Quinoa Flour as a New Foodstuff for Improving Dough and Bread

Naofumi Morita,* Chikako Hirata, Sang Ha Park and Toshio Mitsunaga¹

*Laboratory of Food Chemistry, Graduate School of Agriculture and Biological Sciences,
Osaka Prefecture University (1-1, Gakuencho, Sakai 599-8531, Japan)*

¹*Department of Food and Nutrition, Faculty of Agriculture and Institute for Comprehensive Agriculture,
Kinki University (3327-204, Nakamachi, Nara 631-8505, Japan)*

Characteristics of dough and bread containing quinoa flour as a new foodstuff were studied using a rheometer, a farinograph, differential scanning calorimeter (DSC) and so on. Substitution of 7.5 to 10% of quinoa flour for hard-type wheat flour significantly increased the loaf volume of bread over that of the control, but more than 15% substitution distinctly decreased the volume. However, a combination of microbial lipase (75 ppm) and 15% substitution of quinoa flour resulted in a loaf volume that was distinctly more than that of the control. The hardness of bread crumbs increased in proportion to the amount of quinoa flour substitution. Combining the quinoa flour with lipase suppressed the staling of bread during storage and maintained softness by the liberation of monoglyceride from lipids. DSC data showed that substitution of quinoa flour resulted in a distinctly higher gelatinization temperature and gelatinization enthalpy compared with that of the control. As for the viscoelastic properties of the dough, combining quinoa flour (5 to 10% substitution) and lipase (75 ppm) resulted in reduction of compression stress and the modulus of elasticity, a slight softening of the dough, and slight decreases in the water absorption and the stability of the dough. From these results, a combination of quinoa flour substitution and lipase addition was found to improve bread qualities.

About three thousand species of edible plants in total have been cultivated in the past history of mankind. However, most of them have gradually disappeared, and only a total of 30 species remain in the world.¹⁾ The criteria of the continuation of species might not be due to the quality of crops, but to the yield of production, to the special climate requirement for the production, to the crops cultivated by small communities or tribes, and so on. As the result, plants yielding quite high amounts of crops, such as rice, wheat, corn, *etc.* are selected in the area of sub-tropical or tropical countries.

Quinoa, one of the pseudocereals, belongs to an annual plant of *Chenopodium*, which is mainly produced in the highland of Peru and Bolivia, and the grain contains large amounts of minerals, lipids, and high quality protein, which contain abun-

dant essential amino acids, such as lysine or histidine.^{2,3)} Therefore, the amino acid score of the flour is higher than the scores of rice, wheat and corn. Quinoa flour is rarely allergenic and also the outer layer of the grain lowers the cholesterol level of blood.⁴⁾ Furthermore, NASA has recommended quinoa as one of the plants that are suitable for CELSS (controlled ecological life support system), which can reduce CO₂ concentration in a manned spacecraft atmosphere, and produce foodstuffs, oxygen and water. From these points of view, we focused our attention on using quinoa flour as wheat flour substitute and microbial lipase for a bread improver. This study deals with the effect of quinoa flour substitution on some rheological properties of wheat flour dough and on the loaf volume of bread.

* To whom correspondence should be addressed.

MATERIALS AND METHODS

Flour and chemicals. The wheat flour used for bread making was a hard-type "Camelia" (Nishin Flour Milling Co., Ltd., Kobe) as described previously.⁵⁾ The protein and ash contents were 13.6% and 0.44%, respectively, on a dry flour basis.

Quinoa flour (*Chenopodium quinoa* Real) was obtained from Asai Co., Ltd. (Nagoya), which had imported it from Peru. The flour contained 14.9% protein, 75.1% starch, 5.5% lipids, 2.4% fiber, 2.1% ash on a dry flour basis. The crude lipase (7.5×10^3 U/g) of *Aspergillus niger* was provided by Amano Pharmaceutical Co., Ltd. (Nagoya). The enzyme contained negligible amount of protease and about 5×10^{-2} U of amylase per one unit of lipase. But, the amylase was heat-unstable and did not degrade the raw starch distinctly during the mixing and breadmaking process. The amount of lipase used was 75 ppm on a flour basis. Grain fatty acid methyl ester-mixture was purchased from Suppelco (USA). Other chemicals used were of analytical grade.

Bread baking. The bread making formula was 280 g of flour, 5 g of sodium chloride, 17 g of sucrose, 3 g of dry baker's yeast (Asahi Kasei, Co., Ltd., Tokyo), and 210 g of water in the presence or absence of additives.

Test loaves were baked with five automatic bread makers in the same manner described previously.⁶⁾ The total time for the entire process was 4 h (20 min mixing, 10 min aging, 10 min additional mixing after addition of yeast, 155 min fermentation and 45 min baking). The volumes of loaves were measured by the rapeseed displacement method.

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

Analytical methods.

Rheological tests. Farinograms were recorded in a Brabender Farinograph equipped with a 300 g stainless steel bowl. The standard speed of mixing

was 63 rpm at 30°C. The farinograph data including the percentage of water absorption, arrival time, development time, and stability time were obtained on maximum resistance, centered on 500 B.U.

Viscoelastic properties of the dough and the bread crumbs were measured using a Fudoh rheometer (Rheotech Co., Ltd., Tokyo), as described previously.⁵⁾ For the dough sample which was obtained by mixing for 30 min in the home baker, a 1-cm diameter plunger for viscoelastic measurement and a 3-cm i.d. $\times$ 5 cm sample were used, and the penetration depth was controlled at 2 cm. On the other hand, for the bread crumbs, a 3-cm diameter plunger for compression stress and a $4 \times 4 \times 3$ cm³ sample of stale bread was used, and the compression depth was controlled at 7 mm. For both samples, the speed of the plunger was 6 cm/min, and the data were processed using a computer program, Rheosoft TR-06 (Rheotech Co., Ltd., Tokyo).

Differential scanning calorimetry (DSC).

DSC was taken with a Shimadzu DSC instrument (Model DSC-50, Tokyo) as described previously,^{5,7)} with liquid paraffin as a reference. The initial temperature (T_i), peak temperature (T_p) and recovery temperature (T_r) of gelatinization of starch and melting of starch-lipid complex, and enthalpy values for starch (Δh_1) and starch-lipid complex (Δh_2) were measured to characterize the thermal properties of dough. The temperature was raised from room temperature to 120°C at the rate of 5°C/min.

Scanning electron microscopy (SEM). SEM in a Hitachi SEM apparatus (Model S-800) was essentially the same as described previously.⁶⁾

Image analysis of crumb grains. The procedure was the same as reported previously,⁵⁾ using a Pias Computer Image Analyzer PIAS LA555 equipped with a CCD camera PX-380 and a Victor color monitor AV-M150S. Xerox photocopies of bread crumbs (7×7 cm²) were placed under a non-reflective holding mask, and cell size information was stored in the computer memory. For an analysis of the mean diameter of gas cells, a 6×6 -cm² area in the middle part of a slice was used. A group of pixels of more than 0.0308 mm² was

Fig. 1. Effect of lipase on specific volume of bread substituted with quinoa flour.

Amount of lipase (ppm/g flour basis): ○, 0; □, 50; ▲, 75; △, 100; ●, 200. ($N=3$)

counted as one gas cell, and the data were processed as the area-equivalent diameter when gas cells were assumed to be a circle.

Gas-liquid chromatography (GLC). GLC was carried out with a Yanagimoto GCG-equipped with a flame ionization detector, fitted with a glass column (0.34 cm i.d. $\times$ 2 m) packed with 3% DEGS on Chromosorb WAW DMCS (80–100 mesh) (GL Sciences Inc., Tokyo). Analytical conditions were as follows: The column was programmed at a rate of 5°C/min from 160°C to 210°C; the detector and injector, 250°C; N_2 gas was at a flow rate of 2.1 cm³/min. Free lipids extracted from lyophilized dough sample using *n*-hexane was methylated for 5 min with BF₃ in methanol, as reported.^{8,9)} An aliquot of lauric acid was used for an internal standard.

RESULTS AND DISCUSSION

Baking results.

Substitution of 7.5% and 10% of quinoa flour for wheat flour increased the loaf volume of bread significantly compared with that of the control without quinoa flour (Fig. 1). This suggests that substitution of quinoa flour improves the balance of starch, protein and lipids. Fifteen percent or more of replacement with quinoa flour resulted in a distinct decrease in the loaf volume. Addition of lipase alone to the ingredients increased the spe-

Table 1. Average diameter of gas cells of bread baked with quinoa flour substitution.

Quinoa flour (%)	Lipase 0 (ppm)	Lipase 75 (ppm)
0	1.16 $\pm$ 0.01	0.99 $\pm$ 0.04
5.0	1.04 $\pm$ 0.01	0.99 $\pm$ 0.16
7.5	0.88 $\pm$ 0.07	0.97 $\pm$ 0.24
10.0	1.04 $\pm$ 0.13	1.10 $\pm$ 0.03
15.0	1.07 $\pm$ 0.15	1.17 $\pm$ 0.11
20.0	1.07 $\pm$ 0.23	1.09 $\pm$ 0.06

Number of experiment: 3. Each value shows in mm.

cific volume of bread in proportion to the amount of lipase tested. Combinations of 7.5% quinoa flour substitution and 50 ppm or 75 ppm lipase addition increased the loaf volume significantly as compared with the control.

Table 1 shows the average diameter of the gas cells of bread baked with various amounts of quinoa flour. Substitution of 7.5% quinoa flour with (0.97 $\pm$ 0.24 mm) or without (0.88 $\pm$ 0.07 mm) 75 ppm lipase showed the lowest diameter of gas cells.

Figure 2 shows the change of firmness of bread crumbs during storage at 25°C in a wrapped vessel. In the bread crumbs baked with various amounts of quinoa flour substitution without addition of lipase, the firmness was proportional to the amount of quinoa flour substitution and number of storage days. Addition of 50 ppm lipase to various amounts of quinoa flour distinctly suppressed the firmness as compared with the control. The suppression of firmness was obvious at 15% or more of quinoa flour substitution containing 50 ppm or more of lipase. Therefore, staling of bread crumbs was obviously improved by use of lipase.

As a result, substitution of about 7.5% quinoa flour for wheat flour improved the specific volume and gas cell distribution of the bread. Also, addition of lipase improved the staling of bread crumbs during storage.

Dough property.

The viscoelastic properties, such as relaxation time, stress, modulus of elasticity, and viscosity coefficient of wheat dough containing various amounts of quinoa flour was measured using a

Fig. 2. Staling of bread crumbs baked with various amounts of quinoa flour containing lipase, as measured by a rheometer. ($N=3$)

Table 2. Viscoelastic properties of dough substituted with quinoa flour.

Quinoa flour (%)	Relaxation time (s)	Stress (10^2 N/m^2)	Modulus of elasticity (10^4 N/m^2)	Viscosity coefficient ($10^4 \text{ N} \cdot \text{s/m}^2$)
0	0.6	101.4 ± 6.9	4.39 ± 0.25	2.63 ± 0.11
5.0	0.7	103.6 ± 1.2	4.54 ± 0.11	2.86 ± 0.11
7.5	0.6	113.8 ± 12.4	5.12 ± 0.27	3.07 ± 0.20
10.0	0.6	125.1 ± 13.1	5.63 ± 0.32	3.65 ± 0.21
15.0	0.6	121.6 ± 3.4	5.09 ± 0.17	3.01 ± 0.24

Number of experiment: 3.

rheometer. As is obvious from Table 2, the relaxation time did not change, but the other parameters were the highest in the case of 10% quinoa flour substitution when compared with the control or other amounts of quinoa flour substitution. The addition of 75 ppm lipase to the same dough also showed a result similar to that without lipase addition. This increase may be caused by the strengthening effect of the gluten network or dough structure. However, in the case of 15% quinoa flour substitution, the stress, elasticity and viscosity coefficient of the dough was reduced distinctly. This result is surely due to the decrease in the amount of glutenin and gliadin in the dough, and the subsequent decrease in the amount of gluten forma-

tion. Furthermore, addition of lipase might increase the amounts of monoglyceride and/or free fatty acids, and the monoglycerides produced could act as an emulsifier. This would then result in clear decreases in the elasticity and viscosity coefficient.

Figure 3 shows the farinograph data of quinoa flour-substituted wheat dough, including the arrival time, development time of dough, stability of dough, and water absorption. The values of the farinograph parameters, except for the arrival time, decreased distinctly in proportion to the amount of quinoa flour substitution, suggesting that quinoa flour substitution changes the consistency of the dough. In case of 75 ppm lipase addition, all pa-

Fig. 3. Properties of doughs, as measured by a farinograph.

■, arrival time; ■, development time; □, stability time; ●, water absorption. $N=2$.

Table 3. Effect of quinoa flour and lipase concentration on thermal properties of dough.

Quinoa flour (%)	Lipase (ppm)	T_i (°C)	T_p (°C)	T_r (°C)	Δh_1 (J/g)	Δh_2 (J/g)
0	0	57.7	63.7	90.7	2.68	1.05
0	75	58.7	65.5	89.9	3.22	1.00
7.5	0	58.4	64.9	87.9	1.92	1.13
7.5	75	59.2	66.1	87.4	2.26	1.00
20	0	58.5	66.7	87.2	1.76	1.30
20	75	59.8	66.8	86.3	1.97	1.46
100	0	60.2	67.4	83.3	2.13	1.34

Number of experiment: 2.

rameters tested became lower than the control without lipase. In particular, lipase addition resulted in a distinctly shorter development time compared with that of the control without lipase addition. Furthermore, the development time of dough increased in proportion to the amount of quinoa flour substitution.

Thermal properties of starch in dough substituted with quinoa flour.

Table 3 shows the results of thermal properties of dough after mixing for 30 min using DSC. Regardless of the addition of lipase, T_i and T_p of the dough increased and T_r decreased slightly, according to the increase in the quinoa flour substitution. The value of Δh_1 decreased in proportion to the increase in the quinoa flour substitution, while that of Δh_2 increased. The addition of quinoa flour did not improve the favorable gluten formation, but rather deteriorated the formation (as is obvious

from Fig. 4-B and C). Therefore, the gluten sheet surrounding the starch granules was assumed to weaken the dough strength with the addition of quinoa flour. However, the gelatinization temperature of starch in dough increased slightly. This fact may be mainly caused by the presence of quinoa flour having a higher gelatinization temperature (67.4°C) and lower gelatinization enthalpy (2.13 J/g) than those of wheat flour. In general, the Δh_2 peak is considered to be derived from lipid-starch complex.¹⁰ But, Δh_2 was normally smaller in the case of wheat dough than Δh_1 . And also the quinoa starch, having a high water swelling power,¹¹ may increase the water binding-capacity of the substituted dough. The present quinoa flour increased Δh_2 , which might be caused by the formation of starch-lipid complexes; therefore suppression of the staling of bread crumbs was expected. However, no distinct relations between the firmness of bread crumbs baked with 5 or 10% quinoa

Fig. 4. SEM photographs of doughs containing various amounts of quinoa flour with or without lipase.

Quinoa flour substitution: A, quinoa flour 0%; B, quinoa flour 7.5%; C, quinoa flour 20%; D, quinoa flour 100%. The lower line (E, F, and G) contains 75 ppm of lipase. Direct magnification, $\times 1500$.

Table 4. Composition of free fatty acid in dough containing various amounts of quinoa flour with or without lipase.

Lipase (ppm)		0				75			
Quinoa flour (%)		0		7.5		0		7.5	
Time (min)		20	195	20	195	20	195	20	195
C14:0		0.03	0.07	0.12	0.05	0.03	0.06	0.04	0.04
C16:0		0.29	0.57	1.54	0.69	0.38	0.35	0.70	0.53
C18:0		0.03	0.07	0.11	0.05	0.02	0.05	0.04	0.04
C18:1		0.25	0.46	1.52	0.99	0.30	0.27	1.02	0.78
C18:2		0.83	1.59	5.28	2.46	1.09	0.98	2.58	1.98
C18:3		0.05	0.10	0.40	0.30	0.06	0.06	0.34	0.24
C20:0		—	—	—	0.02	—	—	0.01	—
C22:0		—	—	—	0.03	—	—	0.01	—
C22:1		—	—	—	0.01	—	—	0.03	0.02
Total		1.48	2.86	8.97	4.60	1.88	1.77	4.77	3.63

Each value shows mg/g dry flour basis. —: not detected.

flour substitution with or without 75 ppm lipase (Fig. 2) and Δh_2 (Table 3), were observed, except for the case of 20% quinoa flour substitution with or without 75 ppm lipase. Therefore, the increase in the Δh_2 was assumed to be derived from the characteristics of starch in the quinoa flour, and the Δh_2 might not be directly correlated with the staling of bread during storage.

Effects of quinoa flour substitution and lipase addition on the quantity of lipids in the dough.

The amount of free fatty acid in the dough after mixing for 20 min and 195 min in the home baker was determined (Table 4). In the control dough without quinoa flour substitution, the total amount of free fatty acid slightly increased when the mixing was increased from 20 min (1.48 mg/g dry

flour) to 195 min (2.86 mg/g dry flour). On the other hand, in the 7.5% quinoa flour substitution, the total amount of free fatty acid decreased significantly when the mixing time was increased from 20 min (8.97 mg/g dry flour) to 195 min (4.60 mg/g dry flour). The decrease might be mainly due to the incorporation or inclusion of free fatty acids to the starch in dough during mixing and proofing. On the other hand, when 75 ppm lipase was added to the dough without quinoa flour substitution, the amount of free fatty acid in the dough did not change clearly when compared with the control. However, when 75 ppm lipase was added to the 7.5% quinoa flour substituted dough, the total amount of free fatty acid distinctly decreased compared with that without lipase, when the mixing time was 20 min. Therefore, in the case of dough containing 7.5% quinoa flour and 75 ppm lipase, the free fatty acids liberated from lipids by the lipase might be adsorbed or attached to the dough, or incorporated into the helical portions of starch, which could not be easily extracted with *n*-hexane. In addition, lipoxigenase in the quinoa flour dough might be also involved in the degradation of lipids. However, further experiments are needed to make clear the effect of lipase on the lipids in the dough during mixing and proofing.

SEM observation of bread dough.

Wheat dough containing quinoa flour with or without lipase addition after mixing for 30 min in the home baker was observed by SEM (Fig. 4). The dough (B) containing 7.5% quinoa flour showed an irregular gluten network structure and that (C) containing 20% quinoa flour showed a more irregular structure, that is, most gluten layers did not distribute continuously, and they surrounded the large starch granules as shown in (B) and (C). The lipase-enhanced dough (E) showed a well-developed gluten network structure and the gluten covered each starch granule entirely. Therefore, the combined addition of 7.5% quinoa flour with 75 ppm of lipase was tested as shown in F. The mixed dough showed that the well-developed gluten layers wrapped the starch granules more completely than in B, and also large starch gran-

ules are mostly covered with thick gluten layers. The quite small size of the starch granules (around 1 μm in diameter) of quinoa flour might be helpful as a filling material in the gluten matrix. When 20% quinoa flour was used instead of 7.5% quinoa flour, the dough (G) showed that the gluten appeared to become somewhat thinner, and to be scattered continuously around the starch granules in the dough.

Substitution of quinoa flour alone for wheat flour up to 10% improved the baking property, though it was only a slight effect. However, combining the quinoa flour with lipase distinctly improved the dough and baking properties. In fact, a combination of 15% quinoa flour and 75 ppm lipase distinctly improved the baking property of the dough compared with that of the control. The amount of 10% or 20% substitution of quinoa flour may not be low enough to avoid an allergic reaction and it may not be enough to improve physiological activity significantly. Japanese consumers, unlike those in other advanced countries such as Europe and North America, do not like to eat whole-wheat bread.¹²⁾ So, even though the amount of substitution of quinoa flour for wheat flour is low, it is necessary to acquire the daily habit of eating bread made from flour mixed with pseudocereals, such as amaranth, buckwheat, millet, and so on.

In the present study, addition of lipase to wheat flour partially replaced with quinoa flour was found to improve breadmaking properties. We expect the quinoa flour or pseudocereals to be recognized as a functional foodstuff, and to be practically used for breadmaking as a partial substitution for wheat flour. Since quinoa flour contains high amounts of minerals, vitamins, dietary fibers, *etc.*, these kinds of pseudocereals are needed to improve our daily diet patterns.

The authors wish to thank the Nisshin Flour Milling Co., Ltd. (Kobe) for supplying wheat flour; the Asai Co., Ltd. (Nagoya) for providing quinoa flour; the Asahi Kasei Co., Ltd. (Tokyo) for providing dry yeast; and the Amano Pharmaceutical Co., Ltd. (Nagoya) for providing lipase. They also thank Mr. T. Takao, the Dai-Nippon Meiji Sugar Co., Ltd. (Tokyo), for his valuable suggestions and comments on this study.

REFERENCES

- 1) N.D. Vietmeyer: Lesser-known plants of potential use in agriculture and forestry. *Science*, **232**, 1379–1384 (1986).
- 2) G.S. Ranhotra, J.A. Gelroth, B.K. Glaser, K.J. Lorenz and D.L. Johnson: Composition and protein nutritional quality of quinoa. *Cereal Chem.*, **70**, 303–305 (1993).
- 3) G.S. Chauhan, N.A.M. Eskin and R. Tkachuk: Nutrients and antinutrients in quinoa seed. *Cereal Chem.*, **69**, 85–88 (1992).
- 4) T. Takao: Component of quinoa and biological activity. *Food Sci. J.*, **253**, 52–58 (1999) (in Japanese).
- 5) N. Morita, Y. Arishima, N. Tanaka and T. Shiotsubo: Utilization of hemicellulase as bread improver in a home baker. *J. Appl. Glycosci.*, **44**, 143–152 (1997).
- 6) T. Maeda and N. Morita: Effect of polished-graded flour substitution to commonly milled wheat flour on the properties of dough and bread. *J. Appl. Glycosci.*, **47**, 1–12 (2000).
- 7) N. Inouchi, K. Nishi, S. Tanaka, M. Asai, Y. Kawase, Y. Hata, Y. Konishi, S. Yue and H. Fuwa: Characterization of amaranth and quinoa starches. *J. Appl. Glycosci.*, **46**, 233–240 (1999).
- 8) G.K. Gregory, T.-S. Chen and T. Philip: Quantitative analyses of carotenoid and carotenoid fatty acid esters in fruits by HPLC: Red bell peppers. *J. Food Sci.*, **52**, 1071–1073 (1987).
- 9) G.N. Jham, F.F.F. Teles and L.G. Campos: Use of aqueous HCl/MeOH as esterification reagent for analysis of fatty acids derived from soybean lipids. *J. Am. Oil Chem. Soc.*, **59**, 132–133 (1982).
- 10) T.A. Siswoyo and N. Morita: Effect of phospholipids transesterified enzymatically with polyunsaturated fatty acid on gelatinization and retrogradation of starch. *J. Nutr. Sci. Vitaminol.*, **46**, 252–256 (2000).
- 11) K. Lorenz: Quinoa (*Chenopodium quinoa*) starch-Physico-chemical properties and functional characteristics. *Starch/Stärke*, **42**, 81–86 (1990).
- 12) S. Ueki: Present situation of breadmaking in USA and its future in Japan. *Rep. Jpn. Inst. Baking*, 9–10, 38–43 (2000) (in Japanese).

(Received August 30, 2000; Accepted January 12, 2001)

ドウおよびパンの改良新食品素材
としてのキノア粉

森田尚文, 平田智佳子, 朴 商夏, 光永俊郎¹

大阪府立大学大学院農学生命科学研究科
(599-8531 堺市学園町 1-1)

¹ 近畿大学農学部食品栄養学科・食品総合研究所
(631-8505 奈良市中町 3327-204)

新食品素材としてのキノア粉代替のドウおよび製パン特性についてレオメーター、ファリノグラフ、示差走査熱量計等により検討した。硬質小麦粉に7.5–10%のキノアを代替すると、パンの比容積は無添加のコントロールよりも有意に増大したが、15%以上の添加では明らかに減少した。しかしリパーゼ75 ppm（対粉当り）と15%キノアの代替により、その比容積は明らかに無添加のものより増大した。パンのクラムはキノアの代替量の増加に伴い硬くなったが、リパーゼとの併用により保存中の老化は抑制された。示差走査熱量測定によりキノアの代替による澱粉の糊化温度および糊化エンタルピー変化は無添加のものよりも明らかに大きくなった。ドウの粘弾性についてはキノアとリパーゼの併用により圧縮応力、弾性率を減少させドウを軟らかくするとともに吸水率および安定性を低下させる傾向がみられた。以上、キノア粉の代替とリパーゼの併用により製パン性を改良することが明らかとなった。