[Part 2]

Report on storage experiments with DDGS (Distiller's Dry Grains with Solubles)

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I. Objectives

There have been a number of studies on DDGS for evaluating its nutrient composition and nutritional value for different animal species. But there is no reports on the qualitative changes of DDGS during long-term storage and transportation over long distances. We therefore conducted high temperature storage tests and high temperature and high humidity exposure tests with the main objective of detecting quality changes of its lipid components, simulating its passage through high temperature regions during marine transportation. We also examined changes in odor and color and the effect of adding the antioxidant ethoxyquin.

II. Materials and Methods

1. Materials

DDGS imported in January 2004 from the Glacier Lake Energy LLC. South Dakota, USA, was used as the material. It was from the same lot as used in the field study on dairy cattle. It had a golden yellow (orange-tinged yellow) color and a flavor like a mixture of beer and bread.

2. Test period April to August, 2004.

3. Study location Laboratory of Animal Nutrition of Nippon Veterinary and Animal Science University (Tokyo).

4. Experiment I (High temperature storage test)

DDGS was stored at 60oC(140oF) and 40oC(104oF), both for 8 weeks, to accelerate the oxidation of lipids. Control samples were stored for the same period in an ordinary

feed store. Samples containing the antioxidant ethoxyquin (150 ppm) were also stored at 40oC(104oF) to examine its effects. A number of 3-liter plastic containers, equal to the number measurement days, each containing about 700 g DDGS were placed in storage and everyday the container covers were opened for aeration. A part of the specimen was sampled by the sample reduction technique at each observation date for taking measurements.

Table 1 Treatment groups of Experiment 1 (High temperature storage test)

Group	Storage	Storage temperature	Remarks
H60H60		60℃(140ºF)	
H40	Constant temp. chamber	40℃(104ºF)	
H40E	Constant temp. chamber	40℃(104ºF)	150 ppm of ethoxyquin added
N23	Feed store	About 20-25ºC (68-77ºF)	

The storage temperature and the temperature at the center of the stored samples were measured daily during storage. Samples were removed at the start of storage and 1, 2, 4, 6 and 8 weeks of storage and the color (photograph/color meter), odor (comparative subjective assessment), POV (peroxide value), AV (acid value), water content and crude fat content were measured. α, β, γ and δ-tocopherol contents were measured at the start and after 8 weeks of storage and the total tocopherol content (the sum of contents of the individual tocopherols), was calculated. The analysis was carried out basically according to *Shiryo Bunseki Kijun Chukai* (Commentary on Feed Analysis Standards) (published by Japan Scientific Feeds Association). In other words, the standard lipid analysis test was used for AV, the acetic acid-chloroform method for POV and HPLC for tocopherols.

5. Experiment II (Storage test at high temperature and humidity)

Oxidation of lipids not only occurs spontaneously under high temperature in the presence of oxygen but it also proceeds biologically in some cases by the action of enzymes. Therefore, the DDGS samples were stored for 4 weeks under conditions favorable to enzymatic activity, i.e., the high temperature (40oC or 104ºF) and high relative humidity, and changes in the properties of the lipids were measured. About 600 g samples were placed in petri dishes, and the dishes placed in water-containing airtight containers and stored in a constant temperature chamber at 40oC(104ºF).

Samples containing 150 ppm of the antioxidant ethoxyquin were also stored under the same conditions to see its effect.

Table 2 Treatment groups of Experiment II (Storage test at high temperature and humidity)

Group	Storage	Storage temp., RH	Remarks
HH40	In water-containing airtight containers, in a constant temp. chamber	40ºC(104ºF), 75-100%	
HH40E	In water-containing airtight containers, in a constant temp. chamber	40ºC(104ºF), 75-100%	150 ppm ethoxyquin added

The temperature and relative humidity (RH) of the storage environment were measured daily during the storage period. Samples were removed at the start of storage and after 1, 2 and 4 weeks of storage to measure POV, AV and water content.

III. Results and discussion

1. Experiment I (High temperature storage)

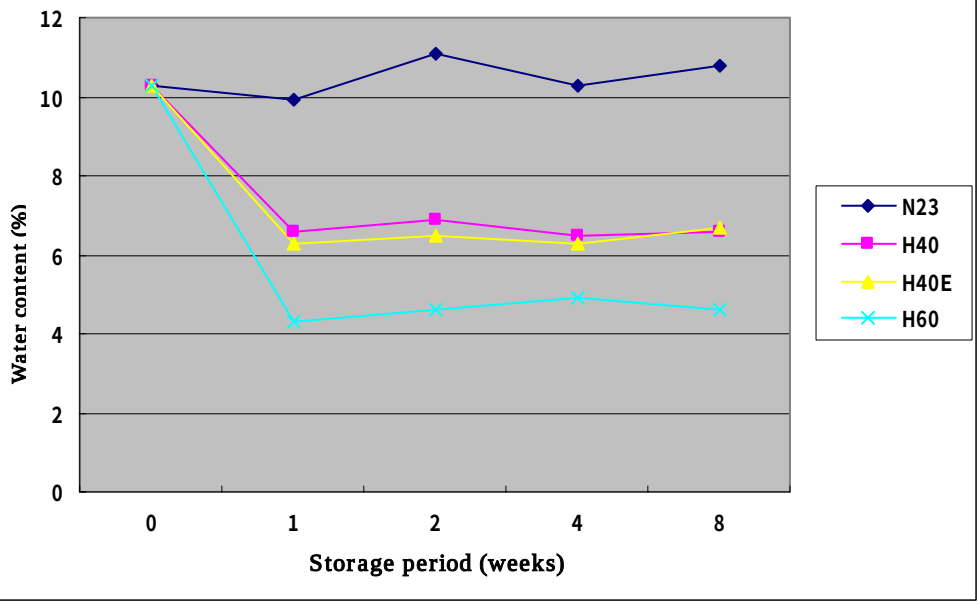
(1) Temperature within the sample

The set storage temperatures were maintained in all the groups. The temperature in the feed store was 20-25oC (mean 23oC) or 68-77ºF(mean73.4ºF). The temperature inside the stored samples remained more or less same as the set ambient temperature and the samples showed no increase in temperature that accompanies lipid oxidation.

(2) Water content (See Fig 1)

The water content in the N23 Group (23oC or 73.4ºF) remained at about 10.5%, the same as at the start of storage. In high temperature storage, the water content decreased one week after storage, reaching about 6.5% in the material stored at 40o C(104ºF) and about 4.5% at 60oC(140ºF). There was little change in water content after that.

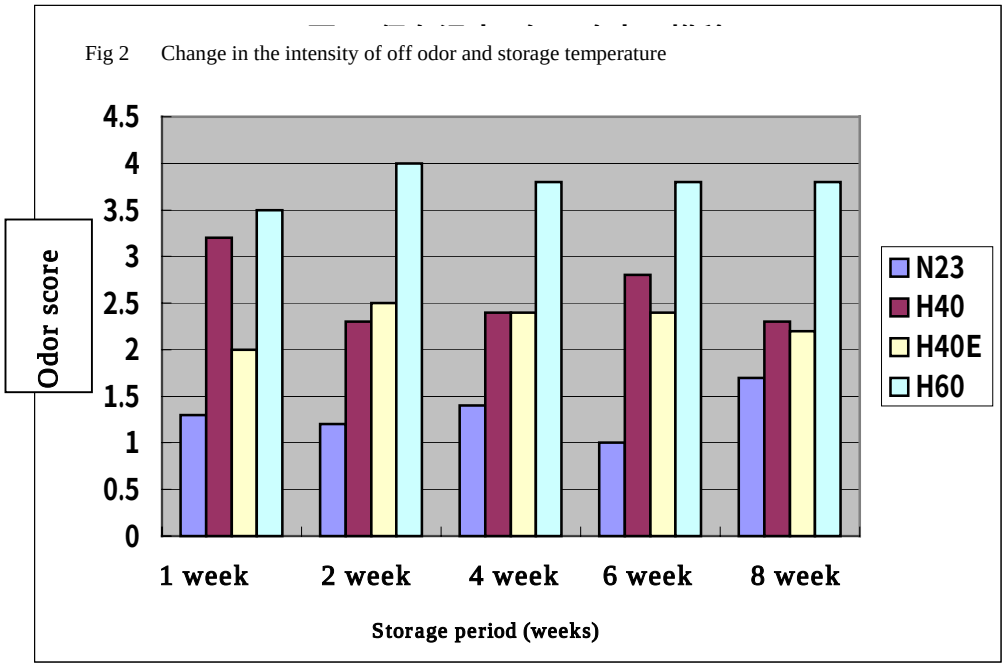
Fig 1 Changes in water content during high temperature storage



(3) Odor (See Fig 2)

Each time the other observations were made, about 7 panelists evaluated the odor and rank scores were assigned (high score for strong off odor) to compare the intensity of off odor. After one week of storage, the high temperature-stored samples clearly had stronger off odor than those stored in the feed store. After 2 weeks, the samples stored at 60oC(140ºF) had clearly stronger off odor than the ones stored at 40oC(104ºF). After that, up to 8 weeks of storage, the odor depended on the storage temperature, the samples stored at 60oC(140ºF) having the strongest odor. Addition of the antioxidant did not affect the odor at 40oC(104ºF). Thus, high temperature storage can lower the commercial value of the material because of the development of off odor. It should be remembered that the odor might affect the palatability of the feed, although this aspect has not been studied in detail.

Fig 2 Change in the intensity of off odor and storage temperature



(4) Color

The DDGS had the so-called golden yellow color, an orange-tinged yellow, at the start of the storage. After 8 weeks of storage, the sample stored at 40oC(104ºF) had the same color as the samples stored in the feed store, as far as eye observation went, with little change in color. The sample stored at 60oC(140ºF) had a clearly darker and more brownish color. This difference was discernible to the eye after 1 week of storage.

Color meter measurements showed that the material stored at 60oC had turned dark because the luminosity (L) decreased, the redness (a) increased and the yellowness (b) decreased (see Figs 3-1 to 3-3). The results thus suggest that DDGS may become brown when stored at a high temperature like 60oC(140ºF) and its commercial value may decrease.

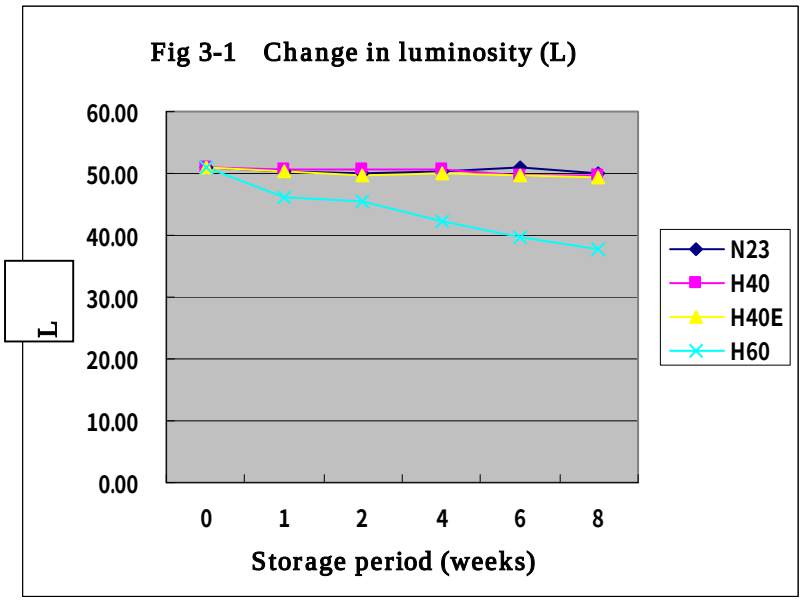


Fig 3-1 Change in luminosity (L)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
L
0
1
2
4
6
8
Storage period (weeks)
N23
H40
H40E
H60

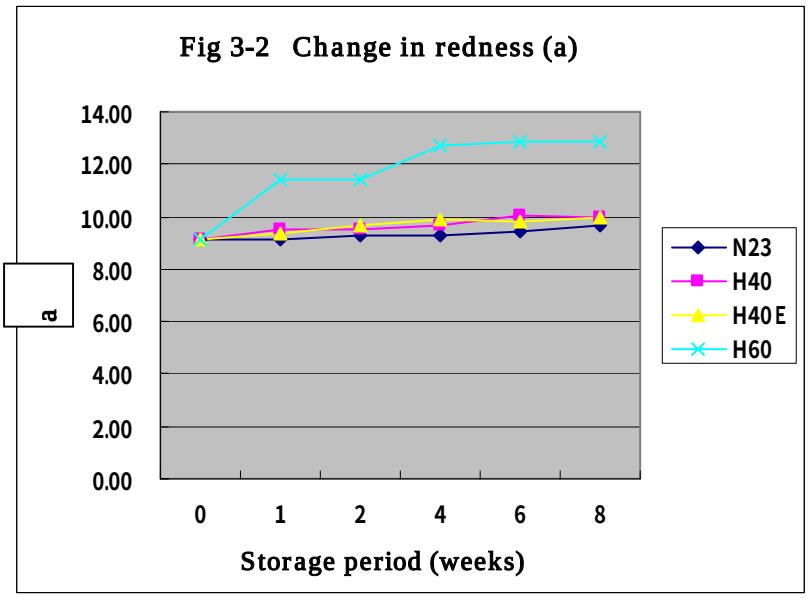
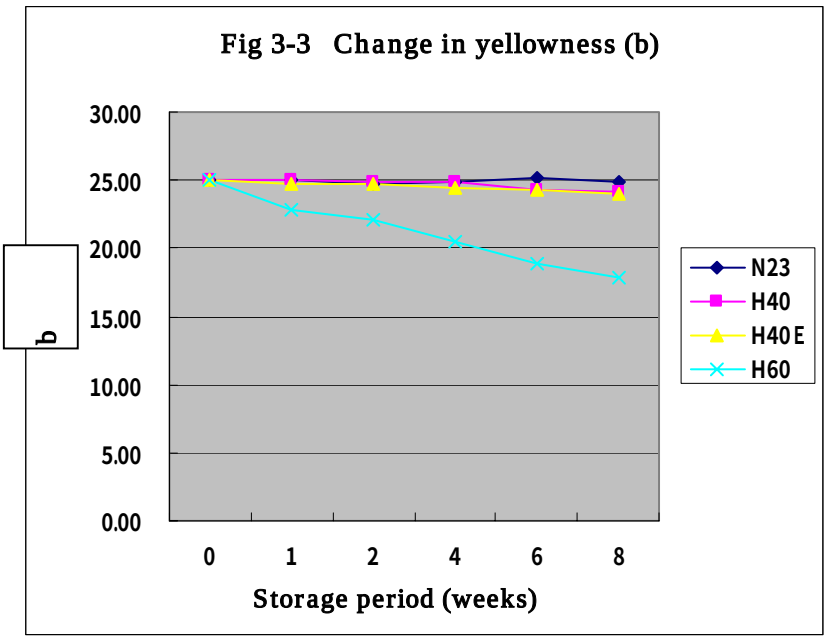
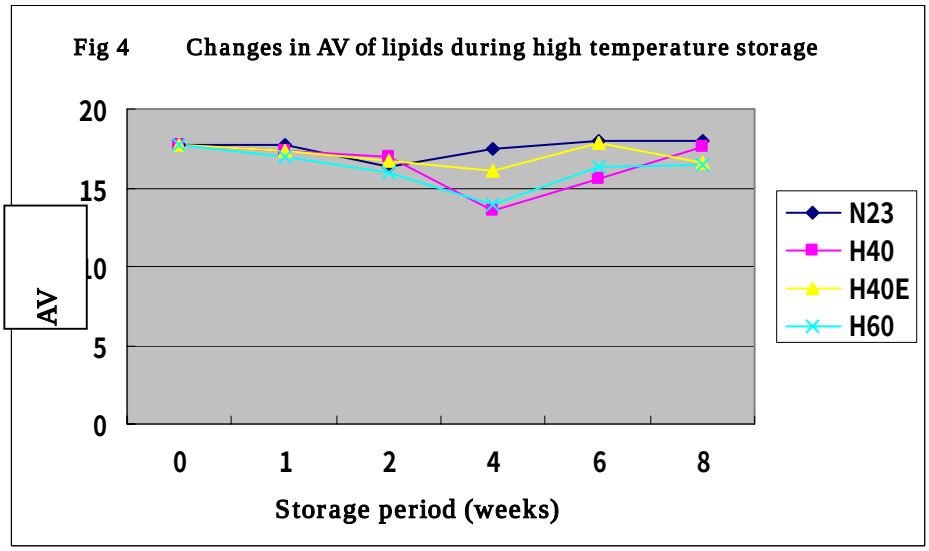


Fig 3-2 Change in redness (a)
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
a
0
1
2
4
6
8
Storage period (weeks)
N23
H40
H40 E
H60



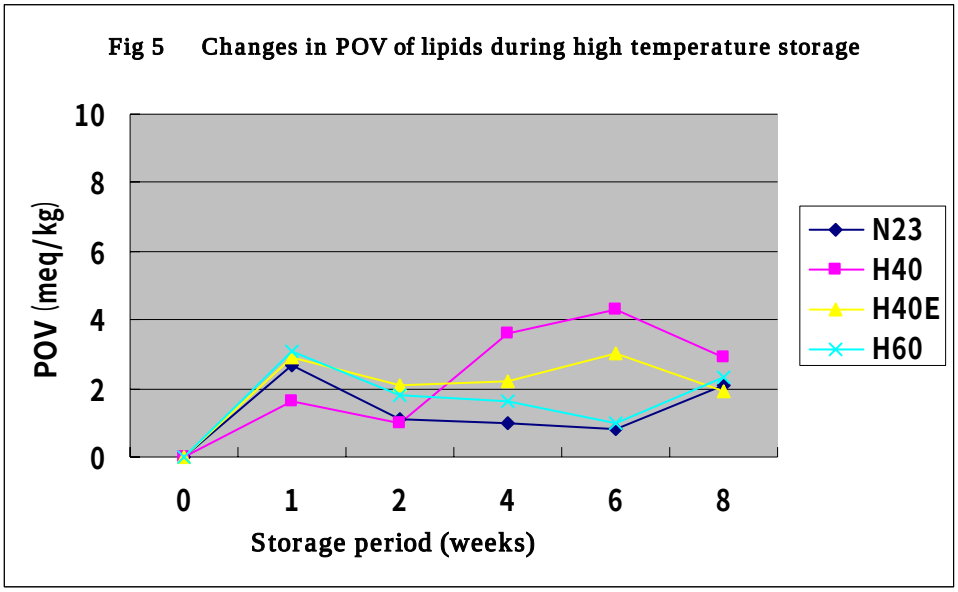
(5) Properties of lipids

The AV (acid value) of lipids was in the range 16-18 at the start and also after 8 weeks of storage at all the storage temperatures. The AV varied by a maximum of 4 units during the storage. But this was not significant and the variation in AV was dependent on the storage temperature or the duration (see Fig 4). The addition of the antioxidant to the material also did not affect the AV. The AV represents the extent of fatty acids released from the fats. Thus, the results suggest that almost no fatty acid was released under the storage conditions of this experiment.



POV (peroxide value) remained below 5 in all the treatments throughout the storage period, although there was some fluctuation. POV was not affected by the addition of the antioxidant either (see Fig 5). In this experiment, POV can be taken as an index of the amount of peroxide produced by lipid oxidation. Thus, it reflects the extent of lipid oxidation. Lipids with high POV are sometimes toxic to animals. The results of this experiment show that very little oxidation of DDGS lipids occurs even when fairly high temperature conditions continue.

Fig 5 Changes in POV of lipids during high temperature storage



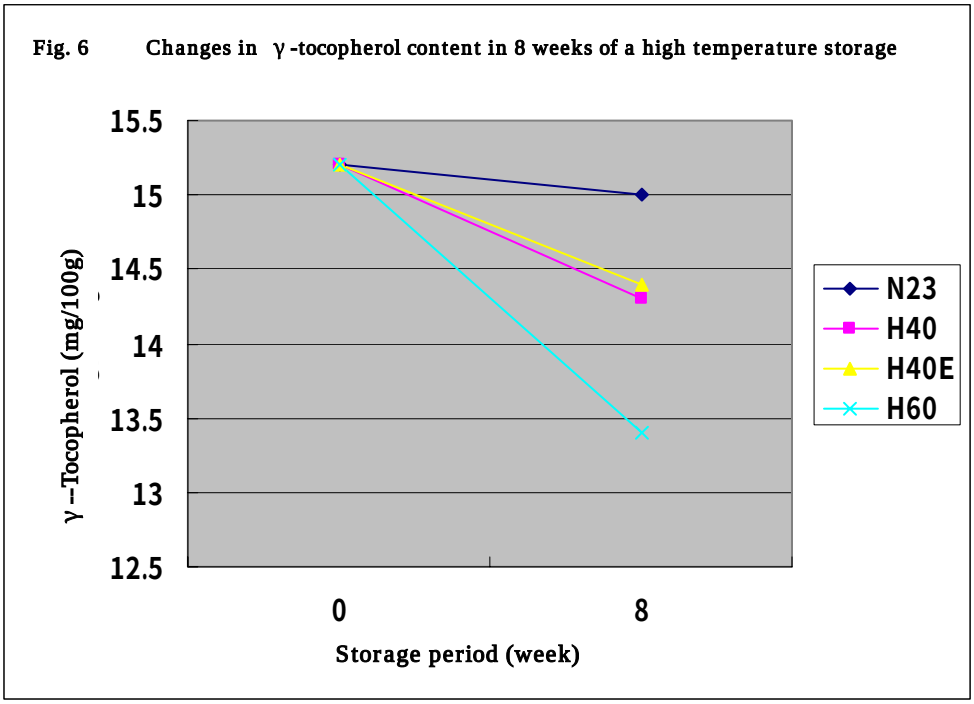
The above results suggest that very little degradation of lipids occurs even when DDGS is stored at a fairly high temperature for a long time.

(6) Tocopherol (vitamin E) content (see Table 3)

The total tocopherol content showed a temperature-dependent decrease after 8 weeks of a high temperature storage. The α-tocopherol showed little change. β-Tocopherol was not detected in any of the treatment groups both before a high temperature storage and after 8 weeks of a high temperature storage. The content of γ-tocopherol showed a clear temperature-dependent decrease during the 8 weeks, which was the main cause of the decrease in total tocopherol (see Fig 6). The δ-tocopherol content decreased slightly during the 8 weeks, depending on the temperature. The addition of ethoxyquin had no effect on the content of any of the tocopherols.

Table 3 Changes in the contents (mg/100 g dry DDGS) of different tocopherols during high temperature storage

Tocopherol	At start	After 8 weeks of storage			
		N23	H40	H40E	H60
Total tocopherol	17.7	17.5	16.9	16.9	16.1
α-Tocopherol	2.0	1.9	2.0	2.0	2.2
β-Tocopherol	Not detected	Not detected	Not detected	Not detected	Not detected
γ-Tocopherol	15.2	15.0	14.3	14.4	13.4
δ-Tocopherol	0.6	0.6	0.5	0.5	0.5



Tocopherols have antioxidant action on the surrounding materials through its own oxidation. This action is said to be strongest in the δ form, followed by γ, β and α. In this experiment, it is likely that these tocopherols suppressed oxidation of lipids present in the DDGS, because very little lipid oxidation occurred and the content of δ-tocopherol, which has a high antioxidant action, showed some decrease and γ-tocopherol content clearly decreased during the storage. Browned substances are also known to have antioxidant action. It is quite likely that this phenomenon occurred in the DDGS studied in this experiment also.

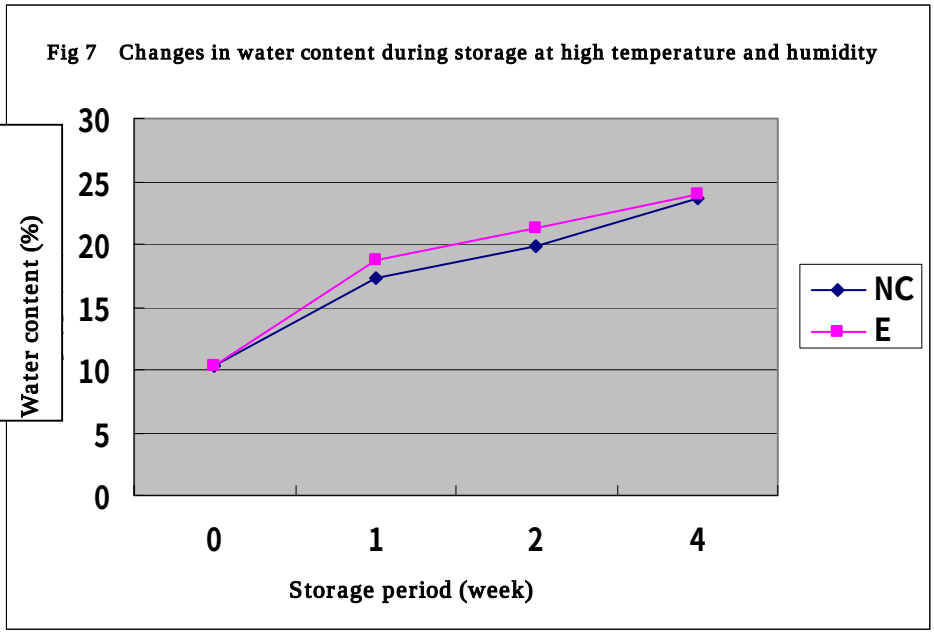
2. Experiment II (Storage at high temperature and humidity)

(1) Changes in temperature and humidity of the storage environment

During storage, the temperature of the samples remained constant at 40oC(104ºF) and the relative humidity was 75-100%. Fungal growth was observed from around day 7 of storage, confirming that the conditions were suitable for enzymatic oxidation in the stored samples.

(2) Water content (See Fig 7)

The water content of DDGS increased with time almost linearly during the storage at 40oC(104ºF) at RH 75-100%. The water content was about 10 % at the start and reached about 24% after 4 weeks.



(3) Properties of lipids (See Figs 8 and 9)

The AV of lipids was in the range 17-20 and showed no change with time during high temperature and high humidity storage (Fig 9). Besides, the AV was no different from that of samples stored at high temperature without high humidity (compare with Table 4). The results suggested that almost no free fatty acid was formed during the high temperature and high humidity storage also. Besides, the addition of the antioxidant had no effect on AV.

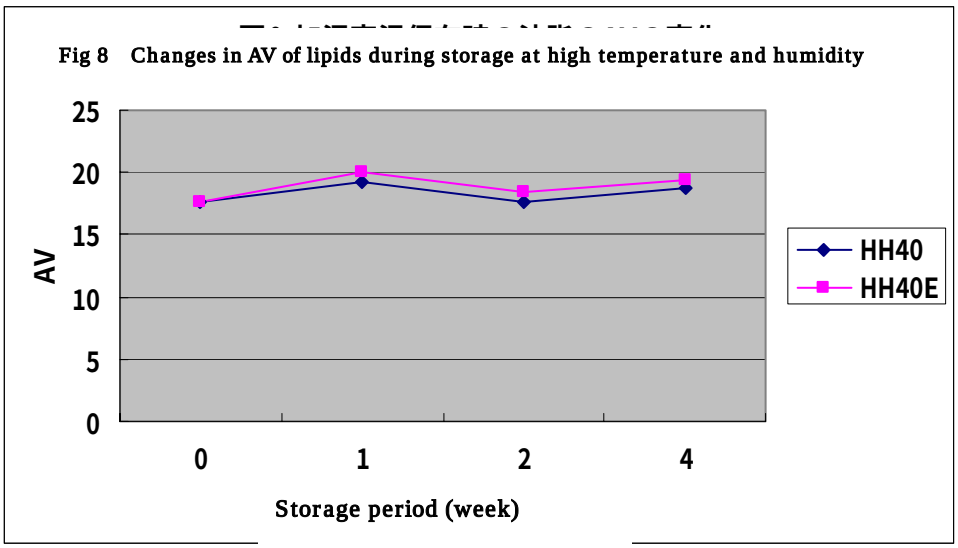


Fig 8 Changes in AV of lipids during storage at high temperature and humidity

The POV (peroxide value) was found to increase with the passage of time during storage. But the increase was less in the samples to which ethoxyquin had been added (see Fig 9). In any case, the POV values remained relatively low suggesting that there was no extensive oxidation of lipids. Thus, an increase in POV, although only slight, believed to be caused by enzymatic oxidation of lipids was observed. The addition of antioxidants suppressed this increase in POV.

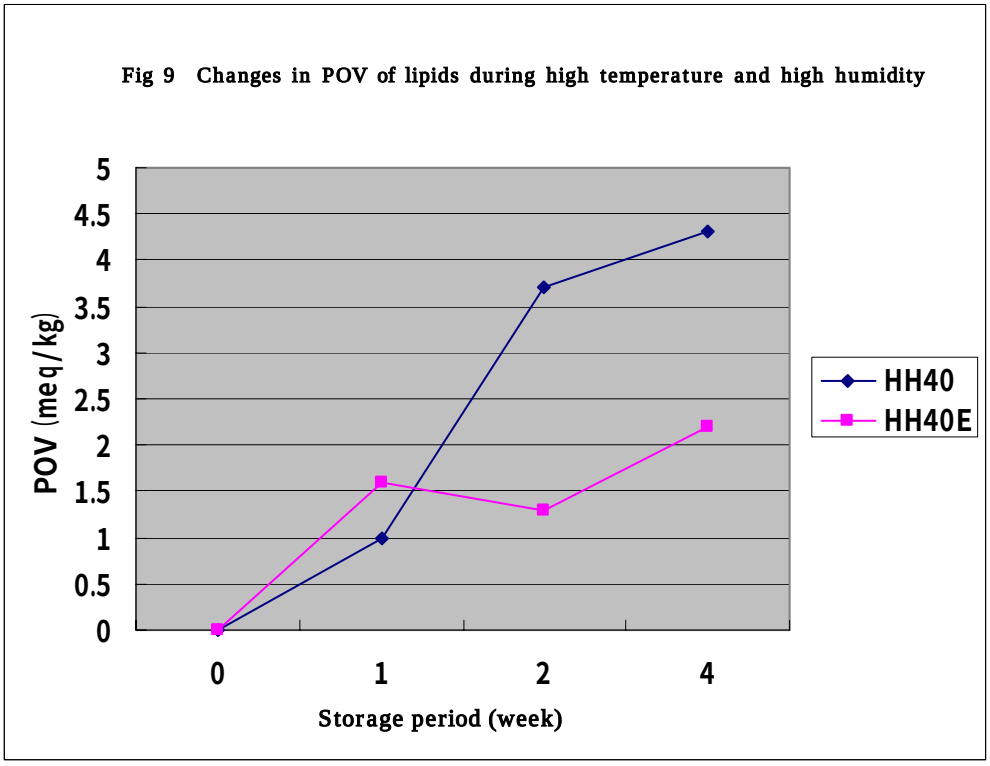


Fig 9 Changes in POV of lipids during high temperature and high humidity

IV. Conclusion

1. The study was conducted at the Laboratory of Animal Nutrition of Nippon Veterinary and Animal Science University (Tokyo).
2. DDGS was stored at high temperature (40oC and 60oC or 104ºF and 140ºF) for 8 weeks, and also at high temperature and high humidity (40oC or 104ºF, RH 75-100%) for 4 weeks to simulate the passage of DDGS through high temperature regions during long duration marine transportation, and qualitative changes in lipids were studied, along with changes in color and odor. Control samples were stored in a feed store (23oC or 73.4ºF). The main objective of storing the samples at high temperature was to examine the autoxidation of lipids at high temperature, whereas the objective of storage tests at high temperature and high humidity was to examine the extent of their enzymatic oxidation.
3. High temperature increased off odor of the samples, depending on the temperature. The off odor could be detected by many persons, by comparing with fresh samples, after only a week of storage at 40oC(104ºF).
4. The color of DDGS changed during high temperature storage. The reddish color increased and the yellowish color decreased at 60oC(140ºF), the color turning darker overall and the material becoming brown in appearance. This change could be seen with the eye after just one week of storage. At 40oC(104ºF), however, there was little change in external appearance even after 8 weeks of storage.
5. In high temperature storage, AV (acid value) and the POV (peroxide value) of the lipids remained low and showed no particular trend. Both AV and POV remained low even under high temperature and high humidity storage, although POV increased slightly with time. This increase in POV was suppressed when an antioxidant was added to the DDGS.
6. It can be stated from the above results that “DDGS changes its color and odor under the high temperature of 60oC but there is no degenerative change of lipids even under such a harsh temperature condition. Under high temperature and high humidity, however, there is oxidation of lipids, although to a small extent. But such oxidation can be suppressed by adding an antioxidant”.
7. DDGS has a high lipid content of 10-13%. The results of the present study suggest that the lipids do not easily degrade and therefore the nutritional value of DDGS does not change much under normal conditions of storage.

[Reference]

Table 6 Comparison of odor of different treatment groups after different periods of storage (Experiment I)

	N23	H40	H40E	H60
1 week	1.3	3.2	2	3.5
2 weeks	1.2	2.3	2.5	4
4 weeks	1.4	2.4	2.4	3.8
6 weeks	1	2.8	2.4	3.8
8 weeks	1.7	2.3	2.2	3.8

The mean rank scores (1 weakest and 4 strongest) in evaluation by ranking of the difference in odor among treatment groups[#1] at each sampling (Fig 2).

Table 7 Comparison of odor after different periods of storage within each treatment group (Experiment I)

	1 [week]	2[weeks]	4[weeks]	6[weeks]	8[weeks]
H40E	0	1.3	2.5	2.8	3.3
H40	0.7	2.2	2.3	2.2	2.7
H60	0	1.3	1.7	3.7	3.3

The mean rank scores (0 weakest, 4 strongest) in evaluation by ranking of change of odor with time in each treatment group at completion of storage (8 weeks)

Table 8 Color values measured by color meter (Experiment I)

		Storage period (weeks)					
L (luminosity)	Treat-ment group	0	1	2	4	6	8
	N23	50.87	50.48	50.14	50.17	50.84	49.96
	H40	50.87	50.75	50.75	50.51	49.75	49.76
	H40E	50.87	50.32	49.82	49.88	49.81	49.33
	H60	50.87	46.25	45.44	42.33	39.74	37.73

a (redness)	Treat-ment group	0	1	2	4	6	8
	N23	9.14	9.16	9.28	9.32	9.43	9.66
	H40	9.14	9.48	9.53	9.70	10.03	9.96
	H40E	9.14	9.37	9.67	9.86	9.85	9.99
	H60	9.14	11.42	11.45	12.73	12.89	12.88

b (yellowness)		0	1	2	4	6	8
	N23	25.03	24.96	24.69	24.92	25.19	24.87
	H40	25.03	25.03	24.82	24.82	24.25	24.12
	H40E	25.03	24.73	24.72	24.50	24.33	23.95
	H60	25.03	22.88	22.11	20.46	18.85	17.84

Table 9 Water content (%) (Experiment I)

Weeks	N23	H40	H40E	H60
0	10.3	10.3	10.3	10.3
1	9.9	6.6	6.3	4.3
2	11.1	6.9	6.5	4.6
4	10.3	6.5	6.3	4.9
8	10.8	6.6	6.7	4.6

Table 10 AV (acid value) (Experiment I)

Weeks	0	1	2	4	6	8
N23	17.7	17.7	16.3	17.5	18.0	18.0
H40	17.7	17.3	16.9	13.5	15.6	17.6
H40E	17.7	17.4	16.7	16.1	17.9	16.6
H60	17.7	17.0	16.0	13.9	16.3	16.4

Table 11 POV (meq/kg) (Experiment I)

Weeks	0	1	2	4	6	8
N23	≦1.0	2.7	1.1	1.0	0.8	2.1
H40	≦1.0	1.6	1.0	3.6	4.3	2.9
H40E	≦1.0	2.9	2.1	2.2	3.0	1.9
H60	≦1.0	3.1	1.8	1.6	1.0	2.3

Table 12 Water content (%) (Experiment II)

Treatment group	Storage period (weeks)			
	0	1	2	4
HH40	10.3	17.3	19.9	23.6
HH40E	10.3	18.8	21.3	24.0

Table 13 AV (acid value) (Experiment II)

Weeks	0	1	2	4
HH40	17.7	19.3	17.6	18.8
HH40E	17.7	20.1	18.5	19.4

Table 14 POV (meq/kg) (Experiment II)

Weeks	0	1	2	4
HH40	≦1.0	1.0	3.7	4.3
HH40E	≦1.0	1.6	1.3	2.2