

Production of Organic Acids and Amino Acids from Fish Meat by Sub-Critical Water Hydrolysis

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Fish meat was easily liquefied by hydrolysis under subcritical conditions without oxidants, and aqueous phase and water-insoluble phase containing oil and fat-like solid were formed. Lactic acid found in the raw fish meat (about 0.03 g/g-dry meat) was stable up to the reaction temperature 513 K (3.35 MPa). Pyroglutamic acid was produced with a yield of 0.095 kg/kg of dry meat by 30 min reaction at 553 K (6.42 MPa). Amino acids such as cystine, alanine, glycine, and leucine were produced in the temperature range 513–623 K with a maximum peak at 543 K. Amounts of cystine, alanine, glycine, and leucine produced in 5 min at 543 K (5.51 MPa) were 0.024, 0.013, 0.009, and 0.004 kg/kg of dry meat, respectively. The oil extracted with hexane contained useful fatty acids such as eicosapentanoic acid (EPA) and docosahexanoic acid (DHA). Thus, subcritical water hydrolysis would be an efficient process for recovering useful substances from organic waste such as fish waste discarded from fish market.

Introduction

In Japan, fish waste mainly discharged from fish markets and food industries had been dumped into the sea until it was banned in 1996 by the London treaty. Since then, processing of the fish waste has become a serious problem because about 40–45% of the fish mass is waste material such as bones, bony parts, entrails, and waste meats. In Osaka prefecture, whose population is about 8 million, for example, only 190 ton out of 320 ton of the waste discharged every day is converted to fish feed. Since the production cost of the fish feed from the fish waste is 2–3-fold as high as those of imported products, the Osaka Prefecture Government has to give financial assistance to this project. However, in other Prefectures, most of the fish waste is incinerated with kitchen garbage from ordinary houses, and its cost becomes a heavy burden not only to the Prefectures' finances but also to the finances of fish markets, restaurants, food industries, and so on. Therefore, an efficient process for converting the fish waste to useful materials is strongly desired.

Supercritical water oxidation has attracted scientists' attentions for a decade because it would be an environment-friendly technology for decomposing sludge from wastewater treatment facilities, harmful substances such as polychlorinated biphenyls (PCBs) and dioxins (Barner et al., 1992; Caruana, 1995; Goto et al., 1997; Sako et al., 1997; Rogak and Teshima, 1999). Above the critical point of water (647 K, 22.1 MPa), water forms a single homogeneous phase, which allows the oxidation reaction to proceed rapidly. Most organic carbons are rapidly and completely decomposed to carbon dioxide via organic acids and most of the nitrogen compounds to gaseous nitrogen via ammonia due to strong oxidation by water with some oxidants in supercritical conditions (Marutino and Savage, 1999). We have extended the idea of the

supercritical water oxidation to subcritical regions (473–647 K) in order to provide much wider and milder reaction conditions. Since hydrolysis becomes a dominant reaction under subcritical conditions without oxidants, organic acids, amino acids, and fatty acids are expected to be produced from the fish waste by choosing proper reaction conditions. These substances would be used as the resources for many other industries. The waste decomposition under subcritical conditions would be also useful for processing biomass, sludge discharged from food industries, and any other organic waste. For these reasons, the production of organic acids and amino acids from the fish meats by the subcritical water hydrolysis has been examined.

Experimental Section

Hydrolysis of Fish Meat under Subcritical Conditions. Fish meat (a horse mackerel) purchased from a local fish store was homogenized with a Warring Blender (Model 31 BL 92, Dynamic Corporation of America) at top speed for 2 min. A stainless tube (SUS 316, i.d. 0.007 m × 0.15 m, reactor volume 7.0 cm³) with Swedgelok caps was used as a reactor. In the subcritical conditions, about 1.0 × 10⁻³ kg of the fish meat (water content, 69–73%) and Milli-Q water (3.36 cm³) was charged to the reactor tube. For supercritical conditions, 3.06 cm³ (653 K, 30 MPa) or 1.76 cm³ (673 K, 30 MPa) of Milli-Q water was added to 1.0 × 10⁻³ kg of the fish meat. After dissolved oxygen in the sample was purged with argon gas, the reactor was sealed and immersed in a preheated molten salt bath (Thomas Kagaku Co. Ltd.) containing a mixture of potassium nitrate and sodium nitrate. The reactions were carried out in the range 473–673 K, and the pressure in the reaction tube was estimated from a steam table for the subcritical conditions and Redlich-Kwong equation for the supercritical conditions. At a predetermined time (from 1 to 30 min), the reactor tube was

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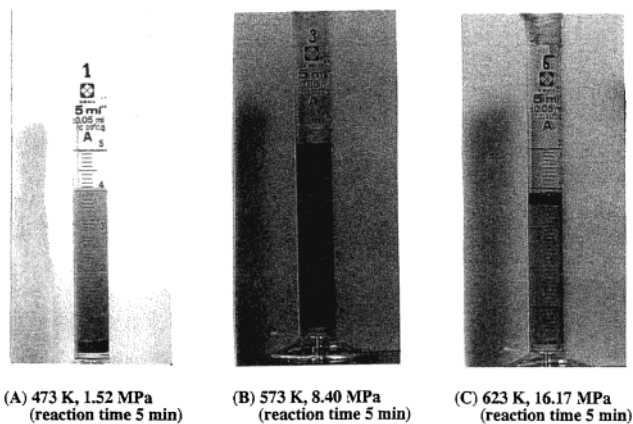


Figure 1. Liquefaction of fish meat: (A) 473 K (1.52 MPa), (B) 573 K (8.40 MPa), (C) 623 K (16.17 MPa). Reaction time, 5 min.

immediately cooled to room temperature by soaking into a water bath.

Analysis of Organic Acids and Amino Acids with HPLC. The reaction product was diluted to 50–200 cm³ with Milli-Q water and was filtered through Millipore membranes (2.2×10^{-7} m) to remove oil droplets and insoluble solids. An HPLC (Shimadzu LC-6A) with an ion-exclusion column (Shim-pack SCR-102H, i.d. 0.008 m \times 0.30 m \times 2) was used to determine each organic acid concentration. As a mobile phase, 10 mol/m³ *p*-toluenesulfonic acid solution was pumped into the column and maintained at 318 K with a column oven (Shimadzu CTO-6A) at the flow rate of 0.8 cm³/min. By injecting a diluted sample (0.020 cm³), we measured concentrations of organic acids with a conductivity detector (Shimadzu CDD-6A). The retention time of each organic acid was confirmed by injecting a sample in which a known amount of the authentic acid was added as an internal standard. Concentrations of amino acids were determined by postcolumn labeling methods with an HPLC system (Shimadzu LC-10A, ISC-07/S 1504 column) using a spectrofluorophotometer (Shimadzu RF535).

TOC Measurement of Aqueous Products. The total organic carbon (TOC) of reaction products was measured with the TOC analyzer (Shimadzu TOC-500). According to the manufacturer's standard procedure, 0.010 cm³ of the aqueous phase was injected to the TOC analyzer. TOC was calculated by subtracting inorganic carbon (IC) from total carbon (TC). The IC values were less than 10% of the TC values for all samples examined.

Carbon, Nitrogen, and Hydrogen Contents of Raw Material and Solid Product. Carbon, nitrogen, and hydrogen contents of the raw fish meat and solid products were with a CHN corder (Yanaco, MT-3, Japan). The CHN corder oxidizes organic samples and quantifies generated gaseous components and ash. Samples were dried in an oven (348 K) for 2 days prior to the analysis. A few milligrams of the sample was loaded on the CHN corder.

Results and Discussion

Liquefaction of Fish Meat under Subcritical Conditions. Figure 1 shows photographs of reaction products at 473, 573, and 623 K (reaction time, 5 min). Aqueous phase and solids were formed as the reaction products at 473 K (1.52 MPa). At 573 K (8.40 MPa), the amount of solids decreased, and water-insoluble phase was formed above the aqueous phase. This water-insoluble phase contains oil and fat-like solids, and the

Table 1. CHN Contents of Dry Fish Meat and Dry Solids

	carbon [wt %]	nitrogen [wt %]	hydrogen [wt %]	others [wt %]	ash [wt %]
fish meat	58.45	11.06	8.62	21.85	0.007
solid product	59.35	11.40	8.65	20.16	0.430

oil could be extracted with hexane. The density of the oil, 0.922, determined by measuring its volume and weight, is similar to the reported values for animal oil. When the reaction temperature was further increased to 623 K (16.17 MPa), the solids disappeared, and the amount of water-insoluble phase decreased. Formation of gaseous products such as carbon dioxide and ammonia was not significant under the employed reaction conditions. As shown in Table 1, carbon, nitrogen, and hydrogen contents of the dry raw fish meat were 58.74%, 11.11%, and 8.66%, respectively. Others (21.48%) contain sulfur and oxygen atoms. The ratio of the components in the solid products was close to that of the raw fish meat. Figure 2 shows the amount of the solids decreased with the increase of the reaction temperature. These results show that the solids are unreacted fish meat. Figure 2 also shows that the volume of the water-insoluble phase increased with the increase of the reaction temperature and then decreased above 580 K. The water-insoluble phase was produced from a part of the solids as the reaction temperature increased. Then, the water-insoluble was degraded to other organic compounds in the high reaction temperatures. Our preliminary analysis with a GC/MS showed that the oil extracted with hexane contains many useful fatty acids such as arachidonic acid, eicosapentanoic acid (EPA), and docosahexanoic acid (DHA) (data not shown). These results show that the fish meat can be easily liquefied and converted to the aqueous phase and the water-insoluble phase in a short time with subcritical water hydrolysis.

Total Organic Carbon (TOC) in Aqueous Phase. The TOC yield of the aqueous phase was defined by the following equation:

$$\text{TOC yield} = \frac{\text{TOC} \cdot V}{m(1 - w)\text{TOC}_{\text{df}}}$$

where TOC, V , m , w , and TOC_{df} were TOCs of the aqueous phase [kg/m³], volume of the aqueous phase [m³], fish wet weight [kg], water content of fish meat [-], and TOC of 1 kg of dry meat [kg/kg of dry meat] obtained by the CHN corder, respectively. The effect of the reaction temperature on the TOC yield is shown in Figure 3. The TOC of the aqueous phase gradually increased from 0.2 at 473 K (1.55 MPa) and reached a plateau (0.58) above 573 K, indicating that about 60% of the organic carbon in the raw fish meat was recovered in the aqueous phase. The TOC was decreased at the supercritical conditions (673 K, 30.0 MPa). The increase of TOC with the reaction temperature is well explained by the decrease of the solids with the reaction temperature shown in Figure 2. The change of the TOC in the aqueous phase at 513 K with reaction time is shown in Figure 4. The TOC steeply increased and reached a plateau in 10 min, suggesting that the hydrolysis reaction under subcritical conditions is rapid and that carbon dioxide was not formed under employed conditions. The organic acids and amino acids in the aqueous phase were then analyzed with HPLC systems.

Organic Acid Production in the Aqueous Phase. In Figure 5, yields of the organic acids produced in the aqueous phase (reaction time, 5 min) are plotted against the reaction temperature. The yield was defined as the

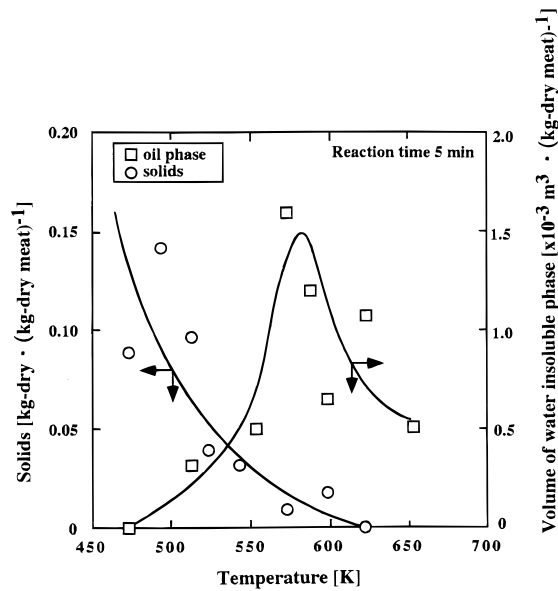


Figure 2. Effect of reaction temperature on amount of solids and volume of water insoluble phase. Reaction time, 5 min.

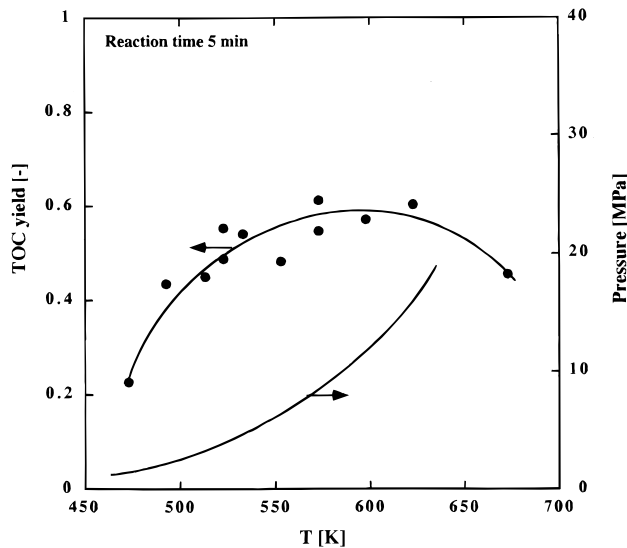


Figure 3. Effect of reaction temperature on TOC of aqueous phase. Reaction time, 5 min.

weight of organic acids (kg) produced from 1 kg of dry fish meat. Lactic acid (0.03 kg/kg of dry meat) was found in 5 min at 513 K (3.35 MPa), while the raw fish meat contained 0.027 kg/kg of dry meat. Degradation of lactic acid become significant above 513 K. In the supercritical conditions (653 and 673 K, 30.0 MPa), most of lactic acid was degraded in 5 min. Pyroglutamic acid showed the highest yield (0.043 kg/kg of dry meat) among the identified organic acids with an optimum temperature at 553 K (6.42 MPa). As will be described later, the optimum temperature was almost the same as that of the amino acid production (Figure 7), indicating that pyroglutamic acid was formed by the hydrolysis of the fish protein. Acetic acid, on the other hand, was produced above 533 K, and the maximum yield was 0.01 kg/kg of dry meat at 653 K (30.0 MPa). Figure 6 shows the effect of reaction time on the organic acid yield at 513 K. Lactic acid was gradually degraded under this condition. Rapid liquefaction of fish meat under subcritical conditions will be advantageous for the recovery of lactic acid from the fish waste meat. The time course of the organic acid

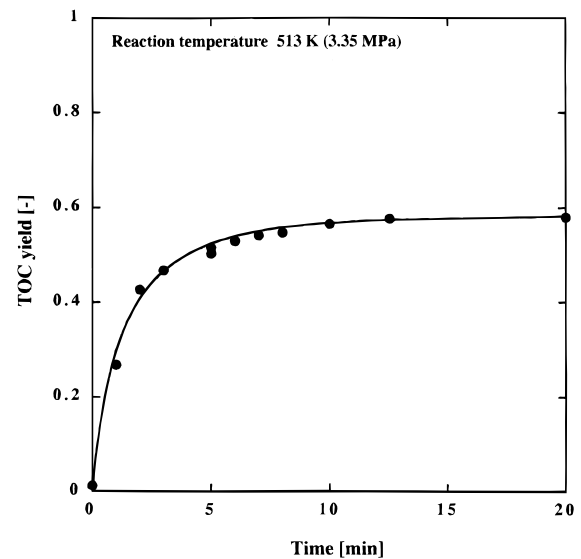


Figure 4. Effect of reaction time on TOC of aqueous phase. Reaction temperature, 513 K (3.35 MPa).

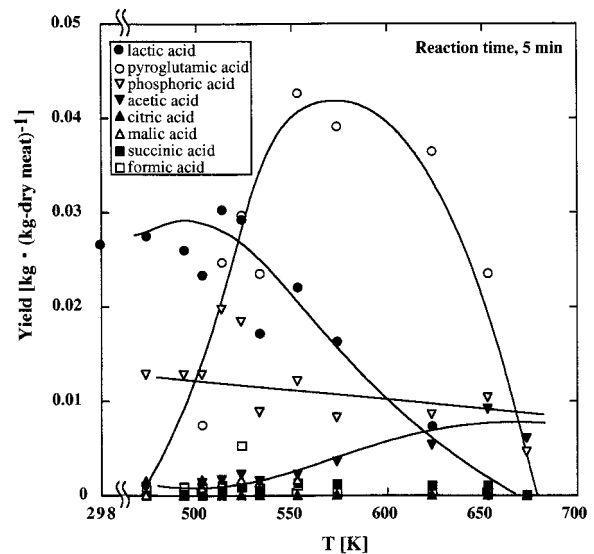


Figure 5. Effect of reaction temperature on organic acid production. Reaction time, 5 min.

production at 543 K (5.51 MPa) is shown in Figure 7. The production rate of the pyroglutamic acid increased, and its yield reached 0.095 kg/kg of dry meat by 30 min at 543 K. Since pyroglutamic acid is readily hydrolyzed to L-glutamic acid using caustic soda, the fish meat will be an inexpensive source for L-glutamic acid.

Amino Acid Production in the Aqueous Phase.

Figure 8 shows the effect of reaction temperature on the amino acid yields at a reaction time of 5 min. Cystine, alanine, glycine, and leucine were produced in the temperature range from 513 to 623 K, while histidine yield was decreased with the reaction temperature. Maximum yields were obtained about 540 K. Amounts of cystine, alanine, glycine, and leucine produced by a 5 min reaction at 543 K (5.51 MPa) were 0.024, 0.013, 0.009, and 0.004 kg/kg of dry meat, respectively. Yields of other amino acids were less than 0.001 kg/kg of dry meat. The temperature dependence of ionization constant of water K_w [(mol/kg⁻¹)²] for the coexisting liquid at saturated vapor pressure (IAPES, 1980) is also shown in Figure 8. The temperature which gives the maximum yields for the amino acids shows a good agreement to that

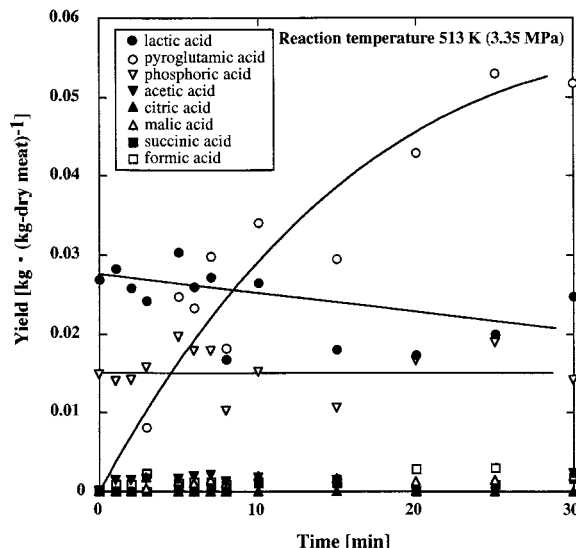


Figure 6. A time course of organic acid production. Reaction temperature, 513 K (3.35 MPa).

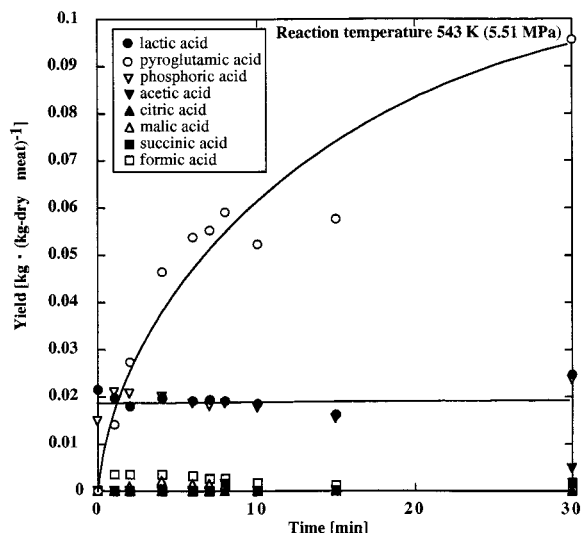


Figure 7. A time course of organic acid production. Reaction temperature, 543 K (5.51 MPa).

for the maximum K_w . This result shows that the production of amino acids by the hydrolysis becomes maximum due to the large K_w value. Time courses of the amino acid production at 513 K are shown in Figure 9. The yields of cysteine, alanine, glycine, and leucine almost linearly increased with the reaction time up to 15 min and reached plateaus at 30 min. The yield of histidine, on the other hand, decreased with the reaction time. The raw fish meat contained histidine at 0.0124–0.0215 kg/kg of dry meat. Figure 10 shows the time course of amino acid production at 543 K. The yields of cysteine, alanine, glycine, and leucine reached plateaus by 6 min, and the yields did not change up to 30 min. This result suggests that those amino acids are stable under the employed reaction conditions. The decomposition of histidine, however, was facilitated.

On the basis of these results, an efficient recovery process from the fish meat can be proposed because the optimal conditions for the production of the various components are different. For example, the fish meat is liquefied at 473 K, and lactic acid, phosphoric acid, and histidine can be recovered by a 5 min reaction. The purification system would become simple because pro-

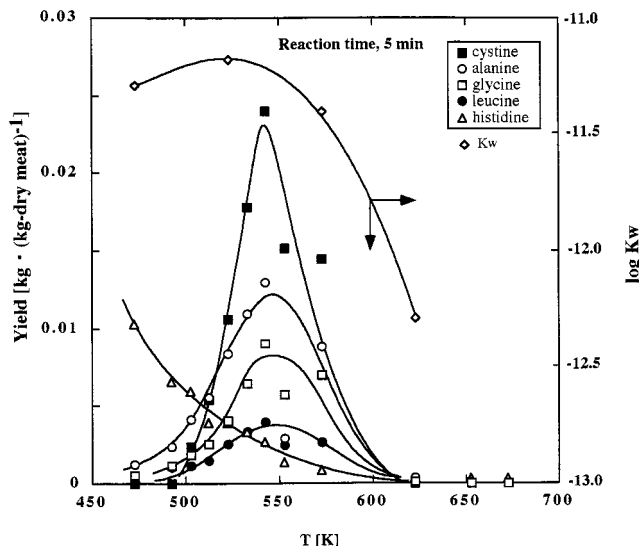


Figure 8. Effect of reaction temperature on amino acid production. Reaction time, 5 min.

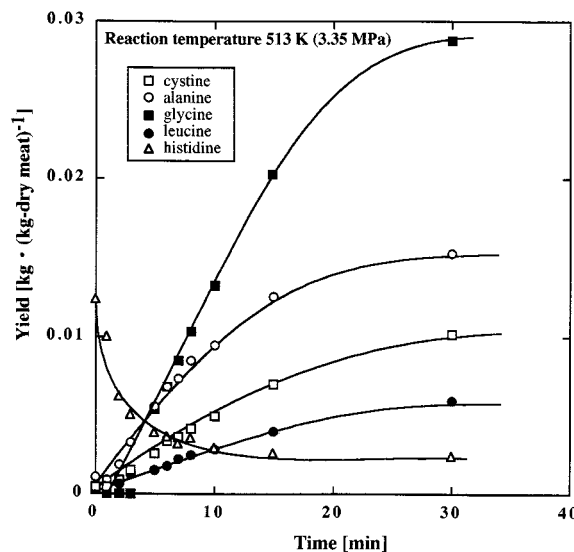


Figure 9. Effect of reaction time on amino acid production. Reaction temperature, 513 K (3.35 MPa).

duction of other components is very low at these conditions. Then, cysteine, alanine, glycine, and leucine are produced in 6 min at 543 K. Pyroglutamic acid production can be produced at high yield by a 30 min reaction at 543 K. Further, oil and fat can be produced at 573 K. The expected yields of these compounds from 100 tons of the fish meat is shown in Table 2. A large amount of the useful resources can be produced in short reaction time.

Thus, it was confirmed that the subcritical water hydrolysis is an effective process to recover the useful substances from the meat of fish waste. Rapid liquefaction and hydrolysis reaction are favorable for processing a large amount of the fish waste. Other waste from many industries can be starting materials for this process. The reaction mechanism and process design to improve the yields of the products will be discussed in the next paper.

Conclusion

Fish meat was rapidly liquefied by subcritical water hydrolysis. By choosing proper conditions, we could produce a variety of useful substances. For example,

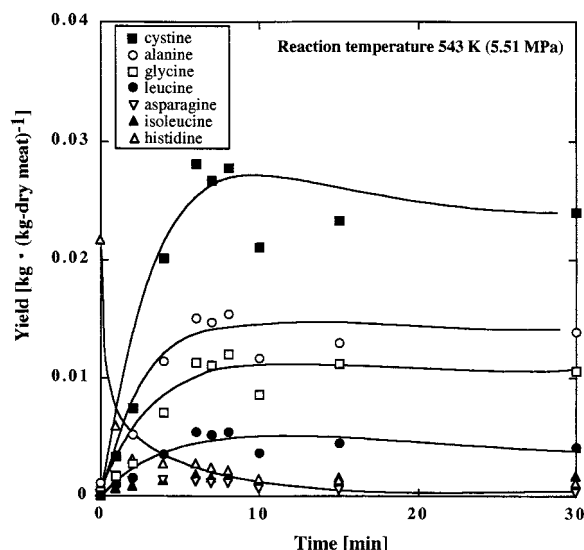


Figure 10. Effect of reaction time on amino acid production. Reaction temperature, 543 K (5.51 MPa).

Table 2. Expected Maximum Yields for Various Compounds from 100 Tons of Fish Meat^a

compound	yield (ton)	temp. (pressure), reaction time
lactic acid	0.81	
phosphoric acid	0.36	473 K (3.35 MPa), 5 min
histidine	0.30	
cysteine	1.11	
alanine	0.42	543 K (5.51 MPa), 6 min
glycine	0.30	
leucine	0.14	
pyroglutamic acid	2.85	553 K (6.42 MPa), 30 min
oil & fat	48 (m ³)	573 K (8.40 MPa), 5 min

^a Water contents of fish meat: 70%.

lactic acid (0.027 kg/kg of dry meat), phosphoric acid (0.120 kg/kg of dry meat), and histidine (0.010 kg/kg of dry meat) were obtained by a 5 min reaction at 473 K (3.35 MPa). Pyroglutamic acid (0.095 kg/kg of dry meat), on the other hand, was produced by a 30 min reaction at

553 K (6.42 MPa). Optimum conditions for the production of cystine, alanine, glycine, and leucine were 543 K (5.51 MPa). The oil phase contained useful fatty acids such as EPA and DHA. Thus, the hydrolysis under the subcritical conditions will be an efficient process for converting organic waste such as fish meat to useful resources.

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