New Chlorine-Containing Organic Compounds in Protein Hydrolysates

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Esters of 1,3-dichloro-2-propanol and diesters and 1-esters of 3-chloro-1,2-propanediol were identified in samples of protein hydrolysates from their mass, infrared, and NMR spectra. Their content was 47 mg/kg in neutralized protein hydrolysate and 305 mg/kg in filter cake obtained by its filtration. All of the three types of esters contained the same fatty acids as the original material employed for the production of protein hydrolysate. The main acids were palmitic, stearic, oleic, linoleic, and linolenic acids. Myristic, palmitooleic, arachidic, gadoleic, and behenic acids were present in lower quantities. The content of unsaturated acids bound in the esters of glycerol chlorohydrins was lower than in that of the original raw material. The identified compounds, esters of glycerol chlorohydrins, represent a new class of chlorine-containing organic compounds in foodstuffs.

Chemical hydrolysates of proteins have become important commodities in many countries all over the world for the improvement of the flavor of various foods and enhancement of their meaty flavor. Currently, vegetable raw materials, e.g., wheat and maize glutens, soybean meal, flour, and others, have been employed in the process of hydrolysis.

All of these vegetable materials contain residual lipids mainly in the form of triacylglycerols and phospholipids. Their role and fate during the hydrolysis process were not established. Recently, the neutral fractions of several types of commercial and laboratory-made protein hydrolysates were analyzed in this laboratory and three not previously reported chlorine-containing alcohols were identified. 1,3-Dichloro-2-propanol, the main chlorine-containing organic compound, was present in concentrations of 0.17–0.94 mg/kg. Two other compounds, i.e., 3-chloro-1-propanol and 2,3-dichloro-1-propanol, were present in lower concentrations (Velíšek et al., 1978).

Beside was shown, all three alcohols together with some new compounds, e.g., 3-chloro-1,2-propanediol, were also formed in the reaction of glycerol with hydrochloric acid under conditions used for the manufacture of protein hydrolysates (Velíšek et al., 1979). 1,3-Dichloro-2-propanol as well as its isomer 2,3-dichloro-1-propanol and 3-chloro-1,2-propanediol were also identified as the hydrolytic products of triacylglycerols and phospholipids with hydrochloric acid (Davídek et al., 1978, 1980; Velíšek et al., 1979).

Besides these alcohols and diols, their corresponding esters with fatty acids are also formed. For example, acetates of 1,3-dichloro-2-propanol and 2,3-dichloro-1-propanol and the diacetate and 1-acetate of 3-chloro-1,2-propanediol were identified as reaction products of triacetin with hydrochloric acid (Velíšek et al., 1979). Tripalmitin, tristearin, and triolein were the precursors of corresponding diesters of 3-chloro-1,2-propanediol, the main reaction products followed by 1-esters of the same alcohol and esters of 1,3-dichloro-2-propanol (Davídek et al., 1980).

Dichlorohydrins as well as monochlorohydrins of glycerol found in protein hydrolysates are only intermediate products of the hydrolysis of lipids with hydrochloric acid; therefore, it could be expected that their immediate precursors, their esters with higher fatty acids, might also form during the production of protein hydrolysates analogically with the model samples of triacylglycerols and hydrochloric acid.

This work was therefore directed at finding the esters of glycerol chlorohydrins with higher fatty acids in protein hydrolysates and following their changes in various stages of protein hydrolysate production.

EXPERIMENTAL SECTION

Materials and Chemicals. Raw materials currently used for the production of protein hydrolysates including wheat and maize glutens and soybean meal, neutralized protein hydrolysate, and its filtrate and the corresponding filter cake (humin) as well as the final product were received from reliable commerical sources.

Liquid (neutralized hydrolysate, 1.5 kg; its filtrate, 3 kg; final commercial hydrolysate, 10 kg) or solid (filter cake, 0.5 kg, mixed with 500 mL of water) samples were repeatedly extracted with 200-mL portions of diethyl ether. The combined extracts were reextracted with 5% sodium bicarbonate to remove levulinic acid and lower fatty acids and dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure at 40 °C. Phenols were removed from an aliquot by extraction with 5% sodium hydroxide. The extract dissolved in chloroform was used for thin-layer and column chromatographic separations.

Glycerol chlorohydrins and their esters with fatty acids were synthesized (Conant and Quayle, 1946a,b; Hartman, 1957) and purified by distillation (Velíšek et al., 1979) and by column chromatography (Davídek et al., 1980), respectively.

Methods. Crude fat was determined according to the procedure of Folch et al. (1957). Fatty acid composition was determined by gas–liquid chromatography (IUPAC, 1974). Ethyl palmitate was used as an internal standard in the gas chromatographic determination of free fatty acids which were converted into their methyl esters with a diethyl ether solution of diazomethane.

Gas chromatographic measurements were performed on a Chrom 4 apparatus (Laboratorní přístroje n.p., Prague, Czechoslovakia) equipped with a flame ionization detector. A 2500 × 3 mm (i.d.) glass column packed with 15% (w/w) Carbowax 20 M on 0.125–0.16 mm Chromaton N-AW-DMCS (Lachema n.p., Brno, Czechoslovakia) and a 1200 × 3 mm glass column packed with 3% OV-101 on the same solid support were employed for the separation of fatty acid methyl esters and esters of glycerol chlorohydrins with fatty acids, respectively. Prior to the gas chromatographic analysis, 1-esters of 3-chloro-1,2-propanediol were converted into their acetates by reaction with acetic anhydride.

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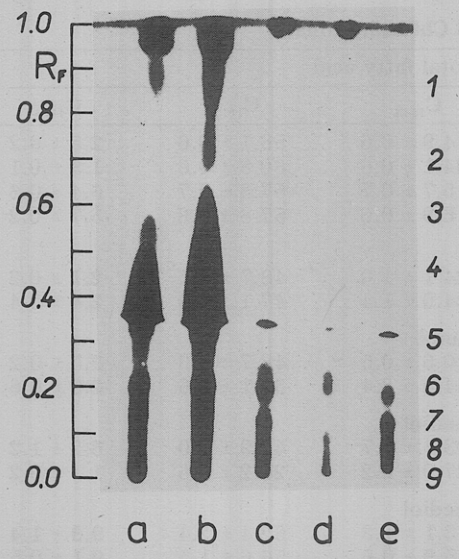

Figure 1. Thin-layer chromatogram of diethyl ether extracts of samples from various phases of protein hydrolysate production: (a) neutralized hydrolysate (1.7 mg), (b) filter cake (2.6 mg), (c) filtrate (0.3 mg), (d) filtrate without phenols (0.1 mg), (e) phenols from filtrate (0.2 mg). (1) Esters of 1,3-dichloro-2-propanol, (2) diesters of 3-chloro-1,2-propanediol, (3) triacylglycerols, (4) free fatty acids, (5) 1,3-dichloro-2-propanol, (6) 1-esters of 3-chloro-1,2-propanediol, (7) 1,3-diacylglycerols, (8) 1,2-diacylglycerols, and (9) monoacylglycerols

Mass and NMR spectrometric and infrared spectrophotometric measurements were performed on the same instruments and under the same conditions as described previously (Davídek et al., 1980).

The contents of esters of glycerol chlorohydrins and triacylglycerols were estimated by thin-layer chromatography (Davídek et al., 1980) using corresponding palmitates as standards. The known concentrations of the standards were chromatographed alongside portions of the sampless. Air-dried chromatograms were sprayed with a 0.01% ethanolic solution of dichlorofluorescein and observed under ultraviolet light (254 and 366 nm). The fluorescent intensities of the spots were compared visually.

RESULTS AND DISCUSSION

A thin-layer chromatographic separation of diethyl ether extracts of the neutralized hydrolysate, the corresponding filter cake (humin), and the filtrate is presented on Figure 1. The compounds representing the individual spots were separated and purified by repeated column chromatography on silica gel, and their mass, infrared, and NMR spectra were recorded and compared with those of the synthesized standards (Davídek et al., 1980).

On the basis of the obtained spectral data as well as by the comparison of the R_f values on thin-layer chromatograms and also by comparison of the retention indexes on gas chromatograms, it was found that samples a and b (Figure 1) contained esters of 1,3-dichloro-2-propanol and diesters and 1-esters of 3-chloro-1,2-propanediol with higher fatty acids. These chlorine-containing esters represent spot no. 1, 2, and 6 in Figure 1. Besides these compounds found for the first time as constituents of protein hydrolysates, some ordinary hydrolytic products of triacylglycerols (spot no. 3) such as 1,3-diacylglycerols (spot no. 7), 1,2-diacylglycerols (spot no. 8), monoacylglycerols (spot no. 9), and free fatty acids (spot no. 4) were also identified.

The above-mentioned esters of glycerol chlorohydrins with fatty acids were not identified in the sample of filtrate of the neutralized protein hydrolysate nor in the final commercial product which is used as soup seasoning. However, traces of glycerol chlorohydrins esters might be present in commercial protein hydrolysates since small amounts of free acids and di- and monoacylglycerols were identified there. The main components, however, were phenols and furans which represent the principal flavor-active consituents of protein hydrolysates (Manley and Fagerson, 1970a,b).

Table I. Concentrations in mg/kg of Some Constituents of the Analyzed Samples

compd	neutralized hydrolysate	filter cake
esters of 1,3-dichloro-2-propanol	8	65
diesters of 3-chloro-1,2-propanediol	4	35
1-esters of 3-chloro-1,2-propanediol	35	205
triacylglycerols	40	315
free fatty acids	1290	14000

All of the four analyzed samples also contained 1,3-dichloro-2-propanol (spot no. 5 in Figure 1) which has been identified as a volatile constituent of protein hydrolysates (Velíšek et al., 1978) and arises also in the reaction of triacylglycerols with hydrochloride acid (Velíšek et al., 1979; Davídek et al., 1980).

Table I presents the amounts of glycerol chlorohydrin esters. It is evident that the hydrolysis of lipids was not complete because the original triacylglycerols were also present in the analyzed samples. The content of free fatty acids was, however, lower than the original one owing to the fact that a part of them was coextracted together with levulinic acid and lower fatty acids. In filter cake the contents of all of the analyzed compounds were higher (approximately 10 times) than those in the neutralized hydrolyzate. Filter cake represents about 0.1 of the weight of the neutralized hydrolyzate, and therefore it is possible that glycerol chlorohydrins esters remain on the filter together with melanoidinss and other solids. In fact, the esters are nonpolar compounds, practically undissolved in the hydrolysate where they form a solid layer on its surface on cooling.

The content of lipids in raw materials employed for the production of protein hydrolysates was 2.09 ± 0.10 (wheat gluten), 2.24 ± 0.11 (maize gluten), and 3.03 ± 0.11 mg/kg (soybean meal), respectively. Depending on the proportions of the individual materials to be hydrolyzed, the mixture prepared for the production of 1000 kg of the final hydrolysate contained 9–11 kg of lipids, i.e., the average content of lipids was approximately 1%.

According to the classification of Ullmann (1956), lipids of the employed raw materials belong to the same group of lipids which contain linoleic and oleic acids as the major ones. The fatty acid composition of the used wheat and maize glutens and soybean meal as well as that of their mixture to be hydrolyzed can be seen in Table II.

Table II also presents the composition of free fatty acids as well as that of the fatty acids bound in glycerol chlorohydrins. It is evident that the fatty acids bound in glycerol chlorohydrins are qualitatively the same as those of the original mixture of raw materials. Some other fatty acids, i.e., myristic, palmitooleic, arachidic, gadoleic, and behenic acids, were present in small quantities and did not exceed 1%. In the hydrolysis process, unsaturated fatty acids (mainly linolenic and linoleic acids) decomposed or were oxidized and a significant loss also occurred during a further technological operation (filtration, as it can be seen by comparing their contents in neutralized hydrolysate and filter cake, respectively.

Qualitative and approximately quantitative results on the composition of fatty acids bound in glycerol chloro-

Table II. Fatty Acid Composition of Raw Materials and Esters of Glycerol Chlorohydrins

sample	% of total fatty acid				
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
wheat gluten	23.4 ± 0.5	1.2 ± 0.1	14.9 ± 0.6	58.1 ± 0.6	2.5 ± 0.2
maize gluten	11.6 ± 0.3	2.2 ± 0.3	24.1 ± 0.6	60.8 ± 0.6	1.3 ± 0.1
soybean meal	16.6 ± 0.6	4.0 ± 0.3	15.7 ± 0.7	57.3 ± 0.7	6.4 ± 0.6
mixture to be hydrolyzed	15.9 ± 0.2	3.7 ± 0.2	16.9 ± 0.6	57.8 ± 0.6	5.7 ± 0.2
	Free Fatty Acids				
neutralized hydrolysate	20.8 ± 0.5	4.3 ± 0.5	24.1 ± 1.0	48.7 ± 1.1	2.1 ± 0.2
filter cake	21.3 ± 1.0	4.3 ± 0.3	24.0 ± 1.4	47.1 ± 1.5	2.4 ± 0.4
	Esters of 1,3-Dichloro-2-propanol				
neutralized hydrolysate	22.8 ± 0.7	5.5 ± 0.4	20.6 ± 0.5	48.7 ± 0.3	2.5 ± 0.2
filter cake	24.4 ± 2.8	7.0 ± 1.2	34.5 ± 3.4	32.1 ± 2.6	2.3 ± 0.6
	Diesters of 3-Chloro-1,2-propanediol				
neutralized hydrolysate	22.0 ± 1.2	8.8 ± 0.2	23.1 ± 0.7	42.9 ± 1.0	3.2 ± 1.2
filter cake	33.0 ± 0.7	11.8 ± 0.6	27.9 ± 0.2	26.2 ± 0.6	1.1 ± 0.2
	1-Esters of 3-Chloro-1,2-propanediol				
neutralized hydrolysate	12.1 ± 0.7	2.5 ± 0.5	13.1 ± 1.3	62.4 ± 2.4	9.9 ± 1.4
filter cake	15.3 ± 0.5	3.2 ± 0.4	15.9 ± 0.6	56.6 ± 1.5	9.1 ± 0.5

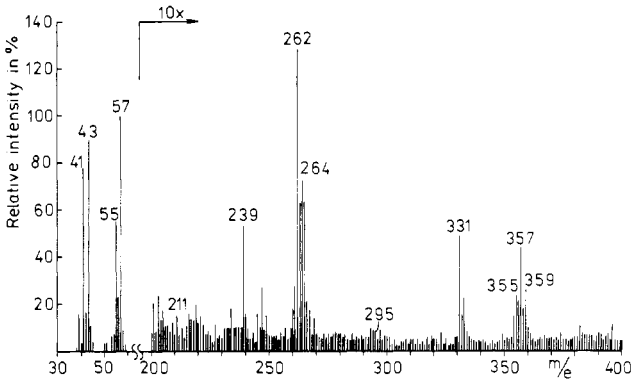

Figure 2. Mass spectrum of diesters of 3-chloro-1,2-propanediol from neutralized hydrolysate.

hydrins can also be gained from their mass spectra. For illustration, in Figure 2 a mass spectrum of 3-chloro-1,2-propanediol diesters isolated from neutralized protein hydrolysate is presented. These compounds correspond to spot no. 2 on Figure 1. Only the ions of higher intensities are diagnostically significant. In the case of diesters of saturated as well as unsaturated fatty acids, ions $(RCO)^+$ and $(M - RCO)^+$ are the most useful. Diesters of unsaturated fatty acids also have significant $(RCO - H)^+$ ions. By comparison of the relative intensities of the $(RCO)^+$ ions, it results that the main constituent is linoleic acid (m/e 263). Palmitic (m/e 239) and oleic (m/e 265) acids are present approximately in the same proportion. Stearic (m/e 267) and linolenic (m/e 261) acids are present in lower quantities. Myristic (m/e 211) and arachidic (m/e 295) acids could be present in traces as the ions at m/e 211 and 295 may, for example, also result by cleavage of C_5H_{11} and $C_{11}H_{22}$ from the molecule of palmitate. Analogous conclusions also result from the presence of $(M - RCO)^+$ ions at m/e 331 (palmitic acid), 355 (linoleic acid), 357 (oleic acid), and 359 (stearic acid) which have, unfortunately, lower intensities.

Esters of glycerol chlorohydrins represent a new class of endogenous food contaminants. It can be supposed that these compounds are not present at all (they might, eventually, be present in traces) in the final protein hydrolysates as well as in other foodstuff production of which is based upon the addition of protein hydrolysates (e.g., soy sauce, dehydrated soups, soup cubes, bouillon cubes, etc.). Quite different problems bring about polar glycerol chlorohydrins (further hydrolytic products of the above-mentioned esters that readily dissolve in protein hydrolysates and, therefore, are present in significant quantities in the hydrolysates). It is necessary to appreciate them from a hygienic–toxicologic point of view, owing to the fact that these substances are chlorine-containing organic compounds contaminants.

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