

Effects of Microcapsulated Docosahexaenoic Acid Preparation on Properties of Dough and Bread

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Summary The effects of microcapsulated docosahexaenoic acid powder (hereinafter DHA powder) on some viscoelastic properties of wheat flour dough and loaf volume were studied. The addition of DHA powder in the range of 0.1 to 0.3% caused a significant increase in the loaf volume of bread after baking. Dough containing 0.2 or 0.3% DHA powder had greater modulus of elasticity, viscosity coefficient and relaxation time than that not containing DHA powder (control). Farinography revealed that DHA powder alone did not decrease the arrival and development times more than those of the control, but increased the stability time significantly. There was no considerable change in the gelatinization temperature or enthalpy of starch in the dough. The size of gas cells in crumbs baked with the DHA powder increased slightly. DHA powder suspension did not appreciably decompose on heating at 120°C for 30 min, nor in the fermentation or baking processes in a home baker.

Key Words microcapsulated docosahexaenoic acid powder, calcium gluconate, viscoelasticity of dough, staleness of bread, image analysis of bread crumbs

Docosahexaenoic acid, 22:6(*n*-3) (DHA), is one of *n*-3 series polyunsaturated fatty acids that are known to have such physiologically important functions as to strengthen the vascular-tract and decrease the level of cholesterol in the blood (1). Though grown-up persons can synthesize DHA in their body when excess amounts of *n*-3 linolenic acid are taken from food stuffs, newborn babies are unable to synthesize it. Therefore, DHA is considered an important fatty acid. However, since the amount of DHA synthesized in the body is not sufficient, it may be better to ingest DHA directly from various food stuffs (2), as recommended by a guideline of the Ministry of Health and Welfare.

A recent survey (3) showed that people who consume fish or shellfish daily

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have significantly lower incidences of heart disease (4), cerebral hemorrhage (5), cerebral tumor, and so on (6, 7). Nutritional surveys in Japan have shown that Japanese do not take enough calcium, and that the composition of fatty acids in the diet is becoming westernized (i.e., low in polyunsaturated fatty acids found in fish) (8, 9). Since children don't want to eat fish, it is difficult to return to a fish-based diet (2, 8).

Though DHA can be solubilized by emulsifier or cyclodextrin trapping, applications for it as a food ingredient have been considerably restricted since DHA is not soluble in water. Recently, microcapsulated DHA powder coated with calcium gluconate (GCA) has become commercially available in Japan. Since GCA has been already applied to breadmaking as a calcium fortifier, it can be expected that this powder, containing mainly GCA, will serve to improve the quality of bread as well. Supplementation of bread with the DHA powder will bring about fortunate selection for people who dislike eating fish.

There are several studies in which unsaturated fatty acids (linoleic acid, microcapsulated linoleic acid, linolenic acid, eicosapentanoic acid, DHA from fish) or marine oils were used as ingredients for breadmaking (10–13). However, microcapsulated DHA powder has not yet been tested. For this reason, this study focused on the use of DHA powder as an ingredient in breadmaking. Especially, we studied the effects of DHA powder on the volume of a loaf, rheological properties of wheat flour dough, and the staleness of bread.

MATERIALS AND METHODS

Flour and chemicals. The wheat flour used for breadmaking was the same "Hermes" (Okumoto Flour Milling, Osaka, Japan) as described previously (14). Its protein and ash contents were 11.8% and 0.38%, respectively, on a 13.8% moisture basis.

Microcapsulated DHA powder and calcium gluconate (GCA) were provided by Fujisawa Pharmaceutical (Tokyo, Japan) and calcium stearoyl-2-lactylate (CSL) and sodium stearoyl-2-lactylate (SSL), both of which were of food grade, were obtained from Musashino Shoji (Osaka, Japan). The DHA powder was composed of 4% DHA in 20% refined fish oil and 73.4% GCA. Other chemicals of analytical grade were used without further purification.

Bread baking. The breadmaking formula was 280 g of flour, 5 g of sodium chloride, 17 g of sucrose, 3 g of dry baker's yeast (from Asahi Kasei, Tokyo, Japan), and 210 g of water containing DHA powder in the presence or absence of GCA, CSL or SSL.

Test loaves were baked with five automatic breadmakers (15, 16) in the same manner described previously (17). The total time for the entire process was 2 h 45 min; comprised of mixing (25 min), additional mixing after addition of yeast (5 min), fermentation (90 min) and baking (45 min). The volumes of loaves were measured by the rapeseed displacement method.

Data were analyzed using ANOVA, and Duncan's multiple-range test (18) was used to compare treatment means; differences were considered significant at $p < 0.05$.

Analytical methods

Rheological tests. For determination of the physico-chemical properties of the dough, the DHA powder was set at 0.2% or 0.3% on a dryweight basis of flour, and CSL or SSL at 0.3% unless otherwise stated.

Farinograms were recorded in a Brabender Farinograph equipped with a 300-g stainless steel bowl. Mixing was at the standard speed of 63 rpm using the slower blade at 30°C.

The viscoelastic properties of bread were measured using a Fudoh rheometer (Rheotec, Tokyo, Japan), as described previously (14). The data were processed using a Rheosoft TR-06 (Rheotec). The staleness of bread as a measure of compression stress, failure strength and Young's modulus were also determined by the rheometer. A $4 \times 4 \times 3 \text{ cm}^3$ sample of stale bread was used.

Differential scanning calorimetry (DSC). DSC measurement was done with a Seiko DSC instrument (Model DSC-100, Tokyo, Japan) as described previously (16, 17), with liquid paraffin as a reference. The heat of gelatinization (Δh), the initial temperature (T_i), the peak temperature (T_p), and the recovery temperature (T_r) were used to characterize the thermal transition of starch.

Image analysis of crumb grains. The procedure used was a modification of that reported by Gohtani et al (19) using a Pias PIAS LA555 computer image analyzer equipped with a PX-380 CCD camera and a Victor AV-M150S color monitor. Xerox photocopies of bread crumbs ($7 \times 7 \text{ cm}^2$) were placed under a non-reflective holding mask and the cell size information was stored in the computer memory. For the analysis of the mean diameter of gas cells, a $6 \times 6 \text{ cm}^2$ area in the middle part of a slice was used. A group of pixels of more than 0.0308 mm^2 was counted as one gas cell, and the data were processed as the area-equivalent diameter when gas cells were assumed to be a circle.

Heat stability of the DHA powder. The DHA powder (10 g) was suspended in 100 mL of deionized water, and heated at 120°C for 30 min. A POV value of the heat-treated sample was determined, and the remaining amount of DHA was also determined by gas-liquid chromatography (GLC) as described below.

Analysis of DHA remaining in the bread. All operations were performed under bubbling N_2 . Lyophilized powder bread crumbs (1 g) were suspended in 2 mL EtOH containing 20 mg heptadecanoic acid as an internal standard. Then 10 mL of 5 N hydrochloric acid was added and the mixture hydrolyzed at 80°C for 30 min. Eight milliliters of EtOH was added to the hydrolyzate and the mixture was gently vortexed. The solution was transferred into a separate funnel, and then DHA was extracted with 50 mL of a diethyl ether-petroleum ether (1:1, v/v) mixture. After shaking vigorously, the upper layer containing the lipids was transferred into another funnel, and the lower solution was extracted twice with 30 mL of the same solvent. Combined solvents were washed with water, dehydrated with solid MgSO_4 , and

then the solvents were evaporated to dryness under reduced pressure. The residue thus obtained was saponified using the AOCS official method (Ce 1B-89): 1.5 mL of 0.5 N NaOH was added to the residue and heated for 7 min under deoxygenated conditions in a screw-capped test tube. After cooling, 2 mL BF_3 /methanol reagent was added to the residue, and then heated at 100°C for 5 min. After cooling, 1 mL of iso-octane was added to the solution, and the solution vortexed vigorously for 30 min. Immediately following, the reaction was stopped with 5 mL of saturated NaCl solution, and the solution was shaken thoroughly. The iso-octane layer was transferred into a separate tube, and the lower layer was repeatedly extracted with iso-octane. Combined solvents were concentrated to about 1 mL. A portion of the sample (1–2 μL) was analyzed by GLC. The peak area corresponding to DHA was calculated by comparison with that of authentic DHA using a Shimadzu C-R6A Chromatopak.

GLC. GLC was carried out with a Shimadzu GC-17A apparatus equipped with a flame ionization detector, fitted with a Quadrex CPS-1 glass capillary column (0.32 mm i.d. \times 15 m; depth of the liquid film, 0.25 μm). Analytical conditions were as follows: column temperature, holding at 60°C for 1 min, then programmed at a rate of 6°C/min until 160°C, following 1.8°C/min until 200°C; the detector and injector, 250°C; helium at a flow rate of 2.1 cm^3/min ; and mode of sample introduction, non-splitting mode.

RESULTS AND DISCUSSION

Effect of DHA powder on the loaf volume of bread

The effect of DHA powder on the loaf volume of baked bread was tested. Figure 1 shows the cross-sectional views of baked bread in which the DHA powder was at levels of 0.1 to 0.5% by way of example. Of the varying amounts added (up to 0.5% w/w flour basis), the loaf volume increased with increasing amounts of DHA powder in the range of 0.1 to 0.3%. The loaf volume reached a maximum (4.48 cm^3/g) at 0.2% and rather decreased at more than 0.3%.

The appearance of crumbs of the baked bread seemed to be fairly good at the 0.5% level of DHA powder. Baked bread containing the DHA powder did not

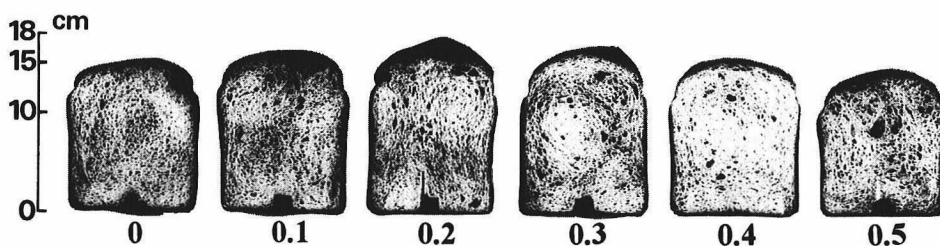


Fig. 1. Cross-sectional views of baked bread containing DHA powder. The amount of DHA powder added was the weight percent to the amount of wheat flour.

develop a fish odor, probably because most of the DHA remained embedded in the microcapsule.

Rheological results

Dough was mixed with different additives in the home baker for 30 min, and then its viscoelastic properties were measured. The modulus of elasticity and the viscosity coefficient of dough mixed with a single additive (0.2 or 0.3% DHA powder or SSL) or with combined additives of DHA powder and CSL or SSL were significantly ($p < 0.05$) higher than those of the control without any additive (Table 1). However, the viscoelastic parameters of dough containing DHA powder with CSL or SSL were not different when compared with dough containing a single additive.

Farinographic data are shown in Table 2. The arrival and development times

Table 1. Effect of DNA powder on the viscoelasticity of dough.

Additive	Viscoelastic parameters ¹			
	τ	g	γ	η
Control	0.55 ^{a+}	169.3 ^a	6.4 ^a	3.49 ^a
DHA powder 0.2%	0.65 ^b	262.1 ^b	10.4 ^b	7.08 ^{cd}
DHA powder 0.3%	0.75 ^c	292.3 ^b	10.7 ^b	8.20 ^d
DHA powder 0.2% + SSL 0.3%	0.63 ^{ab}	247.1 ^b	9.74 ^b	6.03 ^{bc}
DHA powder 0.3% + SSL 0.3%	0.63 ^{ab}	253.5 ^b	10.0 ^b	6.32 ^{cd}
DHA powder 0.2% + CSL 0.3%	0.65 ^{bc}	265.7 ^b	10.4 ^b	6.76 ^{cd}
DHA powder 0.3% + CSL 0.3%	0.73 ^{bc}	276.4 ^b	10.9 ^b	7.72 ^{cd}
SSL 0.3%	0.65 ^{bc}	254.7 ^b	9.8 ^b	6.27 ^{cd}
CSL 0.3%	0.60 ^{ab}	186.8 ^a	7.5 ^a	4.32 ^{ab}

¹ g , stress (10^2 N m^{-2}); γ , modulus of elasticity (10^4 N m^{-2}); τ , relaxation time (s); η , viscosity coefficient (10^5 N s m^{-2}). Each value shows the average of 4 trials.

+ Numbers followed by the same letter are not significantly different at $p < 0.05$ (Duncan's multiple range test).

Table 2. Farinograph data of dough containing DHA powder and other additives.

	Control	DHA powder	DHA powder + CSL	DHA powder + SSL	DHA powder + GCA	CSL	SSL	GCA
Arrival time (min)	1.6	1.5	1.6	1.3	1.5	1.5	1.7	1.5
Development time (min)	2.7	2.2	2.4	2.5	14.5	2.6	2.5	13.0
Stability time (min)	18.2	22.8	21.9	27.7	23.5	18.0	20.0	21.1
Water absorption (%)	66.0	65.5	67.0	66.2	66.0	66.8	66.3	66.0

Amount of additives added: 0.3% (w/w flour basis). Values are means of two trials.

Table 3. Summary of gelatinization temperature and the enthalpy of starch in wheat flour dough with DHA powder after mixing for 30 min in a home baker.

Additive	T_i	T_p	T_r	Δh
	(°C)			J/g wet dough
Control	51.7	66.7	96.9	2.0
DHA powder 0.3%	51.5	65.4	96.5	2.0
DHA powder 0.3% + CSL	49.9	64.7	94.7	2.2
DHA powder 0.2% + GCA	49.5	66.1	93.3	1.7
DHA powder 0.3% + GCA	52.9	65.6	95.1	1.7
CSL 0.3%	51.9	65.1	94.7	2.0

T_i , initial temp.; T_p , peak temp.; T_r , recovery temp.; Δh , enthalpy. Number of trials: 2.

of the dough containing DHA powder alone did not change when compared with those of the control. However, the addition of DHA powder alone or together with SSL or GCA increased the stability time significantly. The rheological properties of dough containing DHA powder may have been improved by the action of GCA and/or unsaturated fatty acid (20) attached to the surface of the microcapsules.

DSC results

The gelatinization temperature and enthalpies of wheat dough mixed for 30 min in the baker are shown in Table 3. The T_p value of gelatinization temperature of starch in the dough containing DHA powder alone or together with CSL or GCA tended to decrease. On the other hand, the gelatinization enthalpy was not appreciably changed by the single addition of DHA powder, but decreased with the combined addition of GCA and DHA powder.

Staleness of bread

The staleness or firmness of bread crumbs stored at 20°C were tested daily using a rheometer (Table 4). The data for the crumbs containing DHA powder were somewhat scattered as compared to the control values. As a whole, the compression stress of the bread containing DHA powder tended to decrease. This suggests that DHA or GCA may retard the rate at which the bread crumbs became stale although there were no significant changes in the firmness of bread crumbs during storage.

After 3 days' storage, the appearance of crumbs in the bread containing DHA powder was considered to be similar to that of the control bread, because of the absence of fish odor.

Table 4. Effect of DHA powder on the firmness of bread, as measured with a rheometer.¹

Storage (day)	Amount of additives					
	0%	0.2%	0.3%	0.5%	1.0%	2.0%
0	140.2	103.3	106.9	124.9	102.8	102.4
1	322.2	252.8	273.3	292.2	252.7	275.3
2	441.4	353.7	380.1	435.1	341.5	372.1
3	550.1	425.6	469.4	530.7	413.1	411.3

¹ Values are stress (10^2 N m^{-2}) of 4 trials.

Table 5. Effect of DHA powder on the size of gas cells of bread, as counted with an image analyzer.

Amount of DHA powder added					
0%	0.1%	0.2%	0.3%	0.4%	0.5%
1.87 ^{a+}	1.97 ^{ab}	2.02 ^b	2.09 ^b	2.02 ^{ab}	2.01 ^{ab}

Each value shows the average diameter (mm) of gas cells of 4 trials.

⁺ Numbers followed by the same letter are not significantly different at $p < 0.05$.

Gas cell distribution in bread crumbs

The effect of DHA powder as a breadmaking ingredient on the gas cell distribution in bread crumbs is shown in Table 5. The addition of DHA powder up to 0.5% slightly increased the mean diameter of the gas cells as compared to those of the control. This increase roughly corresponded to the increase in loaf volume, probably implying with improvement of CO_2 entrapment in the dough. The addition of DHA powder at 0.3% provided bread crumbs with the largest gas cells (2.09 mm).

Stability of DHA powder in heating and bread crumbs

The color of microcapsulated DHA powder suspension heated at 120°C for 30 min was slightly brown, while that of the refined fish oil changed dark brown. The POV value for the heat-treated microcapsulated DHA powder [(2.1 ± 0.2) meq/kg] was significantly different from that of the refined fish oil [(7.9 ± 0.2) meq/kg] at the level of $p < 0.01$. Moreover, the remaining amount of DHA, which was determined by GLC for the heat-treated DHA powder [$(99.8 \pm 0.1)\%$], was also significantly different from that of the refined fish oil [$(98.0 \pm 0.1)\%$]. Therefore, the microcapsulated DHA powder proved to be relatively stable. This result indicates that DHA remains intact in the microcapsulated particles, and does not give out an unpleasant odor even after baking.

The amount of DHA remaining in the crumbs after breadbaking of dough supplemented with 0.1 and 0.5% DHA powder was determined by GLC according to the AOCS official method. In the addition of 0.1% DHA powder, only a trace amount of DHA was detected from 1 g of bread crumbs, since it was the lower limit for the GLC detection, whereas that of 0.5% DHA powder yielded 100%.

DHA embedded in the microcapsulated particles was found to be stable, as was the case with linoleic acid (11). Furthermore, encapsulated DHA powder is expected to be absorbed from the intestine without any problems. When dough containing 0.3% DHA powder was used, the baked bread contained 0.02% calcium as calcium gluconate and 0.012% DHA on a dry weight basis. Two slices of this bread (about 200 g), which is the typical amount that is eaten by one person at one meal, is estimated to contain 40 mg of calcium and 25 mg of DHA. Since an excess amount of linolic acid inhibits the synthesis of DHA from linolenic acid, this amount is not sufficient for daily DHA requirements, but it is possible to obtain DHA directly from bread every meal. The bread supplemented with microcapsulated DHA powder did not have any fish odor after baking. Therefore, there is a possibility of extensive use of DHA powder in food stuffs as an improver of the ratio of *n*-3/*n*-6 polyunsaturated fatty acid (8, 9, 21).

CONCLUSION

The addition of microcapsulated DHA powder to bread dough at a concentration of about 0.3% increased the loaf volume distinctly and the size of gas cells slightly, without affecting the appearance of the bread. The gelatinization temperature and enthalpy of the starch of the dough did not change significantly. Since the DHA powder was relatively stable even when heated under extreme conditions at 120°C for 30 min, DHA might be stabilized by the coating of GCA in the microcapsules. Also, the GCA attached to the surface of the DHA powder might improve the rheological properties of the dough (14). The dough became distinctly more viscous and somewhat rigid with the combined action of calcium ions or fatty acid in fish oil. As a result, the dough containing DHA powder developed well, probably because of the entrapment of CO₂ in the dough during the mixing and baking processes, and improved the baking performance and softness of the bread. Furthermore, there was no fish odor. From the above results, DHA powder appears to have the potential for improving both the loaf volume and viscoelastic properties of bread.

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