

Impact of Processing Conditions and Membrane Fractionation on Chloropropanol Formation during Protein Hydrolysate Production from Meat Trimming By-products

Elif Ekiz Terzioğlu

Ataturk University

Emel Oz

Ataturk University

Fatih Oz

fatihoz@atauni.edu.tr

Ataturk University

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Abstract

The valorization of meat processing by-products into value-added protein ingredients offers a sustainable approach to improving resource efficiency in food processing. However, processing operations used for protein recovery and hydrolysis may also influence the occurrence of process-induced contaminants. In the present study, the effects of processing conditions and membrane fractionation on the occurrence of the chloropropanols 3-chloropropane-1,2-diol (3-MCPD) and 2-monochloropropane-1,3-diol (2-MCPD) were investigated during protein hydrolysate production from meat trimming by-products. Proteins were extracted under different acidic and alkaline conditions (pH 2.5, 3.0, 3.5, 4.0, 10.0, 10.5, 11.0, and 12.0), enzymatically hydrolyzed using Alcalase, and subsequently subjected to membrane fractionation (< 3 kDa). Chloropropanols were evaluated at successive processing stages, including protein extraction, enzymatic hydrolysis, and membrane fractionation. The production process significantly affected 2-MCPD levels ($P < 0.01$), whereas extraction pH had no significant effect ($P > 0.05$). 3-MCPD remained below the detection limit in all analyzed samples, while 2-MCPD was detected in non-fractionated protein hydrolysates but not in the protein extracts or < 3 kDa fractions. These findings indicate that individual processing stages influence the occurrence of chloropropanols during protein hydrolysate production and suggest that membrane fractionation may contribute to reducing 2-MCPD occurrence, potentially through the removal of precursor-associated components. Overall, the study provides new insights into the relationship between processing conditions and process-induced contaminant occurrence during the sustainable production of value-added protein ingredients from meat processing by-products.

1. Introduction

Proteins are essential macromolecules that play fundamental roles in cellular structure, metabolism, and physiological regulation. They are biopolymers composed of amino acids linked by peptide bonds and constitute key components of enzymes, hormones, and transport systems involved in numerous biological processes (Institute of Medicine, 2005; Hettiarachchy et al., 2012; Khan et al., 2017; Chalamaiah et al., 2018; Wu et al., 2020). Because of their critical physiological functions, the Institute of Medicine (2005) recommends a daily protein intake of approximately 0.8 g per kilogram of body weight. In this context, meat and meat products represent an important source of high-quality dietary proteins. However, the production and processing of meat generate large quantities of protein-rich by-products. Reports indicate that approximately 18.4 million tons of slaughterhouse waste were generated in the United States in 2016, while nearly 100 million tons of protein-rich by-products are generated globally each year (Ling et al., 2022; Zhao et al., 2022). The improper management or underutilization of these protein-rich by-products contributes significantly to environmental pollution, greenhouse gas emissions, and economic losses due to disposal costs (Etemadian et al., 2021). According to the Food and Agriculture Organization (FAO, 2023), global meat production is projected to reach approximately 364 million tons in the coming years. Given the substantial volume of by-products generated during meat processing, together with natural resource depletion, global population growth, and rising food demand, their efficient valorization is increasingly recognized as an essential component of sustainable food

systems. Among the potential valorization strategies, the production of protein hydrolysates from protein-rich meat by-products has attracted considerable attention. Protein hydrolysates are produced through enzymatic or chemical hydrolysis processes that convert complex proteins into peptides and free amino acids with improved functional and biological properties. These products can contribute to environmental sustainability by enabling the valorization of protein-rich by-products and transforming low-value materials into high-value functional ingredients (Romero-Garay et al., 2022). Moreover, protein hydrolysates have broad applications in the food, pharmaceutical, cosmetic, and agricultural industries. In addition to their nutritional value, protein hydrolysates may exhibit various bioactive properties, including antioxidant, antihypertensive, antimicrobial, and immunomodulatory activities. Several studies have also reported their potential anticarcinogenic, anti-inflammatory, and hypocholesterolemic effects. Furthermore, protein hydrolysates can improve several techno-functional properties of foods, such as foaming capacity, emulsification, solubility, gelation, oil-binding capacity, and water-holding capacity (Karami & Akbari, 2019; Ramakrishnan et al., 2023). These functional properties are largely influenced by the raw material, hydrolysis conditions, and processing parameters. Despite these advantages, certain processing conditions applied during protein hydrolysate production may lead to the formation of process-induced contaminants. Among these contaminants, chloropropanols have attracted considerable attention due to their potential toxicological effects and their occurrence in thermally processed foods.

Chloropropanols were first identified in vegetable protein hydrolysates by Czechoslovakian scientists in 1978 and were classified as chlorohydrins (Velíšek et al., 1978). In a subsequent model system study conducted in 1979, one of the chloropropanol compounds, 3-chloropropane-1,2-diol (3-MCPD), was detected in lipids hydrolyzed with hydrochloric acid. Later studies reported the occurrence of 3-MCPD in acid-hydrolyzed vegetable proteins (Velíšek et al., 2003; Abu-El-Haj et al., 2007; Yildirim & Yorulmaz, 2017; Turan et al., 2018). Over time, chloropropanols have been detected in a variety of food products and have attracted increasing attention due to their potential carcinogenic and genotoxic effects. These compounds are contaminants formed during the hydrolysis of lipids in the presence of chloride ions in fats or fat-containing foods during thermal processing (Araujo et al., 2020; Sim et al., 2020; Martin et al., 2021).

Chloropropanols are generally classified into two main groups: monochloropropanediols (MCPDs) and dichloropropanediols (DCPs), depending on the number and position of chlorine atoms attached to the propanol backbone. Typical examples include 3-MCPD, 2-monochloropropane-1,3-diol (2-MCPD), and 1,3-dichloropropane-2-ol (1,3-DCP) (Turan et al., 2018). MCPDs may occur in monoester and diester forms, whereas DCPs generally occur in monoester forms (Eisenreich et al., 2023; Mou et al., 2023). In addition, chloropropanols may exist in both free and bound forms when esterified with fatty acids (Arris et al., 2020).

Among these compounds, 3-MCPD is the chloropropanol most frequently detected in foods. It is a colorless, slightly oily liquid with a molecular formula of $C_3H_7ClO_2$, a boiling point of 213°C, a molecular weight of 110.539 g/mol, and a density of 1.32 g/cm³ (Hamlet et al., 2002a; Arris et al., 2020).

Chloropropanols are generally formed during thermal processing as a result of reactions between lipid-derived precursors—such as triglycerides, glycerol, and phospholipids—and chlorine-containing compounds in foods (Velíšek et al., 2003; Önal et al., 2016; Martin et al., 2021). In particular, the presence of precursors such as glycerol, lipids, carbohydrates, and hydrochloric acid under low water activity conditions may promote the formation of free 3-MCPD in thermally processed foods. In addition to vegetable protein hydrolysates, chloropropanols have been reported in several food products including edible oils, baked goods, soy sauce, and thermally processed meat products (Taghizadeh et al., 2023). Similarly, 2-MCPD can be formed when lipid-containing systems such as glycerol, lecithin, or triolein are subjected to acidic conditions and thermal treatment (Mou et al., 2023).

In recent years, protein extracts and protein hydrolysates have attracted considerable attention as functional food ingredients due to their high nutritional value and desirable techno-functional properties. These ingredients are increasingly incorporated into various food formulations, including soups, sauces, meat products, infant foods, and nutritionally enriched foods. Although chloropropanol formation is generally associated with lipid-derived precursors, protein extracts and protein hydrolysates produced from meat processing by-products may still contain residual lipid components originating from the raw material. These residual lipids may act as precursor compounds for chloropropanol formation during processing.

Processing steps applied during protein extraction and enzymatic hydrolysis, such as heating, pH adjustments, and enzymatic treatments, may create conditions favorable for the formation of certain process-induced contaminants. In systems containing residual lipids, monoacylglycerols, diacylglycerols, and glycerol may serve as important precursors for chloropropanol formation. Under acidic or thermal processing conditions, interactions between glycerol-based lipid structures and chloride ions may lead to the formation of chlorinated propanol derivatives such as 2-MCPD and 3-MCPD (Velíšek et al., 2002). Similar reaction pathways have been reported in several food matrices, including vegetable oils, hydrolyzed vegetable proteins, and thermally processed meat products (Collier et al., 1991; Velíšek et al., 2002).

Chloropropanol compounds have also raised significant toxicological concerns. Experimental studies have shown that chronic exposure to 3-MCPD may lead to adverse effects in several organs, particularly the kidneys, causing nephropathy and renal tubular hyperplasia (JECFA, 2002; EFSA, 2013). Additional animal studies have reported reproductive, neurological, and immunological effects associated with prolonged exposure to this compound (Kirton et al., 1970; Samojlik & Chang, 1970; Eisenreich et al., 2023). Due to these toxicological concerns, the occurrence of chloropropanol compounds in food systems has been increasingly investigated in food safety research.

Although numerous studies have investigated chloropropanol formation in different food products (Vicente et al., 2015; Wong et al., 2020; Zhong et al., 2022), to the best of our knowledge, the occurrence of chloropropanol compounds in protein hydrolysates derived from meat processing by-products has not yet been systematically investigated. Considering that protein hydrolysates may still contain residual

lipid components that could act as potential precursors for chloropropanol formation during processing, evaluating these compounds in such systems is important from a food safety perspective. Therefore, the aim of the present study was twofold: (i) to utilize nutrient-rich meat trimming by-products for the production of nutritionally valuable and functionally active protein hydrolysates, and (ii) to evaluate the formation of chloropropanol compounds (2-MCPD and 3-MCPD) during different stages of protein hydrolysate production. To achieve this, proteins were extracted from meat trimming by-products at eight different pH values (2.5, 3.0, 3.5, 4.0, 10.0, 10.5, 11.0, and 12.0), followed by enzymatic hydrolysis using Alcalase. The obtained protein hydrolysates were subsequently fractionated into fractions with molecular weights below 3 kDa. The occurrence of 2-MCPD and 3-MCPD was investigated at three stages of production: protein extracts obtained at different pH values, non-fractionated protein hydrolysates, and fractionated protein hydrolysates. Accordingly, this study provides a food safety-oriented evaluation of chloropropanol formation during the production of protein hydrolysates derived from meat processing by-products.

2. Materials and methods

2.1. Material

This study investigated the production of protein hydrolysates from meat trimming by-products, including meat, adipose tissue, fascia, and connective tissue, collected from slaughtered cattle carcasses. The samples were obtained from the Erzurum Meat and Dairy Institution slaughterhouse after routine ante-mortem and post-mortem veterinary inspections. The samples were transported to the laboratory under cold chain conditions (maintained at 4°C using insulated containers with ice packs) and immediately processed. All experimental procedures were performed in triplicate (n = 3).

2.2. Method

2.2.1. Protein extraction and hydrolysate production

To extract the proteins, the minced meat trimmings were homogenized with distilled water for 45 s at a ratio of 1:7 (w:v). The pH of the homogenate was then adjusted using 1 M HCl for acidic pH values (2.5, 3.0, 3.5, and 4.0) and 2 M NaOH for alkaline pH values (10.0, 10.5, 11.0, and 12.0), and the mixture was stirred for 30 min at room temperature to allow protein solubilization. Soluble protein extracts were then obtained by centrifugation (Lab312, TGL16) at 10,000 g for 20 min (Neves et al., 2017). To produce the protein hydrolysate, the protein extracts were equilibrated at the optimum enzyme temperature (50°C) for 10 min, and the pH was adjusted to 8.0. Then, the alcalase enzyme (Merck, Germany; 100% purity and ≥ 0.75 AU/mL activity) was added to achieve an enzyme-to-substrate ratio of 1% (w/w) (Taheri et al., 2013; Pires et al., 2012; Meshginfar et al., 2016). Hydrolysis was performed at 50°C for 4 h (JSR, JSSB-30T). After hydrolysis, the mixture was heat-treated at 90°C for 10 min to inactivate the enzymes, then cooled to room temperature. The resulting hydrolysates were then subjected to ultrafiltration (EZ-Fit® Manifold and Filtration Units, Millipore) to obtain peptide fractions with molecular weights below 3 kDa.

(Ultracel 3 kDa, Ultrafiltration Discs, Millipore). The obtained fractions were lyophilized and stored in vacuum-sealed bags at -18°C (Li-Chan et al., 2012; Neves et al., 2016; Neves et al., 2017). Figure 1 presents the production flow diagram of protein hydrolysates, and Fig. 2 presents images of protein hydrolysates.

Insert Fig. 1 and Fig. 2 here please!

2.2.2. Determination of chloropropanol compounds

Following the methodology described by Brereton and others (2001), the presence of chloropropanol compounds (2-MCPD and 3-MCPD) in protein hydrolysates was investigated. In this study, an internal standard (1,3-DCP) and Extrelut NT packing material were added to the protein hydrolysate samples and mixed thoroughly. A mixture of hexane and diethyl ether was then used to remove nonpolar compounds. The extraction process was finished by washing the esters with diethyl ether. After derivatization with heptafluorobutyryl imidazole (HFBI) at 70°C for 20 min, the chromatographic method was validated, and the amounts of the chloropropanol esters were determined using Gas Chromatography-Mass Spectrometry (GC-MS, Shimadzu, QP2010 Plus).

In the GC-MS system, analysis was conducted using a DB-5MS column (30 m × 0.25 mm, 0.25 µm film thickness) in splitless injection mode with a flow rate of 1 mL/min of high-purity helium gas as the carrier gas. The injection temperature was set to 270°C, the ion source temperature to 230°C, and the interface temperature to 270°C. The initial temperature in the program was set to 50°C and held for one min. Then, it was increased to 90°C at a rate of 1°C/min and held at this temperature for two min. Then, the temperature increased to 300°C at a rate of 50°C/min and was held for five min. The ions determined by GC-MS (SCAN method) in the standards used to analyze chloropropanol compounds are provided in Table 1.

Insert Table 1 here please!

2.3. Statistical analysis

The study was designed using a completely randomized design with three replications. Data were analyzed using SPSS statistical software, and the Duncan's multiple range test was used to evaluate differences between the results.

3. Results and Discussion

3.1. Method validation

The use of heptafluorobutyryl imidazole (HFBI) as a derivatizing agent enabled the sensitive detection of trace levels of 2-MCPD and 3-MCPD. This reagent readily forms stable derivatives with polar compounds, thereby improving their volatility and reducing contamination of the GC-MS system (Hamlet & Sutton, 1997; Hamlet, 1998). The method validation results, including the limit of detection (LOD), the limit of quantification (LOQ), the regression coefficient, and the recovery values, are presented in Table 2. The

determined LOD values were 164.97 ppb for 3-MCPD and 52.22 ppb for 2-MCPD. Although the LOD for 3-MCPD is relatively higher, these values fall within the broad range (1–1000 ppb) reported by EFSA (2013), where LOD and LOQ values for MCPD analysis vary substantially depending on the analytical method and food matrix (1–1000 µg/kg). The obtained validation parameters were consistent with those previously reported in the literature (Kim et al., 2008; Wang et al., 2016; Zheng et al., 2021). On the other hand, it is important to remember that “not detected (nd)” results do not indicate the complete absence of toxic compounds, but rather that their concentration, if present, remained below the detection capacity of the applied method.

Table 2

The LOD, LOQ, regression coefficients and recovery values of 3-MCPD and 2-MCPD.

Compounds	LOD (ppb)	LOQ (ppb)	Regression Coefficient	Recovery (%)
3-MCPD	164.97	499.92	0.9944	98.48
2-MCPD	52.22	158.23	0.9971	102.25

Insert Table 2 here please!

3.2. Chloropropanol levels

The formation of chloropropanol compounds was evaluated at different stages of protein hydrolysate production, including protein extraction, pre-fractionation (non-fractionated hydrolysate), and post-fractionation (< 3kDa fractionated hydrolysate). This approach allowed identification of the stage(s) at which the chloropropanol compounds may be generated. Table 3 presents the effects of processing steps and extraction pH on the levels of 3-MCPD and 2-MCPD in protein hydrolysates produced from meat trimming by-products. As shown in Table 3, neither the processing stages nor the extraction pH had a statistically significant effect ($P > 0.05$) on 3-MCPD levels. In contrast, the production process steps had a statistically significant effect ($P < 0.01$) on 2-MCPD, while the extraction pH itself did not significantly influence its formation ($P > 0.05$). Relatively large standard deviations were observed in some samples. All analyses were performed in triplicate, and MCPD compounds were detected at trace concentration levels (ppb range); therefore, small analytical variations together with matrix heterogeneity may contribute to increased variability in replicate analyses. It should also be noted that the data presented in Table 3 reflect the combined effects of both processing stages and different pH values on MCPD formation, rather than a single factor. Accordingly, this variability likely reflects the interactive influence of processing conditions and pH, as well as the presence of both quantifiable and below-detection-limit (ND) values within the dataset. Potential variations in raw material composition between batches may have also contributed to the observed dispersion.

No 3-MCPD was detected in protein extracts obtained from meat trimming by-products at different pH values, nor in the non-fractionated or fractionated protein hydrolysates (< 3kDa) produced from these extracts. However, the absence of detectable 3-MCPD should be interpreted in relation to the detection limits of the analytical method applied in this study. The determined LOD values were 164.97 ppb for 3-

MCPD and 52.22 ppb for 2-MCPD. Although the LOD for 3-MCPD is relatively higher, these values fall within the broad range reported by EFSA (2013), where LOD and LOQ values for MCPD analysis vary substantially depending on the analytical method and food matrix (1–1000 µg/kg). The determined LOD and LOQ values indicate that low concentrations of 3-MCPD, if present, may remain below the detection capability of the method. Therefore, the term “not detected” does not necessarily indicate the complete absence of this compound but rather suggests that its concentration may be below the detection limit of the analytical method. Although 2-MCPD was not detected in the analyzed protein extracts or in the fractionated protein hydrolysates.

The main precursors of chloropropanols are reported to be mono-, di-, and triglycerides, as well as chlorine-containing compounds, and their formation is strongly influenced by processing conditions such as time, temperature, and pH (Chung et al., 2008; Albuquerque et al., 2019). Although defatting, centrifugation, and filtration steps remove a substantial portion of lipids during protein hydrolysate production, complete lipid removal is difficult, and residual lipids may remain in the system (Chalamaiah et al., 2012). Similarly, Klompong and others (2007) reported that residual lipids remained in minced fish meat even after defatting treatment, indicating that complete lipid removal is difficult in protein recovery processes. In the present study, a visible fat layer was observed in the supernatant phase during centrifugation at the protein extraction stage. Although this layer was removed during protein recovery, it is likely that small amounts of residual lipids remained in the resulting protein hydrolysates. In addition, the heat treatments applied during enzymatic hydrolysis (4 h incubation at 50°C and 10 min enzyme inactivation at 90°C) may have contributed to the formation of 2-MCPD. The formation of chloropropanol compounds in protein hydrolysate systems may therefore be explained by the interaction between lipid-derived precursors, chloride ions, and thermal processing conditions. Residual lipids present in the raw material may contain monoacylglycerols, diacylglycerols, and glycerol, which are recognized precursors of chloropropanol formation. In the presence of chloride ions and heat, these compounds may undergo reactions leading to the formation of chlorinated propanol derivatives such as 2-MCPD and 3-MCPD (Velíšek et al., 2002). Similar reaction pathways have been reported in other food matrices including vegetable oils, hydrolyzed vegetable proteins, and thermally processed meat products. Interestingly, 2-MCPD was detected only in the non-fractionated hydrolysates and not in the protein extracts or fractionated (< 3 kDa) hydrolysates. This observation may be associated with the presence of lipid-related precursors or peptide–lipid complexes that remain in the non-fractionated hydrolysates but are partially removed during membrane fractionation. Thus, ultrafiltration (< 3 kDa) appears to reduce the concentration of compounds potentially involved in 2-MCPD formation. The different behavior observed between 2-MCPD and 3-MCPD in the present study may also be related not only to differences in precursor availability and but also to distinct formation pathways and matrix-specific factors. Although MCPD formation is generally associated with lipid-derived precursors, emerging evidence indicates that amino acid composition may also influence isomer distribution. Certain amino acids have been reported to differentially affect MCPD formation; for example, glycine has been associated with promoting 2-MCPD formation, whereas tryptophan may enhance 3-MCPD formation while suppressing 2-MCPD, and glutamic acid may contribute to the formation of both isomers, in some cases favoring 3-MCPD (Li et al.,

2025). Furthermore, MCPD formation may proceed via common reactive intermediates such as acyloxonium ions or glycidol, and processes such as acyl migration between diacylglycerol isomers may influence the regioselectivity of chlorination, thereby affecting the relative formation of 2-MCPD and 3-MCPD (Mou et al., 2023; Li et al., 2025; Perestrelo et al., 2025; Shaari et al., 2025). Therefore, the predominance of one isomer over the other is strongly dependent on matrix composition and processing conditions. In this context, the selective detection of 2-MCPD in the present study may be attributed to the combined effects of residual lipid composition, amino acid profile of the hydrolysate matrix, and the applied processing conditions, which may have favored pathways leading to 2-MCPD formation while not promoting detectable levels of 3-MCPD. However, the present study was not designed to experimentally isolate these pathways, and thus the proposed explanations are based on interpretation of existing literature. Previous studies have shown that MCPD formation strongly depends on both lipid composition and processing conditions. Therefore, the formation of 2-MCPD in the non-fractionated hydrolysates may be associated with the presence of lipid-derived precursors and processing conditions favorable for its formation, whereas the conditions applied in the present study may not have favored the formation of detectable levels of 3-MCPD. From a food processing perspective, these findings suggest that membrane-based separation processes such as ultrafiltration may contribute to reducing chloropropanol contamination in protein hydrolysate systems by removing lipid-associated precursors or related compounds. Therefore, membrane fractionation may represent a potential strategy to minimize the presence of process-induced contaminants in protein hydrolysate-based ingredients. Although HCl was used to adjust the pH of the protein extracts, the absence of heat treatment during the extraction stage may have limited the formation of chloropropanol compounds. The relatively higher levels of 2-MCPD detected in hydrolysates produced from proteins extracted at alkaline pH values (10.0–12.0) may therefore be associated with the combined effects of heat treatment and the use of HCl to adjust the optimal pH for the alcalase activity. Hamlet and others (2002b) detected 0.014 mg/kg of 3-MCPD in one out of five samples of chicken meat extract, attributing its formation to precursors originating from post-slaughter chlorination or washing, even though the production process did not exceed 100°C. They also detected 0.078 mg/kg of 3-MCPD in vegetable protein hydrolyzed using three enzymes and associated its formation with chemical processes such as the use of organic acids or alkalis during enzyme inactivation.

As shown in Table 3, no 3-MCPD compounds were detected in the protein hydrolysates produced from protein extracts obtained from meat trimming by-products at different pH values. Similarly, 2-MCPD was not detected in protein hydrolysates produced from extracts obtained at pH values of 2.5 and 3.0. However, the 2-MCPD content of the hydrolysates obtained from extracts at other pH values (3.5, 4.0, 10.0, 10.5, 11.0, and 12.0) ranged from 19.50 to 101.37 ppb. No statistically significant differences were observed among these values.

Insert Table 3 here please!

Due to the absence of similar studies or comparable matrices, it was not possible to directly compare the 3-MCPD and 2-MCPD contents of the produced protein extracts with previously reported values in

the literature. In addition, studies focusing on 2-MCPD are considerably more limited than those investigating 3-MCPD. Nevertheless, several studies have reported the presence of 2-MCPD in various food products, including infant formulas, vegetable oils, and bakery products (Wang et al., 2016; Stauff et al., 2020; Cui et al., 2021a; Goh et al., 2021; Zheng et al., 2021; Liao et al., 2024).

In acid-catalyzed hydrolysis processes of vegetable proteins, acylglycerols, glycerols, and phospholipids may react with hydrochloric acid, leading to the formation of monochloropropanediol esters, which subsequently hydrolyze to produce monochloropropanediols (Stauff et al., 2020). In addition, the formation of 3-MCPD can be reduced when an acid hydrolysis process is followed by an alkaline treatment, because 3-MCPD is unstable and degrades at pH values above 6 (Lee & Khor, 2015). Starski and others (2013) reported that the formation mechanism of 3-MCPD is better understood than that of other chloropropanol compounds. Consequently, 3-MCPD is the most frequently identified chloropropanol in food systems. It is soluble in water and ethanol but unstable in alkaline environments. In triacylglycerol-containing model systems, temperature-dependent interconversion between 2-MCPD and 3-MCPD has also been reported, with glycidol acting as a reaction intermediate (Rahn & Yaylayan, 2011).

Hydrochloric acid is widely used in acidic hydrolysis processes and is commonly applied to adjust acidic pH values during the production of protein extracts, isolates, and concentrates, as well as during enzymatic hydrolysis to adjust the optimum pH of enzymes (Panpipat & Chaijan, 2016; Abdollahi & Undeland, 2019; Pezeshk et al., 2021). The results of the present study suggest that HCl may act as a chlorine donor during pH adjustment in the extraction and hydrolysis stages of protein hydrolysate production. In the present study, HCl was used both to prepare protein extracts at acidic pH values (2.5, 3.0, 3.5, and 4.0) and to adjust the optimal pH of the alcalase enzyme (pH 8.0) during the hydrolysis of proteins extracted at alkaline pH values (10.0, 10.5, 11.0, and 12.0). Although sulfuric acid (H_2SO_4) is sometimes preferred in acid hydrolysis processes to reduce the formation of 3-MCPD, the second proton in H_2SO_4 may negatively affect amino acid stability and consequently the functional properties of proteins (Flork, 1989).

Crews and others (2002) reported maximum 3-MCPD levels of 0.047 mg/kg in smoked bacon, 0.071 mg/kg in charcoal-cooked beef burgers, 0.028 mg/kg in packaged beef burgers, 0.011 mg/kg in various cooked meats, and 0.069 mg/kg in salami. Vicente and others (2015) reported that 3-MCPD levels in Brazilian products containing hydrolyzed vegetable protein ranged from nd to 2529 $\mu\text{g/kg}$, while levels in meat samples ranged from nd to 500 $\mu\text{g/kg}$. In addition, 3-MCPD levels ranged from nd to 15 $\mu\text{g/kg}$ in sausage samples, nd to 12 $\mu\text{g/kg}$ in ham samples, nd to 6 $\mu\text{g/kg}$ in turkey breast meat, and less than 5.9 $\mu\text{g/kg}$ in chicken breast meat. According to the EFSA report (2016), the average 3-MCPD levels were 25 $\mu\text{g/kg}$ in hydrolyzed vegetable proteins, 42 $\mu\text{g/kg}$ in oven-cooked or fried fish, 23 $\mu\text{g/kg}$ in oven-cooked or fried meats, 18 $\mu\text{g/kg}$ in smoked fish, and 24 $\mu\text{g/kg}$ in smoked meat products. The same report indicated that the average 2-MCPD levels were 14 $\mu\text{g/kg}$ in oven-cooked or fried fish, 8.38 $\mu\text{g/kg}$ in oven-cooked or fried meats, 6.1 $\mu\text{g/kg}$ in smoked fish, and 6.8 $\mu\text{g/kg}$ in smoked meat products.

EFSA has established a tolerable daily intake (TDI) of 2 µg/kg body weight/day for 3-MCPD (Cui et al., 2021b). The absence of detectable 3-MCPD in the analyzed samples may therefore indicate that the processing conditions applied in the present study did not favor the formation of this compound. However, due to limited toxicological data and the absence of an established tolerable daily intake value for 2-MCPD (Wöhrlin et al., 2015), no assessment of daily intake could be conducted in the present study.

4. Conclusion

Protein hydrolysates are increasingly used in various industries due to their nutritional value and functional properties. Therefore, evaluating potential food safety risks associated with their production remains important. In the present study, the occurrence of chloropropanol compounds (2-MCPD and 3-MCPD), which are considered potential toxic contaminants, was investigated in protein hydrolysates produced from meat trimming by-products under different processing conditions. The results showed that the hydrolysate production process may influence the formation of 2-MCPD, whereas 3-MCPD was not detected in the analyzed samples (< LOD). While 2-MCPD was not detected in protein extracts or ultrafiltrated fractions (< 3 kDa), it was detected in non-fractionated protein hydrolysates, with an average level of 183.73 ppb. In addition, depending on extraction pH conditions, 2-MCPD levels in the hydrolysates ranged from nd to 101.37 ppb (average values). These findings suggest that certain stages of hydrolysate production, particularly enzymatic hydrolysis and associated thermal treatments, may contribute to the formation of 2-MCPD under specific conditions. To the best of our knowledge, this is among the first studies evaluating chloropropanol formation in protein hydrolysates produced from meat trimming by-products. The results indicate that chloropropanol formation may occur at different stages of the production process and may be influenced by processing conditions and the presence of residual lipid-related precursors. From a food processing perspective, these findings suggest that parameters such as pH adjustment, heat treatment conditions, and the extent of lipid removal could play a role in controlling the formation of process-induced contaminants such as 2-MCPD. Further studies are needed to better define critical control points and optimize processing conditions in protein hydrolysate systems.

Declarations

CRedit authorship contribution statement

Elif Ekiz Terzioğlu: Methodology, Formal analysis, Investigation, Writing-original draft, Visualization; **Emel Oz:** Methodology, Validation, Investigation, Supervision; **Fatih Oz:** Conceptualization, Validation, Data curation, Writing-review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare no conflicts of interest.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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- ## Table 1 and 3

Figures



The flow diagram for the production of protein extracts and hydrolysates.

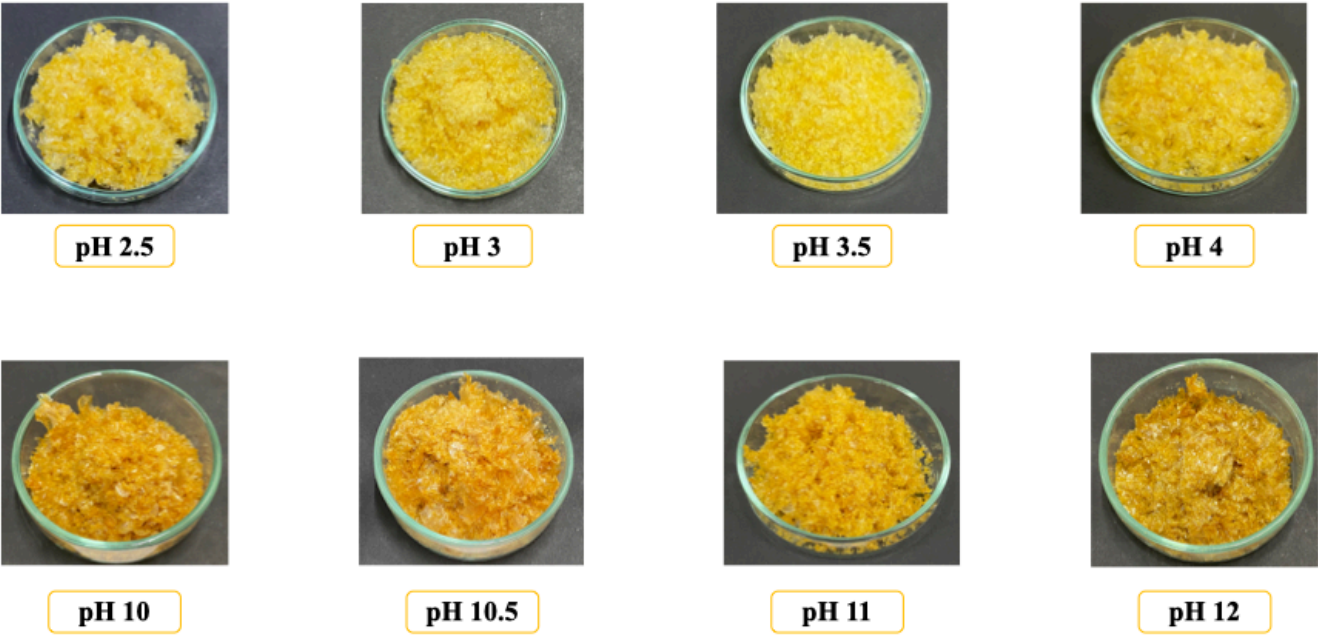


Figure 2

Images of protein hydrolysates produced from meat trimming waste.

Supplementary Files

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- [Table1and3.docx](#)