Preservability and Utilization of Powdered \( \alpha \)-Linolenic Acid with Egg White

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Our previous observations\(^{1,2}\) have revealed that fine droplets of sardine oil simply covered with or virtually embedded in spray-dried egg white powders are good for a long-term storage. The predominant polyunsaturated fatty acids in sardine oil, i.e., eicosapentaenoic and docosahexaenoic acids, have unique nutritional or physiological functions in relation to aging control,\(^{3,4}\) learning ability,\(^{5,6}\) and anti-carcinogenicity.\(^{7,8}\)

In practical eating habits, apprehension exists that excess linoleic acid intake is a risk factor in colonic\(^{9,10}\) or mammary cancer.\(^{11,12}\) Linoleic acid is indispensable to the vital functions in mammals,\(^{13,14}\) so that the effective means must be taken to avoid such risks. For this reason, the action of n-3 polyunsaturated fatty acids has become of much interest in modern lipid nutrition.\(^{9,15,16}\) \( \alpha \)-Linolenic acid in which perilla and linseed oils are much more rich than in linoleic acid, ranks as the first in unsaturation among naturally occurring n-3 series of polyunsaturated fatty acids. In this preliminary paper, the \( \alpha \)-linolenic acid was chosen as a representative of easily available n-3 polyunsaturated fatty acids and examined for its preservability after "microencapsulation" with egg white as a coating material by the process of spray-drying.

\( \alpha \)-Linolenic acid of linseed origin (lot no. 70904—manufactured in 1988) was presented by Amagasaki Research Institute of Nihon Yushi Co., in Hyogo, and was of 75% purity with contamination by linoleic acid (22%) and oleic acid (3%). A fully sonicated oil-in-water emulsion containing \( \alpha \)-linolenic acid and egg white in a 1:9 (w/w) ratio was spray-dried with Yamato-Pulvis GA-1 apparatus in the same manner as previously described.\(^{21}\) For the sake of comparison, egg white powder that had been prepared from the albumen solution by spray-drying was stirred with a solution containing 10% \( \alpha \)-linolenic acid in hexane, and the solvent was evaporated under a nitrogen stream to obtain powdered \( \alpha \)-linolenic acid. A hexane solution of \( \alpha \)-linolenic acid was likewise mixed with \( \alpha \)-corn starch and the air-dried powder was used as another control. The three kinds of samples thus obtained were stored at 25°C in humidity-controlled chambers, in which the relative humidity (RH) levels were adjusted to 30, 50, and 80%, respectively, using various concentrations of sulfuric acid.\(^{22}\)

At prescribed intervals, about 20 mg of a sample was withdrawn three times to extract the \( \alpha \)-linolenic acid with chloroform-methanol (2:1, v/v), and hydroperoxide was determined according to the ferric thiocyanate method.\(^{1,2}\) Seven weeks later, individual extracts of each sample were combined, evaporated to dryness and gas-chromatographically analyzed for the residual fatty acid, after being methylesterified with a 15% BF\(_3\) solution in methanol. As an example of using egg white powder inclusive of \( \alpha \)-linolenic acid, noodles were made from kneaded dough consisting of wheat flour, sodium chloride, egg white (containing 10% \( \alpha \)-linolenic acid), and water in a 100:2:1:1.45 ratio by weight, which were boiled for 10 min. The extraction of \( \alpha \)-linolenic acid from the cooked noodles was carried out with chloroform-methanol (2:1) similarly to that for the ingredient egg white powder.\(^{21}\)

Figure 1 illustrates changes in the peroxide value of the three kinds of samples during storage at three fixed RH levels. With respect to the starch sample, the maximal increase in the peroxide value was observed in the range of 2 weeks (at low RH) to 3.5 weeks (at high RH). On the contrary, the peroxide value of the simultaneously spray-dried egg white sample was unchanged at moderate and high RH values throughout the experimental period, although tending to increase during the last week of storage. \( \alpha \)-Linolenic acid that was merely mixed with egg white powder seemed to

![Fig. 1. Comparison between the Changes in Peroxide Value in Storage at 25°C (RH = 30, 50 and 80%) of "\( \alpha \)-Linolenic Acid" Simultaneously Spray-dried with Egg White and Simply Mixed with the Powder.](image)

The samples were divided into small portions (about 20 mg in conical cups), of which every 60 cups were stored in different humidity-controlled chambers with respective RH levels of 30, 50, and 80%. Each chamber was maintained at 25°C. Three cups were used for hydroperoxide measurements at each point, so that the peroxide value is the mean of three determinations with a standard deviation of no more than 10%. The extraction efficiency of the fatty acid was more than 90%. \( \bigcirc \), starch powder simply mixed with the fatty acid; \( \bigcirc \), egg white powder simply mixed the fatty acid; \( \bigcirc \), egg white powder inclusive of the fatty acid after simultaneous spray-drying.

![Fig. 2. Gas Chromatographic Analysis after 7 Weeks' Storage of "\( \alpha \)-Linolenic Acid" Simply Mixed with Spray-dried Egg White Powder (a) or Simultaneously Spray-dried with Egg White (b).](image)

The corresponding samples in storage at RH = 50% were taken out after the 7th week and analyzed for their residual fatty acids in the usual way. Analytical conditions: apparatus, Shimadzu GC-4BFP equipped with a flame ionization detector; column, 3 m × 3 mm glass capillary; packing, Univolt C; column temp., 220°C; \( \mathrm{N}_2 \) (carrier gas) flow rate, 45 ml/min.
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Fig. 3. Residual \( \alpha \)-Linolenic Acid in Cooked Noodles Supplemented with the Fatty Acid-containing Egg White Powder.

Several pieces of cooked noodles were blended in an electric mixer and extracted with chloroform-methanol (2:1). The residue after evaporating the solvent was methylated, and then used for gas-chromatographic analysis: (a) noodles without \( \alpha \)-linolenate supplementation; (b) noodles with \( \alpha \)-linolenate supplementation. The peaks at the left correspond to "\( \alpha \)-linolenic acid" in the egg white powder used for noodle making.

be more susceptible to oxidation than the spray-dried one with egg white. It was estimated by gas chromatography to what extent the fatty acid was left intact after 7 weeks' storage. As shown in Fig. 2, \( \alpha \)-linolenic acid had been effectively protected against oxidative deterioration in the spray-dried egg white sample, exhibiting no sign of increase in the peroxide value, while \( \alpha \)-linolenic acid and its contaminating 'linoleic acid' was appreciably reduced in the simply mixed sample with egg white powder. It is worth emphasizing that spray-drying prolonged the preservability of \( \alpha \)-linolenic acid beyond a certain period. The antioxidative effect of a food protein (e.g., wheat gliadin) has already been reported to be affected by environmental RH and to be improved by spray-drying. A similar effect was observed for egg white, although the protein being a little more effective at low RH values than gliadin. The improvement in the antioxidative effect of powdered egg white by the process of spray-drying may be explained in terms of the 'microencapsulation' of \( \alpha \)-linolenic acid and the consequent protection from contact with external oxygen. It is highly possible that products of the Maillard reaction in the spray-drying process or during the preservation period are also involved in the elicitation of a prominent antioxidative effect.

Since the powdered \( \alpha \)-linolenic acid was in good preservation at high RH values according to expectation, its application would be directed toward food with a high water content (e.g., noodles). The addition of egg white powder to a dough brings on a desirable change in palatability that is ambiguously expressed in Japanese "koshi". Accordingly, the egg white powder was replaced by the one inclusive of \( \alpha \)-linolenic acid, and was evaluated by a paired preference test for any difference in palatability between noodles with and without \( \alpha \)-linolenic acid supplementation. Most of 15 amateur panelists could not statistically give any preference for either of the two (data not shown), and none were conscious of any grassy odour from the noodles with \( \alpha \)-linolenic acid supplementation after a week of storage in a refrigerator. The results of the fatty acid analysis at that time are depicted in Fig. 3, in which the considerably increased peak by \( \alpha \)-linolenic acid supplementation was in evidence. The gain in quantity was, however, not enough to attain the original level of linoleic acid occurring in wheat flour. The limitation of egg white supplementation without affecting palatability and its accompanying fortification of n-3 polyunsaturated fatty acids remains to be investigated.

References