19. PRESENT STATUS OF JAPANESE RESEARCH ON ANIMAL PRODUCTS

Masao FUJIMAKI*

Studies on animal products, especially on milk and milk products, meat and meat products have much developed in Japan, with the increasing demand for them.

Milk and milk products: It has been long pointed out that cow's raw milk produced in Japan is apt to have a low value of non-fat milk solid, particularly of protein. This tendency may not be on account of lacking in scientific elucidation on milk production, but of practical influence of the legal standard in Japan. Basic studies on casein, a major protein of normal milk, have progressed and revealded it to be chemically heterogeneous. α_s-, β-, κ-caseins were fractionated directly from skimmilk by gel chromatography[1]. All the κ-caseins prepared by various methods are more or less heterogeneous and dissociate into two components, one of which seems to be a para-like κ-casein[2]. By treatment with milk protease, the rate of liberation of tyrosine from unfractionated casein, α_s-casein and κ-casein were similar, but that from β-casein was one half that from any of other caseins. However, β-casein showed a drastic change in its polyacrylamide gel electrophoretic pattern. Two of the decomposed products from β-casein by treatment with milk protease are supposed to be possibly temperature-sensitive (TS.) casein and R- (or γ-) casein, respectively[3]. TS-casein was separated by gel filtration on a Sephadex G-100 column from the temperature sensitive fraction in casein, consisted mainly of two components observable by starch gel electrophoresis[4]. Conformational changes of casein by various treatments (e.g. pH, medium, temperature) were recognized by measuring UV difference spectra[5]. The blue shift of α_s-casein spectra was observed at above 30°. This is considered to mean unfolding of the molecule, its aggregation by heating being observed above ionic strength of 0.4[6].

In Japan, a modified milk powder preparation has been produced with paying special consideration for nursing babies, aiming at ideal human milk substitution. To approximate its protein condition to that of human milk, for example, it will be necessary for the preparation to be enriched with milk whey protein and/or to be reduced in casein content. It has been reported that the mixtures of skimmilk and whey protein solutions are stabilized against heating at 132° for 1 min if the whey protein solutions are added to the skimmilk preheated at 80–95° for 10 min. By preheating the skimmilk separately the mixtures were stabilized against heating in certain narrow pH ranges, possibly owing to the well-known interaction between casein and β-lactoglobulin through disulfide interchange reaction[7].

Recently, long-distance transportation of raw milk under low temperature has become popular. The proteolysis of casein and whey protein by psychrotrophic organisms isolated from bulk cooler milk was examined. After the treatment for 7 days, the peaks of β-casein and α_s-casein were shown to decrease on DEAE-cellulose chromatograms, on the other hand, the whey protein being unchanged[8]. As a new method for transportation of raw milk, a theoretical analysis on the level land milk transportation by means of polyethylene pipeline and compressed air was conducted[9].

* Professor, Department of Agricultural Chemistry, Faculty of Agriculture, Tokyo University, Japan.

The properties of milk lipids, including the relation between feeding conditions and their variations[10], and the presence of tocopherols in milk fat[11] were also revealded. The total tocopherol fraction isolated from milk fat was found to contain 6 of the known naturally occurring tocopherols, that is, α-, β-, γ-, and δ-tocopherols and α-, and γ-tocotrienols. The antioxidant effect of tocopherols on milk fat was discussed.

Owing to severe shortage of beef and improvement of methods of raising small calves for beef with industrially feasible cost, the number of male calves slaughtered has decreased in recent years. This resulted in a severe shortage and a very high price of calf rennet. Because of the difficulty in obtaining sufficient quantities of rennet for the cheese industry, other sources for this enzyme has been sought. Among some 800 strains of microbes investigated, a fungus isolated from soil and identified as Mucor pusillus lindt was found to produce a suitable milk-curdling enzyme named Mucor rennin (rennet). Though there are many similarities and some dissimilarities between Mucor rennin and calf rennin, it has now been proved that Mucor rennin can be used as a substitute for calf rennin, in the processing of almost all important types of cheeses of the world[12].

To elucidate cheese flavor, roles of Brevibacterium linens and yeasts on the development of volatile flavor substances in Limburger cheese were investigated[13]. Also, bitter peptides were isolated from tryptic hydrolysate of casein and identified[14].

Several strains of yeast with the strong proteolytic activity were selected and the semi-soft cheese manufacturing experiment has been carried out with success by using these strains of yeast as the starter together with lactic acid bacteria. Two strains of yeast (Saccharomyces fragilis and Candida pseudotropicalis) isolated from milk or milk products were shown to possess the strong proteolytic activity on milk protein as well as the lactose fermenting ability. The use of S. fragilis and the ripening at 15° for 3–4 weeks gave the best result. The results indicate that the proteolytic activity of yeast is of importance for the ripening of cheese of this kind. Ethanol, isoamylalcohol and ethyl acetate produced by use of S. fragilis were considered to be responsible for the characteristic flavor of yeast-ripened cheese[15].

Freeze-drying has been widely employed both in the preservation of stock cultures and in the distribution of dairy organisms. However, little attention had been paid to the number of organisms that survived the freeze-drying process. Accordingly, freeze-drying of lactic acid bacteria has been quantitatively studied and now frozen concentrated cultures applicable to one-step preparation of bulk starter are being marketed. The choice of an appropriate suspending medium, the clarification of the nature and action of protective solutes, and the factors affecting the survival of lactic acid bacteria in the process of freeze-drying were given[16].

Meat and meat products: The main studies conducted with great efforts in Japan are those in the aspect of protein; the one is on the mechanism of the resolution of post-mortem rigor, and the other on the meat quality ascribed to protein, especially on water-holding capacity and binding quality.

The changes of myosin B which is the main protein in myofibril were studied during storage of rabbit muscle, and the interaction between actin and myosin was found to become less strong when myosin B was stored in the medium of high ionic strength, low temperature and low pH[17),18]. However, the well-known phenomenon that the resolution of rigor is accelerated by high temperature cannot be explained by the result. By the study in the morphological aspect, was found the very important change in myofibril assumed to the cause of tenderization of meat accompanied with the resolution of rigor: myofibril became susceptible to mechanical force and was fragmented by the homogenization procedure[19]. It was shown that

this change was caused by the weakening at the Z-band and/or Z-I junction[20] and accelerated in vitro by the addition of Ca ion[21].

The concerned problem has been approached by comparing the postmortem changes in morphological and chemical properties between red and white muscles. The sarcomeres of red muscle fibers of rabbit highly contract in 24 hours after slaughter and afterwards do not change, whereas those of white muscle fibers show the minimum length after three days with the following extension[22]. Recently, the changes in the tensile strength of chicken muscle fibers during aging were examined and it was found that the strength increased with the progress of rigor and afterwards decreased[23]. Also, the addition of divalent cations and phosphate was effective in the depression of the tensile strength[24].

Beside actin, myosin and tropomyosin, there are troponin and actinins in myofibril as regulatory proteins, but α-actinin and tropomyosin-troponin complex are found not to be the key protein bearing post-mortem modification in the actin-myosin interaction[25].

The contributions of cathepsins, which are well-known proteases existing in muscle, to the post-mortem change in myofibril have been examined. The action of cathepsin D isolated from muscle did not seem to be the primary cause of the post-mortem alteration of myofibrilar structure[26),27]. Recently, was examined the relation between the rate of increase in non-protein nitrogeneous compounds (NPN) of rabbit muscle, and altered muscle pH from 5.9 to 7.2 prepared by the injection of ICH_2COOH[28] and it was evidenced that there was a neutral proteolytic system responsible for post-mortem proteolysis besides the acidic one composed of cathepsins, and that the former was composed of plural enzymes and more abound in exopeptidase activity than the latter[29]. Another work in the United States has indicated that muscle contains the Ca-enhanced neutral protease which is capable of removing Z-band in vitro[30]. Therefore, the post-mortem action of the neutral proteolytic system on myofibril is expected to be revealed in detail. From the standpoint of the flavor improvement, further studies on muscle proteases responsible for the post-mortem liberation of NPN are also expected.

The water-holding capacity, one of main factors determining meat quality, decreases with the progress of rigor mortis and afterwards increases with the resolution of rigor. It has been shown that this change is caused not only by the post-mortem change of pH, but also by the change in the molecular properties of actomyosin system, whereas it remains indistinct what change is ascribed to the improvement of water-holding capacity, although various kinds of information on the post-mortem changes in the molecular properties of actomyosin have been offered.

The studies on binding quality in Japan have brought about notable contributions to meat processing. It was shown that myosin was a key constituent of binding quality of sausage, and had a major influence on gelation, whereas actin little influence[31]. When actin and myosin were combined, however, gel strength is improved, the complex binding more water than myosin alone. A tropomyosin-troponin complex was suggested to contribute to the binding quality of meat[32]. Since the quality of sausage was found to be influenced by the degree of denaturation of myosin B at the emulsification in meat processing, many valuable studies on the denaturation of contractile proteins have been performed.

Effects of some food additives promoting the formation of cured meat color and some metal ions naturally occurring in meat on the behavior of nitrite were investigated. The decomposition of nitrite was fairly promoted by the addition of Na-ascorbate, isoascorbate and Fe^{2+}, respectively, and particularly in the case of Fe^{2+}, a significant decomposition of nitrite took place at pH 6 to 5. The addition of

nicotinamide significantly promoted not only the decomposition of nitrite, but also the formation of nitric oxide from nitrite in the presence of Na-ascorbate[33].

A simple and rapid method has been presented for estimating the freshness of muscle by thin-layer chromatography, using "K-value" proposed by T. Saito et al. The freshness of various kinds of muscle was successfully estimated by this method[34].

Flavor compounds of beef fat were examined. The volatile compounds from beef fats heated under the cooking condition, at 145° for 10 min were isolated and nonacidic compounds separated from them were further fractionated into five fractions by silicic acid column chromatography. The odor of nonacidic compounds significantly resembled the flavor of heated beef fats. Several carbonyl compounds, hydrocarbons, alcohols, lactones and pyrazine compounds, were identified with the techniques of gas chromatography-mass spectrometry. The typical heated flavor could probably ascribed to a proper combination of aldehydes, ketones, esters and S-containing compounds[35].

References

1) C. Nakahori and S. Nakai, J. Dairy Sci., **55**, 24 (1972)
2) M. Kanamori et al., J. Jap. Soc. Fd. & Nutr., **24**, 96 (1971)
3) S. Kaminogawa and K. Yamauchi, Agr. Biol. Chem., **36**, 249 (1972)
4) Y. Igarashi and Z. Saito, Jap. J. Zootech. Sci., **41**, 262 (1970)
5) S. Yoshida, Nippon Nogei Kagaku Kaishi, **44**, 143 (1970)
6) T. Ono et al., Agr. Biol. Chem., **37**, 957 (1973)
7) K. Miyazawa, Nippon Nogei Kagaku Kaishi, **46**, 549 (1972)
8) T. Yanagiya et al., ibid., **47**, 259 (1973)
9) H. Hamada et al., Jap. J. Zootech. Sci., **42**, 570 (1971)
10) F. Tsuchiya et al., ibid., **44**, 105 (1973)
11) C. Kanno et al., Agr. Biol. Chem., **36**, 2067 (1972)
12) K. Arima, Paper presented at the 56th Annual Session of IDF (1972)
13) A. Hosono and F. Tokita, Jap. J. Zootech. Sci., **41**, 137 (1970)
14) T. Matoba et al., Agr. Biol. Chem., **34**, 1235 (1970)
15) T. Tsugo et al., Jap. J. Zootech. Sci., **41**, 445 (1970)
16) T. Morichi, Paper presented at the 56th Annual Session of IDF (1972)
17) M. Fujimaki et al., J. Food Sci., **30**, 937 (1965)
18) A. Okitani et al., Agr. Biol. Chem., **31**, 939 (1967)
19) K. Takahashi et al., J. Food Sci., **32**, 409 (1967)
20) T. Fukazawa et al., ibid., **34**, 606 (1969)
21) O. Hattori et al., unpublished (1972)
22) T. Nakanishi et al., Jap. J. Dairy Sci., **20**, A-114 (1971)
23) R. Nakamura, J. Agr. Food Chem., **20**, 809, 1087 (1972)
24) R. Nakamura, ibid., **20**, 1167 (1972)
25) N. Arakawa et al., J. Food Sci., **35**, 703 (1970)
26) A. Suzuki et al., Agr. Biol. Chem., **33**, 1723 (1969)
27) A. Okitani et al., ibid., **36**, 2135 (1972)
28) A. Okitani et al., ibid., **37**, 321 (1973)
29) A. Okitani et al., ibid., **36**, 1265 (1972)
30) W. A. Busch et al., J. Call Biol., **52**, 367 (1972)
31) K. Samejima et al., J. Food Sci., **34**, 242 (1969)
32) T. Nakayama and Y. Sato, Agr. Biol. Chem., **35**, 208 (1971)
33) Y. Nagata and N. Ando, J. Jap. Soc. Fd. & Nutr., **25**, 28 (1972)
34) T. Tanaka et al., ibid., **23**, 127 (1970)
35) K. Watanabe and Y. Sato, Agr. Biol. Chem., 35, 756 (1971)