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Effect of Storage in Frozen Soil on the Quality of Adzuki Beans [*Vigna angularis* (Willd.) Ohwi et H. Ohashi], Soybean [*Glycine max* (L.) Merr.], Red Kidney Beans [*Phaseolus vulgaris* L.], and Buckwheat [*Fagopyrum esculentum* Moench]

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Abstract Ice and snow, which are important components of Earth's climate system, maintain high humidity and low temperature, and hence can be used to preserve the quality of agricultural products. The purpose of this study was to investigate how the quality of adzuki beans, soybean, red kidney beans, and buckwheat was affected after storage in frozen soil for 15 months. Storage of the grains in frozen soil, in a sealed polyethylene bag, helped in controlling humidity and maintaining a low temperature during the storage period, thus suppressing the increase in the fatty acid values and thiobarbituric acid reactive substances. The fatty acid value was not the sole criterion for evaluating the quality when the moisture content of the grains was too low. Moisture contents of 12.5–13.5%, 9–11.5%, 11–13%, and <14%, combined with low-temperature conditions, were best suited for storing adzuki beans, soybean, red kidney beans, and buckwheat, respectively. Hence, storage of Hokkaido's main agricultural products in frozen soil, in a sealed polyethylene bag, is expected to have promising applications in the storage industry.

Keywords: *bean, buckwheat, storage, frozen soil, moisture content, fatty acid value, thiobarbituric acid reactive substances*

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1. Introduction

Ice and snow, which are important components of Earth's climate system, show a significant feature: they help in maintaining high humidity and low temperature. This feature can be exploited to preserve the quality of agricultural products stored in ice. Moreover, ice and snow are considered low-cost resources that would help in mitigating storage costs. Most importantly, the use of ice and snow will help in saving energy resources (electricity and fuel) and reducing environmental load by minimizing CO_2 emission. Past attempts have been limited to the storage of rice and potatoes using frozen soil, [1] but there is no clear information on the effect of this type of storage on the quality of adzuki beans, soybean, red kidney beans, and buckwheat, which are the main agricultural products of Hokkaido.

Free fatty acids (FFAs) have been reported to have adverse effects on the quality of pinto beans, wheat, and coffee seeds. [2,3,45] During storage, the membrane covering the seeds ruptured, and the constituent lipids were found to undergo oxidation and turn rancid, causing the generation of some compounds such as (*E*)- and (*Z*)-2-nonenal, (*E*,*Z*)-2,6-nonadienal that adversely affected the aroma of raw coffee. [6,7,8,9] Thiobarbituric acid reactive substances (TBARS), formed as a result of lipid peroxidation, can be used to evaluate the extent of lipid oxidation in coffee beans, meat, fish products, etc. [5,10,11]. With an increase in the storage period and moisture content (MC) of the beans, the fatty acid values (FAVs) were found to increase considerably. [12,13,14] Further, as in the case of enzymes in general, the peroxidase and lipase activity increased with an increase in temperature up to about 40 °C [15].

The purpose of this study was to investigate the quality of adzuki beans, soybean, red kidney beans, and buckwheat that were packed in sealed polyethylene bags (SPo) and paper bags (Pa) and then stored in frozen soil; quality assessments were made by measuring the MC, FAV, and TBARS content of the stored samples. The grain samples were also stored in a freezer (F) and a

thermostatic oven (TO), and the quality was compared with that of the grains stored in the frozen soil (FS) for 15 months. In addition, attempts were made to develop a method for evaluating the quality of these grains and identifying the optimal storage conditions (humidity and temperature).

2. Materials and Methods

Sample storage Adzuki beans, soybean, red kidney beans, and buckwheat were packed in paper bags (Pa) and sealed polyethylene bags (Spo), and stored in frozen soil (FS) in Obihiro, Hokkaido, Japan. The frozen soil was prepared by means of an artificial permafrost storage system using heat pipes. The heat pipes act as heat transfer elements that transfer the outdoor cold energy during winter to the underground, without the need for a power source. Some of these samples were also stored in a freezer (F, EV200NXK; Whirlpool, USA) and thermostatic oven (TO, SDN27; Sansyo Co. Ltd., Tokyo, JP) in a school. The storage period was 15 months, from March 2010 to May 2011. Every three months, each sample was ground to powder, sieved (355 µm, Sanpo Co. Ltd., Tokyo, JP), and analyzed for MC, FAV, and TBARS content.

Measurement of moisture content The sample was first ground to powder in a mixer (T-429; Rong Tsong Precision Technology Co., Taiwan), and then, about 10 g of the powder was weighed onto a dish with a constant weight. The dish was heated for 24 h in an oven at 105 ± 1ºC, and then, the combined weight of the sample and the dish was measured. The moisture content was determined from the expression

Moisture content (%) = (W2-W0) / W1×100, where W0 is the weight of the dish, W1 is the weight of the powder sample, and W2 is the combined weight of the sample and the dish.

Measurement of fatty acid value The FAVs of the samples were determined using a modified version of Duncombe's method. [16,17] 1.00 g of the sample was placed in an 11 ml test tube fitted with a screw cap (TST-SCR 16-100; Iwaki Co. Ltd., Tokyo JP) and mixed with 5 ml toluene for extracting the fatty acids. The tube was shaken for 10 s in a vortex mixer, and this process was repeated thrice every 10 min. After subsequent centrifugation at 3000 rpm for 10 min, 0.5 ml of the supernatant was transferred to a clean dry glass tube. Then, 2 ml chloroform and 1.25 ml of triethanolamine (1 mol/l)/acetic acid (1 mol/l)/copper nitrate (6.4%) (9:1:10, v/v/v) mixture were added. After thorough mixing, the resulting solution was centrifuged for 5 min at 3000 rpm to ensure phase separation. The supernatant aqueous phase was removed by using a glass pipette, and 1.2 ml of the chloroform phase was pipetted into a clean dry glass tube; then, 0.2 ml of sodium diethyldithiocarbamate in *n*-butanol (0.1%) was added. The tube was shaken in a vortex mixer and allowed to stand for 5 min. The absorbance of the sample was measured at 440 nm using a U-1800 spectrophotometer (Hitachi. Ltd., JP), and the range of linoleic acid from 50 to 250 µmol/ml as the standard curve (R^2 > 0.99) was employed for the calculations. The FAV was determined as follows:

FAV (mg•KOH/100 g) = 56.1 × 10^3 / 10^{-6} × X × 100 ×100 / (100-MC) = 561 × X / (100-MC), where X (µmol) is the free fatty acid content calculated on the basis of the standard curve, 56.1 is the molecular weight of KOH, and MC (%) is the moisture content of the sample.

Measurement of thiobarbituric acid reactive substances TBARS values were determined using a previously reported method. [18] 1.00 g of the sample was placed in an 11 ml test tube with a screw (TST-SCR 16-100; Iwaki Co. Ltd., Tokey, JP) and mixed with 3 ml of a chloroform/methanol (2:1, v/v) mixture. Then, the tube was shaken for 2 min in a vortex mixer and centrifuged at 3000 rpm for 5 min. This process was repeated thrice to obtain a constant final volume of 10 ml. Then, 2 ml of the extraction liquid was transferred to a clean dry glass tube and dried under nitrogen flow. Subsequently, 2 ml of sodium dodecyl sulfate (SDS, 4%)/20% acetate buffer (pH 3.5)/dibutylhydroxytoluene (BHT, 0.8%)/2-thienylacetic acid (TBA, 0.8%)/distilled water (8:30:1:30:11, v/v/v/v/v) mixture was added to the tube. The reaction mixture was cooled in an ice bath for 1 h, and then heated at 100 ºC for 1 h on a block heater (DTU-2c, Taitec, JP). The mixture was again cooled, and 0.5 ml of distilled water and 2.5 ml of an *n*-butanol/ pyridine solution (15:1, v/v) was added. Then, the tube was shaken for 30 s in a vortex mixer, and centrifuged for at 3000 rpm for 10 min. The absorbance of the supernatant was measured at 532 nm, using 1,1,3,3-tetraethoxypropane (TEP, 25 nmol/ml) solution for plotting the standard curve. The results were expressed as nmol of MDA/g of the sample.

Statistical analysis Dunnett's test (p < 0.01) was performed for comparing the changes in MC, FAV, and TBARS over the storage period. The MC, FAV, and TBARS under different storage conditions were compared by Tukey's test (p < 0.01). Pearson correlation analysis was applied to the results of MC, FAV, and TBARS analyses, independently for each sample. Statistical Analysis System (SAS version 9.1, Statistical Analysis System Institute, Inc. Cary, NC, USA) was used for all the analyses.

3. Results

Moisture content The MC of all the samples was maintained during storage under the FS (SPo) conditions (Table 1). However, under the FS (Pa) conditions, the MC increased notably over the storage period. Under the TO (Pa) conditions, the MC decreased over the storage period.

Fatty acid value Under the FS (Spo) conditions, there was no significant change in the FAVs of adzuki beans and soybean until the 15th month, and in the FAVs of red kidney beans and buckwheat until the 6th and 9th months, respectively, compared to that under the F (Spo) conditions (Table 2). However, the FAVs of the samples under the FS (Pa) conditions were higher than that under F (Spo) conditions between the 3rd or 6th month and the 15th month. Under the TO (Spo) conditions, the FAVs of adzuki beans and red kidney beans did not show any significant difference until the 12th and 9th months, but those of soybean and buckwheat increased considerably from the 3rd month compared to that under the F (Spo) conditions. The FAVs of adzuki beans, soybean, and red kidney beans stored under the TO (Pa) conditions were lower than those under the F (Spo) conditions for the 3rd, 9th, 12th, and 15th months, and in the FAV of buckwheat for the 3rd month.

Table 1. Changes in moisture content of stored grains at F, FS, and TO during 15 months

Storage Condition	Moisture Content (%)											
	Storage Period (Months)											
	0	3	6	9	12	15
Adzuki Bean						
F (SPo)	13.1 ± 0.2	11.5 ± 0.2 ^{b*}	13.2 ± 0.1 ^{b}	12.9 ± 0.1 ^{c}	13.3 ± 0.2 ^{cd}	12.9 ± 0.1 ^{b}
F (Pa)		12.8 ± 0.1 ^{a}	13.5 ± 0.2 ^{b}	13.6 ± 0.1 ^{b}	14.3 ± 0.1 ^{b*}	13.1 ± 0.1 ^{b}
FS (SPo)		11.1 ± 0.1 ^{b*}	12.9 ± 0.1 ^{bc}	11.8 ± 0.2 ^{d*}	13.5 ± 0.2 ^{c}	12.7 ± 0.1 ^{b}
FS (Pa)		13.3 ± 0.1 ^{a}	14.9 ± 0.2 ^{a*}	15.5 ± 0.1 ^{a*}	16.0 ± 0.1 ^{a*}	14.9 ± 0.1 ^{a*}
TO (Spo)		11.4 ± 0.2 ^{b*}	13.1 ± 0.4 ^{bc}	13.1 ± 0.1 ^{bc}	12.6 ± 0.2 ^{d}	12.7 ± 0.1 ^{b}
TO (Pa)		9.6 ± 0.1 ^{c*}	12.2 ± 0.1 ^{c*}	9.0 ± 0.1 ^{e*}	7.6 ± 0.1 ^{e*}	7.4 ± 0.1 ^{c*}
Soybean						
F (SPo)	11.4 ± 0.2	9.1 ± 0.1 ^{c*}	11.3 ± 0.4 ^{b}	12.0 ± 0.2 ^{b}	10.7 ± 0.3 ^{b}	11.1 ± 0.2 ^{b}
F (Pa)		10.1 ± 0.2 ^{b*}	10.6 ± 0.1 ^{bc*}	10.0 ± 0.3 ^{c*}	9.6 ± 0.2 ^{c*}	9.6 ± 0.2 ^{c*}
FS (SPo)		8.8 ± 0.1 ^{c*}	10.0 ± 0.1 ^{cd*}	10.7 ± 0.3 ^{c*}	10.5 ± 0.3 ^{b*}	10.3 ± 0.1 ^{c*}
FS (Pa)		11.1 ± 0.2 ^{a}	13.4 ± 0.4 ^{a*}	14.0 ± 0.2 ^{a*}	15.5 ± 0.1 ^{a*}	15.4 ± 0.3 ^{a*}
TO (Spo)		11.0 ± 0.1 ^{a}	10.9 ± 0.3 ^{bc}	10.7 ± 0.1 ^{c*}	10.0 ± 0.1 ^{bc*}	9.8 ± 0.1 ^{c*}
TO (Pa)		6.0 ± 0.1 ^{d*}	9.3 ± 0.0 ^{d*}	6.0 ± 0.1 ^{d*}	5.1 ± 0.0 ^{d*}	6.0 ± 0.1 ^{d*}
Red Kidney Bean						
F (SPo)	13.1 ± 0.1	10.4 ± 0.1 ^{c*}	12.8 ± 0.3 ^{c}	12.8 ± 0.1 ^{c}	13.3 ± 0.1 ^{b}	13.5 ± 0.1 ^{b}
F (Pa)		11.0 ± 0.1 ^{b*}	13.0 ± 0.1 ^{b}	13.9 ± 0.2 ^{b*}	12.6 ± 0.1 ^{c}	12.8 ± 0.2 ^{b}
FS (SPo)		10.2 ± 0.1 ^{c*}	12.4 ± 0.4 ^{bc*}	13.2 ± 0.2 ^{bc}	11.6 ± 0.0 ^{d*}	10.8 ± 0.2 ^{c*}
FS (Pa)		11.8 ± 0.1 ^{a*}	15.7 ± 0.3 ^{a*}	18.7 ± 0.1 ^{a*}	16.5 ± 0.2 ^{a*}	16.6 ± 0.2 ^{a*}
TO (Spo)		11.5 ± 0.1 ^{a*}	13.4 ± 0.1 ^{c}	12.6 ± 0.2 ^{c}	12.1 ± 0.1 ^{cd*}	11.6 ± 0.2 ^{c*}
TO (Pa)		6.8 ± 0.0 ^{d*}	12.1 ± 0.2 ^{d*}	9.2 ± 0.2 ^{d*}	6.6 ± 0.1 ^{e*}	7.8 ± 0.3 ^{d*}
Buckwheat						
F (SPo)	13.5 ± 0.2	12.1 ± 0.0 ^{bc*}	14.2 ± 0.2 ^{a*}	14.1 ± 0.1 ^{b*}	14.3 ± 0.2 ^{b*}	13.2 ± 0.1 ^{bc}
F (Pa)		12.4 ± 0.1 ^{b*}	13.4 ± 0.1 ^{b}	13.6 ± 0.2 ^{bc}	13.7 ± 0.2 ^{bc}	13.6 ± 0.2 ^{b}
FS (SPo)		11.8 ± 0.1 ^{c*}	13.4 ± 0.2 ^{b}	13.4 ± 0.1 ^{c}	13.3 ± 0.1 ^{c}	13.6 ± 0.1 ^{b}
FS (Pa)		13.0 ± 0.1 ^{a*}	14.8 ± 0.1 ^{a*}	16.2 ± 0.2 ^{a*}	16.5 ± 0.2 ^{a*}	15.6 ± 0.1 ^{a*}
TO (Spo)		12.9 ± 0.1 ^{a*}	14.5 ± 0.2 ^{a*}	13.7 ± 0.0 ^{bc}	13.6 ± 0.2 ^{c}	12.8 ± 0.1 ^{c*}
TO (Pa)		8.6 ± 0.0 ^{d*}	12.4 ± 0.0 ^{c*}	8.5 ± 0.0 ^{d*}	7.4 ± 0.1 ^{d*}	8.1 ± 0.1 ^{d*}

Superscripted letters in the vertical line indicate significant difference (P<0.01)
* in the same line indicates significant difference (P<0.01)

Table 2. Changes in fatty acid values of stored grains at F, FS, and TO during 15 months

Storage Condition	Fatty Acid Value (mg KOH/100 g seed)											
	Storage Period (Months)											
	0	3	6	9	12	15
Adzuki Bean						
F (SPo)	3.9 ± 0.9	5.6 ± 0.1 ^{b}	4.2 ± 0.4 ^{b}	5.3 ± 0.7 ^{b}	2.4 ± 1.2 ^{b}	5.4 ± 0.4 ^{c}
F (Pa)		5.4 ± 0.2 ^{b*}	5.2 ± 0.2 ^{b}	4.4 ± 0.2 ^{bc}	4.2 ± 0.5 ^{b}	6.5 ± 0.1 ^{c*}
FS (SPo)		6.5 ± 0.6 ^{ab*}	4.5 ± 0.5 ^{b}	4.9 ± 0.7 ^{b}	2.7 ± 0.3 ^{b}	6.0 ± 0.2 ^{c}
FS (Pa)		7.5 ± 0.3 ^{a*}	8.7 ± 0.4 ^{a*}	7.7 ± 0.2 ^{a*}	7.6 ± 0.4 ^{a*}	12.2 ± 0.4 ^{a*}
TO (Spo)		5.5 ± 0.5 ^{b}	5.0 ± 0.3 ^{b}	4.8 ± 0.1 ^{b}	4.1 ± 0.9 ^{b}	9.0 ± 0.4 ^{b*}
TO (Pa)		2.3 ± 0.2 ^{c*}	5.1 ± 0.4 ^{b}	2.6 ± 0.4 ^{c}	1.9 ± 0.1 ^{b*}	3.0 ± 0.3 ^{d}
Soybean						
F (SPo)	11.8 ± 0.6	18.2 ± 0.8 ^{b*}	14.4 ± 0.4 ^{c*}	14.5 ± 0.1 ^{c*}	11.5 ± 0.2 ^{b}	15.9 ± 0.5 ^{c*}
F (Pa)		14.6 ± 0.3 ^{b*}	15.8 ± 0.5 ^{bc*}	14.2 ± 0.2 ^{c}	12.2 ± 0.4 ^{b}	15.6 ± 0.1 ^{c*}
FS (SPo)		16.8 ± 0.4 ^{b*}	16.0 ± 0.2 ^{bc*}	14.5 ± 0.5 ^{c*}	13.4 ± 0.4 ^{b}	16.8 ± 0.2 ^{c*}
FS (Pa)		17.2 ± 1.2 ^{b*}	23.8 ± 0.1 ^{a*}	28.0 ± 0.0 ^{a*}	27.8 ± 0.5 ^{a*}	34.8 ± 0.3 ^{a*}
TO (Spo)		23.8 ± 1.3 ^{a*}	20.3 ± 0.3 ^{b*}	23.2 ± 0.4 ^{b*}	24.4 ± 0.2 ^{a*}	31.4 ± 0.1 ^{b*}
TO (Pa)		5.0 ± 1.0 ^{c*}	14.5 ± 1.1 ^{c}	8.2 ± 0.9 ^{d*}	5.0 ± 1.7 ^{c*}	4.7 ± 0.6 ^{d*}
Red Kidney Bean						
F (SPo)	8.6 ± 0.5	8.9 ± 0.4 ^{b}	8.0 ± 0.6 ^{c}	6.9 ± 0.2 ^{c*}	7.1 ± 0.2 ^{c*}	10.9 ± 0.1 ^{b*}
F (Pa)		9.9 ± 0.1 ^{b}	10.4 ± 0.6 ^{b*}	6.3 ± 0.4 ^{c*}	6.5 ± 0.2 ^{cd*}	9.2 ± 0.3 ^{c}
FS (SPo)		9.4 ± 0.1 ^{b}	7.5 ± 0.3 ^{c}	8.4 ± 0.2 ^{b}	5.8 ± 0.1 ^{d*}	8.5 ± 0.3 ^{c}
FS (Pa)		13.4 ± 0.6 ^{a*}	13.2 ± 0.3 ^{a*}	18.3 ± 0.4 ^{a*}	16.7 ± 0.1 ^{a*}	21.2 ± 0.4 ^{a*}
TO (Spo)		10.3 ± 0.6 ^{b*}	9.1 ± 0.2 ^{bc}	6.2 ± 0.5 ^{c*}	8.4 ± 0.2 ^{b}	10.6 ± 0.1 ^{b*}
TO (Pa)		3.6 ± 0.3 ^{c*}	9.3 ± 0.7 ^{bc}	1.3 ± 0.1 ^{d*}	2.3 ± 0.1 ^{e*}	2.9 ± 0.3 ^{d*}
Buckwheat						
F (SPo)	10.7 ± 0.6	16.3 ± 0.4 ^{bc*}	14.4 ± 0.1 ^{d*}	15.3 ± 1.0 ^{b*}	12.9 ± 0.5 ^{d*}	17.9 ± 0.2 ^{d*}
F (Pa)		14.3 ± 0.3 ^{d*}	16.7 ± 0.7 ^{c*}	13.9 ± 0.8 ^{b*}	14.7 ± 0.6 ^{cd*}	17.1 ± 0.5 ^{d*}
FS (SPo)		15.4 ± 0.4 ^{cd*}	15.1 ± 0.2 ^{d*}	15.8 ± 1.1 ^{b*}	16.3 ± 0.5 ^{c*}	20.7 ± 0.3 ^{c*}
FS (Pa)		16.9 ± 0.1 ^{b*}	23.6 ± 0.0 ^{a*}	25.8 ± 0.6 ^{a*}	25.0 ± 0.5 ^{b*}	30.5 ± 0.3 ^{b*}
TO (Spo)		18.8 ± 0.1 ^{a*}	21.3 ± 0.1 ^{b*}	23.8 ± 0.5 ^{a*}	27.6 ± 0.1 ^{a*}	34.0 ± 0.5 ^{a*}
TO (Pa)		10.6 ± 0.1 ^{e}	20.2 ± 0.1 ^{b*}	12.7 ± 0.5 ^{b*}	13.4 ± 0.3 ^{d*}	16.8 ± 0.5 ^{d*}

Superscripted letters in the vertical line indicate significant difference (P<0.01)
* in the same line indicates significant difference (P<0.01)

Thiobarbituric acid reactive substances The TBARS value of adzuki beans showed no significant difference between the FS (SPo) and F (SPo) conditions until the 15th month (Table 3). However, the TBARS of adzuki beans stored under the FS (Pa) conditions differed notably by the 9th month. The TBARS values of soybean, red kidney beans, and buckwheat showed a significant difference under the FS (SPo) conditions as compared to those under the F (SPo) conditions, from the 12th, 6th, and 9th months. For the same storage period, the TBARS of these samples were higher under the FS (Pa) conditions than under the F (SPo) conditions. The TBARS values under the TO conditions were much higher than those under the F and FS conditions over the same storage period. However, there was no notable difference in the TBARS between the F (Pa) and F (Spo) conditions, until the 15th month.

Table 3. Changes in TBARS of stored grains at F, FS, and TO during 15 months

Storage Condition	TBARs (nmol MDA/g seed)					
	Storage Period (Months)					
	0	3	6	9	12	15
Adzuki Bean						
F (SPo)	43.9 ± 0.7	43.3 ± 1.4 ^{c}	48.7 ± 1.2 ^{c}	49.6 ± 1.4 ^{c}	50.9 ± 0.2 ^{d}	51.5 ± 0.3 ^{e}
F (Pa)		41.7 ± 5.0 ^{c}	47.5 ± 3.3 ^{c}	50.2 ± 2.5 ^{c}	53.7 ± 0.9 ^{cd*}	56.5 ± 1.6 ^{cd*}
FS (SPo)		43.8 ± 0.4 ^{c}	49.0 ± 2.3 ^{c}	50.7 ± 3.4 ^{c}	53.9 ± 2.3 ^{cd*}	55.1 ± 1.0 ^{de*}
FS (Pa)		48.1 ± 1.4 ^{bc}	54.3 ± 0.4 ^{c*}	58.2 ± 1.8 ^{bc*}	59.3 ± 0.9 ^{c*}	60.2 ± 0.0 ^{c*}
TO (Spo)		57.4 ± 0.9 ^{ab*}	63.3 ± 0.6 ^{b*}	65.8 ± 0.2 ^{b*}	69.5 ± 0.3 ^{b*}	71.7 ± 0.3 ^{b*}
TO (Pa)		65.1 ± 2.4 ^{a*}	76.8 ± 2.1 ^{a*}	78.8 ± 0.6 ^{a*}	80.9 ± 1.6 ^{a*}	81.9 ± 1.1 ^{a*}
Soybean						
F (SPo)	62.5 ± 3.2	79.6 ± 0.6 ^{b*}	93.1 ± 1.0 ^{d*}	95.5 ± 1.2 ^{c*}	97.6 ± 0.2 ^{e*}	96.7 ± 0.3 ^{e*}
F (Pa)		89.7 ± 0.6 ^{b*}	101.3 ± 2.1 ^{cd*}	99.4 ± 1.1 ^{c*}	99.9 ± 0.9 ^{e*}	99.6 ± 1.0 ^{e*}
FS (SPo)		86.9 ± 7.7 ^{b*}	101.7 ± 0.3 ^{cd*}	104.0 ± 1.8 ^{bc*}	104.7 ± 0.4 ^{d*}	106.2 ± 1.0 ^{d*}
FS (Pa)		87.9 ± 3.6 ^{b*}	106.3 ± 1.0 ^{bc*}	112.4 ± 1.4 ^{b*}	116.3 ± 0.3 ^{c*}	118.1 ± 0.9 ^{c*}
TO (Spo)		96.4 ± 0.7 ^{ab*}	112.7 ± 1.7 ^{b*}	123.0 ± 2.8 ^{a*}	131.4 ± 1.4 ^{b*}	134.7 ± 0.4 ^{b*}
TO (Pa)		110.4 ± 5.0 ^{a*}	128.7 ± 3.2 ^{a*}	131.0 ± 3.3 ^{a*}	137.4 ± 1.2 ^{a*}	140.6 ± 1.4 ^{a*}
Red Kidney Bean						
F (SPo)	53.4 ± 1.4	55.1 ± 8.0 ^{a}	63.0 ± 1.0 ^{b}	70.9 ± 0.8 ^{c*}	72.1 ± 0.4 ^{e*}	73.7 ± 1.8 ^{d*}
F (Pa)		56.5 ± 3.4 ^{a}	68.5 ± 1.4 ^{b*}	74.4 ± 1.4 ^{bc*}	77.4 ± 0.4 ^{d*}	78.2 ± 0.7 ^{d*}
FS (SPo)		53.5 ± 0.8 ^{a}	81.6 ± 0.3 ^{a*}	84.0 ± 1.4 ^{b*}	83.8 ± 1.0 ^{c*}	85.2 ± 0.7 ^{c*}
FS (Pa)		64.5 ± 6.5 ^{a*}	78.9 ± 4.1 ^{a*}	100.5 ± 0.4 ^{a*}	102.1 ± 0.2 ^{b*}	102.1 ± 1.9 ^{b*}
TO (Spo)		54.3 ± 1.5 ^{a}	79.1 ± 0.4 ^{a*}	101.8 ± 4.3 ^{a*}	108.6 ± 1.5 ^{a*}	111.4 ± 0.6 ^{a*}
TO (Pa)		65.3 ± 0.5 ^{a*}	86.0 ± 0.6 ^{a*}	109.3 ± 2.7 ^{a*}	112.0 ± 1.7 ^{a*}	115.9 ± 0.4 ^{a*}
Buckwheat						
F (SPo)	8.0 ± 0.6	13.4 ± 0.4 ^{b*}	12.6 ± 0.5 ^{b*}	12.5 ± 0.2 ^{c*}	13.1 ± 0.3 ^{d*}	13.2 ± 0.2 ^{e*}
F (Pa)		14.4 ± 0.6 ^{ab*}	14.0 ± 0.5 ^{b*}	15.1 ± 0.3 ^{b*}	15.4 ± 0.3 ^{c*}	14.8 ± 0.3 ^{ed*}
FS (SPo)		15.2 ± 0.9 ^{ab*}	15.2 ± 0.2 ^{b*}	15.4 ± 0.3 ^{b*}	16.1 ± 0.4 ^{c*}	16.2 ± 0.3 ^{d*}
FS (Pa)		14.7 ± 2.0 ^{ab*}	14.5 ± 2.0 ^{b*}	14.7 ± 0.6 ^{b*}	15.6 ± 0.3 ^{c*}	23.6 ± 0.4 ^{c*}
TO (Spo)		19.5 ± 2.7 ^{ab*}	20.7 ± 0.3 ^{a*}	22.6 ± 0.6 ^{a*}	23.5 ± 0.3 ^{b*}	26.4 ± 0.2 ^{b*}
TO (Pa)		20.5 ± 0.6 ^{a*}	21.8 ± 0.7 ^{a*}	24.6 ± 0.6 ^{a*}	26.6 ± 0.3 ^{a*}	28.9 ± 0.9 ^{a*}

Superscripted letters in the vertical line indicate significant difference (P<0.01)

* in the same line indicates significant difference (P<0.01)

4. Discussion

During storage under the FS (Pa) and TO (Pa) conditions, the MC of all the samples showed a significant increase or decrease. Previous studies also showed that the storage environment (humidity and temperature) strongly affected the MC. [19,23] Duman reported that hermetic conditions can control the average absolute humidity of the storage environment and the MC of the samples. [24] The average absolute humidity under the FS (Pa) conditions reached 84.8%RH in a year (Table 4). The absolute humidity of adzuki beans, soybean, red kidney beans, and buckwheat stored under the FS (SPo) conditions was 59.9 ± 0.3%, 66.9 ± 0.1%, 50.2 ± 0.2%, and 59.3 ± 0.1%RH, respectively, in February. The results implied that the SPo can control the humidity inside the package well, thus preventing the samples from absorbing moisture even during storage under high-humidity conditions. Although the average absolute humidity under the TO (Pa) conditions was only 35.5 %RH in a year (Table 4), the temperature of 24.9 °C could cause loss of moisture from the samples. However, under the TO (SPo) conditions, such moisture loss can be prevented; indeed, the humidity of adzuki, soybean, red kidney beans, and buckwheat was 59.7 ± 0.2%, 64.7 ± 0.2%, 56.5 ± 0.2%, and 60.6 ± 0.1%RH, respectively. Therefore, under high-humidity or high-temperature conditions, SPo can help in preventing drastic variations (increase or decrease) in the MC.

FFAs produced during the storage of food grains [20] can adversely affect the product quality, especially the flavor. [5] Hence, the FAV is usually used for evaluating the quality of stored grains. [12] Statistical analysis of the obtained data for our samples (Table 5) showed a positive correlation between the MC and the FAV.

Table 4. Date of recording temperature and humidity at F, FS, and TO in a year

	Temp. (ºC)			Humidity (%RH)		
	F (Pa)	FS (Pa)	TO (Pa)	F (Pa)	FS (Pa)	TO (Pa)
3-2010	-21.8	4.4	25.0	46.7	78.4	29.4
4-2010	-21.9	3.9	25.0	46.9	79.6	29.0
5-2010	-20.5	4.0	25.0	44.6	89.3	30.2
6-2010	-18.0	6.9	24.9	40.6	88.5	41.7
7-2010	-17.7	9.5	24.8	42.1	86.9	55.9
8-2010	-17.4	11.8	24.9	44.3	87.3	64.0
9-2010	-18.2	12.8	24.9	51.2	86.3	57.8
10-2010	-18.1	10.7	25.0	41.0	87.1	43.6
11-2010	-18.7	8.3	25.0	36.7	87.5	28.8
12-2010	-18.5	5.6	24.9	33.5	86.0	19.2
1-2011	-18.1	3.7	24.9	34.0	84.6	13.4
2-2011	-18.2	3.9	24.9	33.5	75.7	13.2
AVG	-18.9	7.1	24.9	41.3	84.8	35.5
SD	1.6	3.4	0.1	5.9	4.4	17.1

Table 5. Correlation coefficients between MC, FAV, and TBARS

Adzuki Bean			Correlation Coefficient		
			Moisture<12.5%	12.5%<Moisture<13.5%	Moisture>13.5%
MC	×	FAV	0.81**	-0.22	0.64*
	×	TBARs	-0.69*	-0.07	0.85**
FAV	×	TBARs	-0.79*	0.52	0.87**
Soybean			**Correlation Coefficient**		
			Moisture<9%	9%<Moisture<11.5%	Moisture>11.5%
MC	×	FAV	0.93**	-0.13	0.89**
	×	TBARs	-0.85**	-0.35	0.96**
FAV	×	TBARs	-0.8**	0.58	0.96**
Red Kidney Bean			**Correlation Coefficient**		
			Moisture<11%	11%<Moisture<13%	Moisture>13%
MC	×	FAV	0.77*	-0.33	0.88**
	×	TBARs	-0.50	0.21	0.82**
FAV	×	TBARs	-0.8**	-0.20	0.78*
Buckwheat			**Correlation Coefficient**		
			Moisture<12%	12%<Moisture<14%	Moisture>14%
MC	×	FAV	-0.43	-0.07	0.78*
	×	TBARs	-0.65	-0.10	0.30
FAV	×	TBARs	0.93**	0.88**	0.70*

* Correlation coefficients greater than 0.6 indicate a strong relationship (P<0.01).

** Correlation coefficients greater than 0.8 indicate a very strong relationship (P<0.01).

The positive correlation for the moisture contents of adzuki bean, soybean, and red kidney bean was >12.5–13.5%, 9–11.5%, and 11–13%, respectively, while that for buckwheat was <14%. This implied that the MC had to be within the aforementioned limits for effective storage of the said food grains. This correlation can be explained from two viewpoints. When the MC of adzuki beans, soybean, red kidney beans, and buckwheat was greater than 13.5%, 11.5%, 13%, and 14%, respectively, the FAV increased with the MC. In our results, these MC values were mainly reflected under the FS (Pa) conditions (84.8% RH). A similar correlation was observed for pinto beans stored at the same temperature: that is, the FAV increased with the MC during the storage period. [2] We consider that FFAs may be formed by the hydrolytic reaction triggered by the enzymatic secretions from microorganisms in the stored grains. For example, in the case of stored wheat, the FAV was positively correlated with *Penicillium* and MC. [3] Further, the FAV decreased with decreasing MC when the MCs of adzuki beans, soybean, and red kidney beans were less than 12.5%, 9% and 11%, respectively. In our results, these MCs were mainly reflected under the TO (Pa) conditions. These changes were similar to those observed in wheat stored for 8 weeks at 10°C and 15–20%RH. [21] Some previous studies have proposed that this decrease in the FAV is due to the oxidation of fat, and that the rate of FFA consumption exceeds the rate of FFA production by

triacylglycerol (TAG) hydrolysis during this storage period. [22] We confirmed this hypothesis on the basis of the negative correlation between the FAV and the TBARS of adzuki beans, soybean, and red kidney beans (Table 5). In other words, during storage under the TO (Pa) conditions, the temperature (24.9°C) would cause considerable oxidation of FFAs, and the low MC of the grains would reduce the rate of FFA production, thereby decreasing the FAV. We suggest that the FAV cannot be considered the sole criterion for evaluating the quality of food grains, when the MC of the grains is too low. Thus, the FAV was correlated not only with moisture but also with the storage temperature. [3] During the storage of adzuki beans, soybean, red kidney beans, and buckwheat under the TO (Spa) conditions, the MC showed no significant change, but the FAV increased 2.3, 2.7, 2.4, and 3.2 times, respectively, by the 15th month. The degree of increase was slightly different because of the difference in the nature of the grain samples. Therefore, we suggest that the FS (SPo) condition can suppress the increase in FAV at low humidity and the decrease in FAV at low temperatures (by preventing the oxidation of FFAs); this is because high humidity (84.8%RH) or high temperature (24.9°C) will alter the FAV.

TBARS was used in this study as an indicator of the degree of oxidation of the grains, for comparing the storage conditions. After 15 months, the TBARS values showed different degrees of growth, irrespective of the storage conditions. Rendóna et al. also reported an increase in the TBARS of coffee beans during storage at 22 °C and 64% RH. [5] However, the results revealed that the TBARS increased with an increase in the temperature from -18.9°C (F) to 24.9 °C (TO) in the three storage environments, over the same storage period. The results reported by Wenjiao et al. indicated that the rate of increase in TBARS showed a positive correlation with the storage temperature. [25] Further, the increase in the TBARS was prevented when using the SPo as compared to that when using paper bags, in all the storage environments. This difference can be attributed to differences in the amounts of FFAs generated and the oxygen distribution. Mendes et al. reported that the increase in TBARS may be attributed to the increased oxidation of unsaturated fatty acids. [26] The oxygen level in the Spo was probably lower than that in paper bag, in our study. Weinberga et al. stored maize grains (MC=14%) in sealed glass jars for 75 days, and found that the oxygen concentration decreased from about 20% to less than 5% during the storage period because of aerobic respiration [27].

5. Conclusions

The present study aimed at investigating the effect of storage in frozen soil on the quality of adzuki beans, soybean, red kidney beans, and buckwheat, and at identifying the optimal storage conditions for these food grains. MCs of 12.5–13.5%, 9–11.5%, 11–13%, and <14%, combined with low-temperature conditions, were best suited for the storage of adzuki beans, soybean, red kidney beans, and buckwheat, respectively. In addition, it was found that the FAV cannot be the sole criterion for evaluating the quality of food grains when the MC is too low. Storage of the aforesaid food grains in frozen soil, in a sealed polyethylene bag, can help in controlling humidity and maintaining a low temperature during the storage period, thus suppressing the increase in the FAV and TBARS. Hence, this method has promising applications for the storage of agricultural products in Hokkaido.

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Statement of Competing Interests

The authors have no competing interests

List of Abbreviations

FFA: free fatty acid
TBARS: thiobarbituric acid reactive substances
MC: moisture content
FAV: fatty acid value
SPo: sealed polyethylene bags
Pa: paper bags
FS: frozen soil
F: freezer
TO: thermostatic oven.

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