandara, S. J., Eun, J. B., Shim, J. H., & Abd El-Aty, A. M. (2021). Bioactivites, applications, safety and health benefits of bioactive peptides from food and by-products. Food chemistry, 8, 1-18.

[20] Mokrejs, P., Svoboda, P., Hrncirik, J., Janacova, D., & Vasek, V. (2011). Processing poultry feathers into keratin hydrolysate through alkaline-enzymatic hydrolysis. Waste Management & Research, 29(3), 260–267.

[21] Norgaard, J. V., Blaabjerg, K., & Poulsen, H. D. (2012). Salmon protein hydrolysate as a protein source in feed for young pigs. Animal Feed Science and Technology, 177(1-2), 124–129.

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مقاله علمی_پژوهشی

بررسی افزودن پروتئین هیدرولیز شده احشائی ماهی قزل آلای رنگین کمان بر خواص کیفی همبرگر پخته شده

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چکیده	اطلاعات مقاله
اکسیداسیون چربی در طول دوره نگهداری از عوامل مهم در افت کیفیت مـاده غـذایی است. ضایعات ماهی هیدرولیز شده از مهم ترین منابع پپتید های زیست فعال به عنوان نوعی آنتی اکسیدان طبیعی می باشد. هدف از این پژوهش افزودن پپتید زیست فعال حاصل از هیدرولیز آنزیمی ضایعات ماهی قزل آلای رنگین کمان (ویسرا)، به فرمولاسیون همبرگر و اندازه گیری خصوصیات همبرگر پخته شده است. ضایعات ماهی در شرایط اپتیمم (دمای ۵۹ درجه سانتی گراد، زمان ۱۱۸ دقیقه و غلظت ۲ درصد آنزیم آلکالاز) هیدرولیز شده و سپس، به مقدار ۰/۵ ، ۱ ، ۱/۵ و ۲ درصد وزنی به نمونه های همبرگر، افزوده شد. یک تیمار نیز به عنوان شاهد است. آزمون‌ها در ۳ تکرار و مقایسه ی میانگین ها با آزمون دانکن جهت بررسی معنادار بودن متغیرها در ۰/۰۵ >P و داده ها به صورت میانگین ± انحراف میانگین گزارش شدند. نتایج آزمون ها نشان داد که فرمولاسیون همبرگر پخته ی حاوی ۲ ٪ وزنی ویسرا، دارای بالاترین میزان عملکرد پخت، احتباس آب و چربی و همچنین کمترین میزان فاکتور سختی بافت، افت پخت و کاهش قطر (چروکیدگی) می باشد. کاربرد بهینه از یک منبع ضایعاتی، در جهت تبدیل آن به فرآورده ی با ارزش با خاصیت آنتی اکسیدانی، سبب کاهش حجم ضایعات ماهی و کمک به محیط زیست می شود. با این روش می توان از آنتی اکسیدان های طبیعی به جای منابع سنتزی استفاده نمود.	