USDA Attachment C

PN-ABD-536

VITAMIN A FORTIFICATION OF FOODS FOR DEVELOPING COUNTRIES

FINAL REPORT

SEPTEMBER 15, 1989

COOPERATIVE RESEARCH AGREEMENT NO. 58-319R-5-034

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Introduction

Vitamin A fortification of several foods has been studied over the period of this research agreement. The foods used have been chosen through consultation with OICD and interested countries. The report will be divided into discussions of the progress made with each food fortified. The foods studied for vitamin A fortification were: a) monosodium glutamate (MSG); b) wheat; c) rice; d) synthetic rice (called Ultrarice); e) synthetic wheat (called Ultrawheat); and f) salt. Significant progress has been made in obtaining stable fortified products for MSG, wheat, Ultrarice and salt. MSG, wheat and Ultrarice could go forward with in-country fortification trials (as has been happening with MSG in Indonesia). The level of expenditure and level of effort are reported in part G. Recommendation for future work are included in the discussion for each food fortified during this project.

A. MSG

The major portion of our results with vitamin A fortification of MSG are found in our September 1987 report to Indonesia and is appended (Appendix 1). Vitamin A fortification of MSG in Indonesia principally required suitable size segregation reduction and color masking. Size segregation was effectively reduced through agglomeration of the vitamin A particles by The Coating Place (Verona, WI). By preparing a range of particle sizes slightly larger than the size distribution of the MSG yielded a fortified product were the vitamin A did not segregate away from the MSG upon agitation. The vitamin A was coated with Klucel and TiO_2 to provide an opaque white color. These white agglomerates are visible in fortified MSG upon careful inspection but were judged suitable by the Indonesian government and the MSG manufactures.

Quality assurance guidlines were developed in February 1988 and forwarded to HKI in Indonesia for presentation to the MSG manufacturers. The enormous scope of the market in Indonesia may make quality assurance as we understand it in the US almost impossible. For example, Ajinomoto business managers reported that they have 150,000 agents selling their product to retailers in Indonesia. He admited that probably no one has any idea how many commercial outlets there are in Indonesia for the MSG product. Many of the very remote retailers buy their supplies from less remote retailers. The proposed guidelines are appended.

Production of larger quantities of WVA was accomplished in November, 1988. These materials were evaluated with Ajinomoto and Miwon MSG (received in 4/89 from the MSG manufacturers in Indonesia) for mixing variability and segregation characteristics. The MSG was sized as well. These data were reported to R. Crowley 4/20/89 and the report entitled 'Evaluation fo vitamin A fortified MSG: Miwon and Ajinomoto" is appended (Appendix 2).

All white vitamin A produced prior to September 1988 was on a pilot scale. Production scale was initiated in September 1988. There was a problem with the scale-up at that time. The scale-up material was much more yellow in color than the pilot batches. In Table 1, the Hunter-Color difference meter readings are shown for the product and the amount of dilution with old material required to not observe any color difference. The b reading seems to be the closest to reporting what the human eye perceives for this material. We recommend that the L,a,b readings reported for lot 8710xx or the 4-fold dilution of lot 8811xx with lot 8710xx be used as a standard color reading acceptable prior to shipment to Indonesia.

Of more concern is the ability of the scale-up material to take up water with and without MSG present. At high humidity (75%RH), these material melt or solidify and are no longer free-flowing in marked contrast to earlier white vitamin As. The scale-up white vitamin A (WVA) with MSG takes up water and discolors more rapidly than earlier materials at high temperatures and humidities. We had not observed this discoloration problem in lab experiments prior to the scale up. Limited tests were done using lot 870202 at high temperatures and humidities in 1988. No discoloration (browning) was reported. In-field observations in Indonesia suggest a problem also with water uptake through the package and browning of the product over a 3 month period in the field. On-site examination was conducted August 28-September 1, 1989. The trip report is appended (Appendix 3). Because of the very high humidities in Indonesia and the long turnover period required (>6 months), the current formulation of WVA is not sufficiently nonhydroscopic to yield the shelf-life needed for MSG. A more nonhydroscopic barrier will need to be formulated for the WVA without loss of the agglomeration characteristics and white color.

Further, an experiment was conducted using various combinations of temperature and humidities in order to study the effect on WVA. The conditions for the tests were temperatures at 35 and 55°C and relative humidities of 11, 22, 32, 43, 54, and 65%. The WVA was studied alone and with reference MSG (3000IU/g) and vitamin A formualations parents 87 A, 88 A and 89 A and WVA lots 871020, 880224, 881103 and 8904xx.

During the course of the experiment, (22 days), no browning was observed except at the highest humidity (66%) with the WVA/MSG mixtures. The first browning at 66% RH occured on day 3 for all the vitamin formulations except 871020 and 880224. Lot 880224 did not brown until day 22. Lot 871020 resisted color change throughout the experimental period. The vitamin A formulations alone did not undergo any dramatic color change during the course of the experiment. There was a slight yellowing of the formulations. Lot 871020 was most stable to any color change. None of the mixtures nor vitamin alone show any stickines nor resistance to flowability as observed earlier at 75% RH. These data suggest that a moderate change in the water permeability of the package or slight increase in the water resistance of WVA will problably yield a fortificant that would be suitable for use in Indonesia. These observations also support the lack of any deterioration of fortified MSG in the Bogor trials in 1984. The humidities in Bogor may have been lower than found in most of Java. Lower humidities along with the higher turnover of MSG on Java probably contributed to no reports of water uptake of fortified MSG packets.

B. Wheat

Eighteen batches of wheat have been fortified with vitamin A by Wright Enrichments, Inc. (Crowley, LA) with improving levels of success as judged by concentration of vitamin A in the wheat upon receipt and during accelerated storage testing. Fortification with 250 SD (acacia matrix containing retinyl palmitate) is superior to fortification using retinyl palmitate oil or oil-base formulations despite the ease of handling the latter. The stability of fortified wheat premix has been evaluated in several batches and half-lives (time required for one-half of the original vitamin A to be destroyed) predicted for storage at 25° of more than one year were judged to be suitable premix materials. The use of premixes in fortification of wheat and preparation into chapatis has been evaluated. A comparative trial using premix to prepare chapatis in Bangladesh was conducted with the cooperation of the University of

Dhaka and ISU. Chapatis, flours and meals were prepared in Bangladesh and shipped to US. The results show that the premix could be used in a food fortification program in Bangladesh and achieve the desired level of fortification. A summary of these results is contained in quarterly report (1/88 to 6/88) and is appended (Appendix 4).

Two final fortification premixes were prepared by Wright Enrichment in Spring 1989, batches 17 and 18. These were prepared to confirm that the process could be replicated. The storage stability of these premixes has been evaluated. Although the premixes have apparently been prepared in the same manner, the stability of the two is markedly different. The stability of both are still adequate to meet the needs of a fortification project, but the reasons for the difference in vitamin A losses is not understood at all. The predicted stabilities of batches 17 and 18 are presented in Table 2.

C. Rice

No new experiments have been conducted on fortified rice premixes since data was reported in quarterly report (1/88-6/88)(Appendix 4). Work was put on hold due to rinsing loss observed with fortified rice. Efforts were directed towards a synthetic rice, Ultrarice, the subject of the next section, which is highly rinse resistant. It is felt that the technology used by Wright Enrichment to fortify wheat grains can easily be transfered to rice if an outside coating is decided upon.

D. Ultrarice

Previous work with a synthetic form of rice, Ultrarice, have been reported in the previous quarterly reports and in "Fortification of Ultrarice with Vitamin A" report (1/16/89) (Appendix 5) through Ultrarice III. Stability of the early Ultrarices was not high enough to allow this premix material to be used in the field, although the washing stability was very high compared to fortified rice reported above. After an on-site visit with the Bon Dente Company, the makers of Ultrarice, we agreed upon a series of experiments to improve the product. A pilot plant size pasta machine that was modified to produce rice size and shape grain was lent to ISU by Bon Dente to run concurrent experiments in order to produce a vitamin A stable premix. The products produced by Bon Dente will be refered to as Ultrarice (UR) while the products produced by ISU will be identified as Ames Ultrarice (AUR).

After our discussions with Duffy Cox and others at Bon Dente, several different combinations of lipids, antioxidants and ways of drying the Ultrarice were attempted. These data are reported in Table 3 for Ultrarice 4 through 9. These data are the cooking stabilities of the UR and were used to determine which UR would go into longer term stability testing. UR 7 through 9 were deemed stable enough to warrant further investigation.

UR 7, 8 and 9 were all judged stable enough to be used as a premix. The stabilities of vitamin A in UR 7-9 are presented in Table 4. The stabilities of vitamin A in these three UR ranged from 213 to 773 days at low humidity. Stability was also quite good at high humidity for these UR. In conversations with D.Cox and similar results obtained with AURs, we concluded that more saturated lipids as the fat source and tocopherols as the antioxidant were the best modifications to the original UR recipe.

After learning to process UR in Ames under the guidance of D. Cox, a

number of modifications of UR were tried. Successful products were evaluated for vitamin A stability. The composition of these successful AUR (1, 6-10, 14-18) are presented in Table 5. Tocopherol and combinations of tocopherol and ascorbic acid were used to improve the stability of the vitamin A. A series of oils were used with different levels of unsaturation. This was to determine if more unsaturation provided sacrificial lipids to be oxidized in place of vitamin A, or instead, unsaturated lipids provided a greater source of lipid hydroperoxides, and thus, accelerate vitamin A oxidation or loss. The decreasing order of unsaturation for the oils tried was soy>corn>peanut~olive>coconut>no oil. Initial vitamin A levels, cooking retention and 'quicky' stability (days required to destroy one half the vitamin A at 65°C) tests were done to evaluate the suitability of the various AUR components for AUR 1, 6-10 and 14-18. The results of these experiments are summarized in table 6. In our hands, retention of vitamin A upon cooking was not a good indicator of longer term vitamin A stabilility. We did decide, however, to look for retentions of close to 80% or more if possible. Some of the AUR with the best 'quicky' stability, ie. 8, 14, 16 and 17, did not have the best cooking retention. However, their storage stability was deemed to be excellent. All these AURs contained tocopherol as the antioxidant component. These four AUR had stabilities at 65°C that corresponded with stabilities at this temperature for UR 7-9. Thus, the next step in the experiments was to use tocopherol as the antioxidant and compare stabilities using more saturated oils in the AUR recipe.

Longer, more careful stability studies were conducted for AUR 19-22. The composition of AUR 19-22 are presented in Table 5. AUR 19 had no oil in the recipe to see if this made any difference in the formulation. The stability of vitamin A in AUR 19-22 are presented in Table 7. Stability of AUR 19 with no added oil was quite good at relatively dry humidities but the vitamin A was quite unstable in this formulation at high humidities. Addition of the metal ion chelator, ascorbic acid, to formulations with oil did not greatly improve the good stability already achieved at low humidity storage without this additive. However, at high humdity, ascorbic acid vastly improved the vitamin A stability of these AURs. We recommend that future UR contain tocopherol and ascorbic acid as antioxidants and saturated oils such as peanut and coconut in the formulation. Discussion with D. Cox revealed that Bon Dente had come to a similar conclusions in their formulations. The only negative observation for these formulations is that upon long term storage at the higher humidities, these UR and AUR turn tan to brown in a time-dependent fashion. This may or may not be a problem, depending on the quality of rice used with UR as the premix.

E. Ultrawheat

No new work has been conducted on a synthetic wheat premix, Ultrawheat, since the data that were reported in the quarterly report,(1/88-6/88)(Appendix 4). Because of the success in fortifying whole wheat kernals by Wright Enrichment and because the progress with Ultrarice formulation can be readily translated to a wheat system, no effort was expended in further exploring the area at this time. The prognosis for production of a synthetic wheat product is very good.

F. Salt

Four main experiments have been conducted since the last report (1/88-6/88)(Appendix 4) for "sea salt". These experiments involve: two encapsulated forms of 250 SD vitamin A by Durkee and by Balchem; effect of moisture on vitamin A (Balchem) stability; and attempted protection of vitamin A with calcium stearate. The encapsulated forms of vitamin A show a great deal of promise for intermediate moisture levels in salt.

The results for the Durkee encapsulated products are presented in Table 8. The stability of vitamin A was evaluated at 0, 0.1 and 3.5% moisture for the parent 250 SD and for three levels of coating on the 250 SD, 50, 40 and 25 % coating by weight. There was no significant increase in the stability of vitamin A by encapsulation in the no moisture samples. This was not unexpected because the parent vitamin A formulation is very stable when no moisture is present. A significant increase in stability was observed compared to the parent material at 0.1% moisture. This is the moisture content where salt appears sticky but not visibly wet. The 25% coating yielded the best stability at 0.1%. At high moisture content, 3.5%, the 50 and 40% coating resulted in the most improved stability for vitamin A. The 3.5% moisture content is comparable to saturated salt which would be expected in a very humid environment. These stabilities at high moisture are the best ever observed.

The results of the stability of vitamin A encapsulated by Balchem are presented in Table 9. The Balchem material was also based on a 250 SD parent material. The Balchem material gave the greatest increase in vitamin A stability at modest moisture level, 0.1%, compared to the parent material. The improvement in vitamin A stability at 3.5% moisture was less dramatic. This encapsulated material appears work best at moderate humidities.

In order to determine if there was a cutoff for moisture level where vitamin A dramatically starts to degrade, a moisture versus vitamin A degradation at 45°C experiment was conducted. The vitamin used was the parent 250 SD and the Balchem encapsulated 250 SD. For the parent material, there was a dramatic loss of vitamin A when the moisture content exceeded 0.1%. The results for the Balchem encapsulated vitamin A are less clear cut. The lower moisture content vitamin A stabilities have very large errors of estimation assoiated with them (see Table 9). However, there does appear to be a significant break at 0.4% moisture and again at 2.5% moisture. If moisture contents of salt could be kept below 1%, there may be hope for the development of a suitable salt fortificant. These data coupled with the significant improvements in stabilities for the encapsulated vitamin A reported in Tables 8 and 9 show that vitamin A can be protected from moisture even in a harsh environment such as sodium chloride.

Finally, based on a report of improved vitamin A stability in blocked salt for animals by encorporation of calcium stearate, experiments were designed to evaluate the stability of vitamin A in the presence of 0.25% calcium stearate. Both an acacia based vitamin A (250 SD) and a gelatin based vitamin A (250 S) were used. The vitamin A forms were mixed in a V-mixer with calcium stearate prior to mixing with salt. The results of stability studies of these materials with salt are presented in Table 11. Unfortunately, calcium stearate only improved the stability of 250 SD at 0.1% moisture compared to the parent materials. The earlier report of vitamin A stability in block salt may have been due to the solid nature of this product compared to the free flowing salt used in our model systems.

G. Expenditures

Expenditures on this project from 8/1/85 through 8/31/89 were $312,662. This includes 18% indirect cost of $13,952 applied the last two year of the Cooperative Agreement. Approximately $9600 will be expended during the last month of the agreement. The level of effort has been calculated at 15% for the PI and 100% for the technician on the project. Total budgeted dollars were $372,353.

Table 1

Hunter Color Difference of WVA lot 8711xx and 8811xx

Sample	L	a	b
Lot 8710xx	82.28±0.45	0.18±0.11	7.67±0.50
Lot 8811xx	80.30±1.56	-1.64±0.23	9.69±1.00
Dilution 1[a]	85.68±0.06	-0.58±0.04	9.40±0.18
Dilution 2	84.10±1.68	-0.34±0.06	8.14±0.59
Dilution 3	84.55±0.01	-0.03±0.00	7.93±0.08
Dilution 4	83.45±0.55	0.01±0.01	7.67±0.29
Reference tile	91.18	-5.97	8.21

a. Lot 8811xx was diluted in half with lot 8710xx and color was measured. Humans could still detect a difference. Dilution 2 was dilution 1 mixed in half with lot 8710xx again. Human observers reported no observable difference at dilution 4. The b value appears to be the best indicator.

Table 2

Stability of Fortified Wheat Batches 17 and 18

Batch 17

Temperature	Half-life (weeks) Aw=0.11	Aw=0.75
45	7±0	4±0
55	2±0	1±0
65	0.4±0	0.1±0
*25	123	131

Batch 18

Temperature	Half-life (weeks) Aw=0.11	Aw=0.75
35	9±1	10±1
45	5±0	4±0
55	2±0	1±0
65	0.4±0	0.3±0
*25	32	34

Table 3

VITAMIN A CONTENT AND COOKING STABILITY OF ULTRARICES

Ultrarice	Vitamin A (IU/g) Initial	Cooked	% retention
4a	1909±58	880±86	46
4b	1816±45	938±60	52
5a	5061±195	3906±500	77
5b	5471±96	3004±311	55
7	2320±25	1891±65	82
8	2109±33	1593±173	76
9	1879±25	1778±73	94

Table 4

VITAMIN A STABILIITY OF ULTRARICE 7, 8 & 9

		Half-life (days)	
	Aw	0.11	0.75
Ultrarice	T		
7	35	243±59	146±15
	45	52±5	36±1
	55	13±0	9±0
	65	5±0	2±0
8	45	84±6	91±11
	55	30±1	17±1
9	45	116±13	16±1
	55	123±26	ND
	65	70±2	6±0
Estimated Stability at 25°C (days)			
7		763	517
8		659	2608
9		213	43

Half-life is the time in days required for one half of the vitamin A to degrade. For example, UR7 at 35°C and relative humidity of 75% (Aw=0.75), it would take 146 days for half the vitamin A to be lost. The estimated half-life at 25°C are predicted from a linear regression of ln(half-life) versus temperature. The greater the number of temperatures evaluated, the greater the confidence in the 25° estimate.

Table 5

COMPOSITION OF AMES ULTRARICES (AUR)

AUR	OIL	ANTIOXIDANT	VITAMIN A	OTHER
1	corn	BHT, BHA	250 SD (ACACIA)	
6	CORN	BHT, BHA	250 SD	CARRAGEENAN
7	CORN	BHT, BHA	250 S (GELATIN)	
8	CORN	TOCOPHEROL	250 SD	
9	SOY	BHT, BHA	250 SD	
10	PEANUT	BHT, BHA	250 SD	
14	PEANUT	TOCOPHEROL	250 SD	
15	OLIVE	BHT, BHA	250 SD	
16	OLIVE	TOCOPHEROL	250 SD	
17	PEANUT	T & ASCORBATE	250 SD	
18	PEANUT	BHT, BHA	250 SD	SKIP FREEZE
19	NONE	TOCOPHEROL	250 SD	
20	PEANUT	T & ASCORBATE	250 SD	
21	COCONUT	TOCOPHEROL	250 SD	
22	COCONUT	T & ASCORBATE	250 SD	

NOTE: ANTIOXIDANTS ADDED AT 0.1% FINAL CONCENTRATION FOR EACH ANTIOXIDANT.

TABLE 6

VITAMIN A STABILITY AND COOKING RETENTION IN AURs

	Vitamin A (IU/g)			Half-life (days) 65C	
AUR	Initial	Cooked	% Retention	Aw=0.11	Aw=0.75
1	2387±33	2186±33	92	-	-
6	1948±48	1542±53	79	-	-
7	3352±115	2332±73	70	6.0	3.3
8	2243±90	1557±59	69	10.3	3.9
9	2446±117	1877±230	77	3.5	2.4
10	2241±223	1807±145	81	3.5	2.5
14	1456±49	1061±131	73	20.5	3.7
15	2094±99	1276±35	61	4.8	2.7
16	1959±36	1212±90	62	11.2	6.1
17	2198±149	1376±127	63	14.8	4.6
18	1945±90	1417±21	73	6.1	3.4

Table 7

VITAMIN A STABILITY IN REFINED AURs

AUR	T	Half-life (days) Aw=.11	Aw=.75
19	45	118	9.98
	55	56	6.80
	65	43	4.96
20	45	73	57
	55	79	12
	65	26	5
21	45	70	13
	55	67	6
	65	24	4
22	45	84	84
	55	56	14
	65	19	7

ESTIMATED STABILITY OF AUR AT 25C

AUR	Half-life (days) Aw=.11	Aw=.75
19	297	20
20	252	566
21	238	37
22	408	902

Table 8

DURKEE PROTECTED VITAMIN A SALT STABILITY STUDY

% Moisture	Temperature	Half-Life (weeks)			
			% Coating		
		Parent	50	40	25
0	35	23±4	23±3	24±4	18±4
	45	13±2	28±10	16±1	16±1
	55	5±0	5±0	5±0	5±0
	* 25	52	51	65	39
0.1	35	20±7	18±4	21±8	43±56
	45	119±875	14±3	13±4	17±9
	55	6±13	3±1	4±1	3±1
	* 25	36	58	56	181
3.5	35	1±0	5±0	5±1	2±0
	45	1±0	2±0	3±1	2±0
	55	9±15	1±0	1±0	1±0
	* 25	1	12	13	3

* Extrapolated half-life in weeks from higher temperatures; values with • were excluded from half-life estimation.

Table 9

STABILITY OF BALCHEM ENCAPSULATED VITAMIN A IN SALT

% Moisture	T	Half-life (days) Parent	Half-life (days) Encapsulated
0	35	863±1442	4302±56913
	45	141±29	425±325
	55	58±8	54±8
	65	18±2	13±1
	* 25	1167	12620
0.1	35	40±5	657±902
	45	76±18	161±28
	55	5±0	52±20
	65	6±1	8±2
	* 25	142	3896
3.5	35	17±1	22±1
	45	12±1	14±1
	55	3±0	5±1
	65	3±1	3±0
	* 25	34	48

* Half-life estimates at 25°C were calculated from a linear regression of ln(half-life) on temperature.

Table 10

EFFECT OF MOISTURE ON STABILITY OF VITAMIN A IN SALT

% Moisture	Half-life (days) at 45C	
	Parent	Balchem
0	141	425
0.1	76	161
0.4	3	39
1.0	2	63
2.5	2	22
3.5	12	14

Table 11

EFFECT OF CALCIUM STEARATE AS VITAMIN A STABILIZER IN SALT

		Half-life (days)		
% M	T	250 SD	250 SD & CaSt	250 S & CaSt
0	37	863±1442	115±51	158±54
	45	141±29	520±715	141±38
	55	58±7	76±11	33±10
	* 25	1167	308	572
0.1	37	40±5	119±47	29±6
	45	76±19	113±34	43±11
	55	5±0	28±23	39±17
	* 25	142	394	26
3.5	37	17±1	4±0	18±4
	45	12±1	2±0	11±0
	55	3±0	1±0	3±0
	* 25	34	11	52

FORTIFICATION OF MONOSODIUM GLUTAMATE WITH "WHITE" VITAMIN A IN INDONESIA 1/

by

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FORTIFICATION OF MONOSODIUM GLUTAMATE WITH "WHITE" VITAMIN A IN INDONESIA

Monosodium glutamate (MSG) can be fortified easily and effectively with vitamin A by blending a special "white" vitamin A with MSG in a mechanical mixer. The resulting mixture is chemically and physically stable and is essentially indistinguishable from unfortified MSG.

1.0 MONOSODIUM GLUTAMATE (MSG)

MSG is the sodium salt of glutamic acid, a common amino acid found in virtually all proteins. Small amounts of MSG have been found to enhance the flavor of meats, vegetables and other foods and it is used for that purpose in many parts of the world.

In Indonesia, MSG is manufactured and marketed by several commercial organizations as branded products, the most widely consumed of which are Sasa, Miwon, and Aji-no-moto. MSG is used regularly by up to 90% of the rural Indonesian population. Average consumption by rural users is roughly 0.6 gram per person per day.

Commercial MSG is sold in the form of purified crystals. The crystals have a needle-like habit and are generally 0.1-0.5 mm in diameter and 0.2-4.0 mm long. The product has a snow-white appearance. MSG is packaged for the retail market in sachets, bags, and jars in units from 1.0 gram to one kilogram. Typically, low income users in Indonesia, particularly those in rural areas, purchase MSG in sachets of 5 grams or less.

2.0 "WHITE" VITAMIN A

"White" vitamin A is a granular dry product consisting of vitamin A palmitate oil dispersed in an edible carbohydrate matrix and surrounded by a layer of edible white coating. The coating consists of a white pigment (food grade titanium dioxide) dispersed in a cellulose binder (hydroxypropyl cellulose).

"White" vitamin A is produced in the form of clusters or agglomerates of several coated particles held together by the coating binder. The size of the clusters is adjustable and can be controlled during the manufacturing process.

All ingredients in white vitamin A are considered safe and wholesome and are permitted for use in food in Indonesia.

"White" vitamin A contains 175,000 International Units (IU) of vitamin A per gram of product.

Although the Vitamin A palmitate in "white" vitamin A is stabilized with antioxidants and is partially protected from atmospheric oxygen by embedding it in a protective carbohydrate matrix, it losses potency over time due to oxidation. Loss of potency is more rapid at higher temperature and in the presence of moisture. In addition, light tends to accelerate deterioration.

The storage stability of "white" vitamin A is at least as good as uncoated vitamin A. Both products retain over half their potency when stored under dry, dark conditions for over 75 weeks (25°C) and over 30 weeks under moist, dark conditions (water activity of 0.75 at 25°C), see Table 1. Preliminary tests suggest that the white coating reduces the loss of potency of vitamin A caused by light; as shown in Table 1, the storage life of "white" vitamin A under light conditions is roughly double that of uncoated vitamin A.

"White" vitamin A is dispersible in cold and hot water and has been found to be biologically available (see Appendix A).

"White" vitamin A is manufactured and distributed by:

Coating Place Inc.
Box 248
Verona, Wisconsin 53593
USA

3.0 FORTIFICATION OF MSG

MSG can be fortified with "white" vitamin A by dry blending the "white" vitamin A with MSG in a mechanical mixer. The mixing action should be gentle in order to prevent breakage of the needle-like MSG crystals and to minimize deagglomeration of the "white" vitamin A clusters. A Patterson-Kelley "vee" type mixer or similar, slow acting, batch mixing machine is considered satisfactory. The duration of mixing should be long enough to assure homogeneous dispersal of the vitamin A in the MSG. Excessive mixing should be avoided to minimize damage to the MSG and vitamin A.

The target fortification level in Indonesia is 3000 IU of vitamin A per gram of MSG. To achieve this level, 17.1 kg. of "white" vitamin A (175,000 IU per gram) should be mixed with 982.9 kg. of MSG.

4.0 FORTIFIED MSG

4.1 Chemical Stability

The chemical stability of vitamin A in MSG was tested by storing fortified MSG at 25°C, 35°C, 45°C and 55°C under dry conditions with and without light. The results of storage tests of MSG fortified with "white" vitamin A and

uncoated "yellow" vitamin A (the parent vitamin A), are listed in Table 2 and Figure 1 A-H. Vitamin A potency was lost in an apparent first order rate process which followed the Arrhenius equation. 2/ The results of loss of vitamin A potency with time are reported as the half-life of the vitamin A, which is defined as the storage time corresponding to loss of one half the vitamin A potency. As listed in Table 2, the half-life of white vitamin A is comparable to the yellow A when stored in the dark. Both retain half of their potency for over two years at temperatures up to 35°C. In addition, Table 2 shows that vitamin A looses potency rapidly when exposed to direct light. Although "white" vitamin A is much more stable than yellow vitamin A, exposure to light should be minimized.

Figure 1I-K illustrate the stability of MSG fortified with "white" vitamin A and stored under wet conditions. MSG fortified with white vitamin A and stored at a water activity of 0.75 gave half-lives of 9.3, 2.3, and 1.0 weeks at 35°, 45°, and 55°C. The calculated shelf-life at 25°C was 17.4 weeks. Normally, fortified MSG is expected to be stored under dry conditions in Indonesia and these results suggest that precautions should be taken to assure that fortified MSG is not exposed to wet conditions.

The results of the tests cited above indicate that MSG fortified with "white" vitamin A has stability roughly comparable to that of "yellow" vitamin A under dry conditions and in the dark. Also as noted in section 2.0 above, "white" vitamin A appears to be more stable than "yellow" vitamin A when exposed to light. As shown in Table 2, even under different intensities of light, the white vitamin A had at least twice the half-life of yellow vitamin A.

2/ The Arrhenius equation, $k=A_0 \exp(-E_a/RT)$ expresses the reaction rate constant (k) in terms of absolute temperature (T) and heat of activation (E_a). The Half-life ($t_{\frac{1}{2}}$) equals 0.693/k for first order reactions and thus half-life or shelf-life can be predicted at any temperature from measurements at a specific temperature.

"Yellow" vitamin A of the type used in the tests reported above had been used previously for MSG fortification in the Bogor field trials and had been found to have satisfactory stability under Indonesian conditions 3/. Accordingly, it can be concluded that MSG fortified with "white" vitamin A should also have satisfactory stability for MSG fortification in Indonesia.

4.2 Physical Stability

When coarse, granular products such as MSG are mixed with fine granular products or powders such as commercial vitamin A preparations, the fine products sometimes separate physically from the coarse products during handling and shipping. Generally the fine particles tend to congregate toward the bottom of a container. If vitamin A powder separates from MSG during handling it might lead to: (1) excessive fortification of MSG in some packages or parts of packages, (2) under-fortification of MSG in some packages or parts of packages, and (3) alteration of the color or appearance of the MSG in areas where the vitamin A congregates.

Physical separation of vitamin A from MSG can be prevented by using vitamin A with particle size similar to that of the MSG. Because the particle size of commercial vitamin A preparations is generally considerably smaller than MSG, the vitamin A must be increased in size to prevent segregation. This can be accomplished by agglomerating the vitamin A, for example, by gluing the vitamin A particles together to form clusters 4/.

3/ Muhilal et al. A pioneering project for combatting vitamin A deficiency and xerophthalmia with MSG fortified with vitamin A., Center for Research and Development of Nutrition, Bogor, Indonesia. 1986

4/ Separation of vitamin A from the fortified MSG used in the Bogor field trials was prevented by creating agglomerates or clusters of vitamin A bound with powdered MSG which were roughly the same size as the MSG. The powdered MSG used to form the agglomerates also tended to mask the color of the yellow vitamin A used for fortification.

Table 3 lists the particle size distribution of the three principle types of MSG in Indonesia (Sasa, Aji-no-moto, and Miwon), "yellow" vitamin A, unagglomerated "white" vitamin A, and "white" vitamin A which has been agglomerated to form clusters which are roughly similar in size to the three type of MSG. It can be seen that although the three types of MSG have somewhat different size distributions, they are roughly similar. The "white" and "yellow" unagglomerated products are markedly smaller than the MSG and therefore might tend to separate during fortification, packaging, and shipping. The agglomerated "white" vitamin A, on the other hand, would not be expected to separate during handling because it has a size distribution similar to the three MSG products.

Resistance to separation was evaluated by two laboratory tests. In the first test, the MIXING TEST, the vitamin A and MSG were mixed under controlled conditions and the homogeneity of the mixture measured and reported as the MIXING VARIABILITY or the variance of the vitamin A content in the mixture (see Appendix B for the MIXING TEST procedure). Products which tend not to separate generally would be expected to have a MIXING VARIABILITY about equal to the standard error of analysis or about 15% or less. Correspondingly, products which have a MIXING VARIABILITY of 15% or more are generally expected to be susceptible to physical separation.

Results of the MIXING TEST for agglomerated "white" vitamin A in Sasa, Aji-no-moto and Miwon MSG are listed in Table 4. Also included are results of tests of "yellow" vitamin A, unagglomerated "white" vitamin A, and agglomerated "white" vitamin A in a "standard" MSG used for testing. As shown in Table 4, the MIXING VARIABILITY of all MSGs (Sasa, Aji-no-moto, Miwon, and standard) fortified with agglomerated "white" vitamin A were less than 15%; the MIXING VARIABILITY of standard MSG fortified with yellow vitamin A and unagglomerated "white" vitamin A were about 20% or more.

The second test used to assess resistance to segregation was the SHAKING TEST. In the shaking test, a sample of fortified MSG is placed in a plastic bag, the bag is mechanically shaken for a period of time, and the fortification level is measured in the top, middle and bottom layers of the bag. The resistance to segregation is measured by comparing the means of vitamin A concentration in the top, middle and bottom layers using Scheffe's interval (see Appendix C for segregation test procedure).

The results of the SHAKING TEST for agglomerated white vitamin A are presented in Table 5. The results show that the mean vitamin A content in the top, middle, and bottom of the shaken bags were not significantly different and, therefore, agglomerated "white" vitamin A does not tend to segregate from MSG. In contrast, the yellow vitamin A (the parent material for the white vitamin A), was significantly lower in the top layer than the bottom suggesting that yellow vitamin A tends to segregate from MSG.

It should be noted that the separation tests reported here were designed to simulate procedures used in the manufacture, packaging, and distribution of fortified MSG and can not be interpreted as establishing conclusively the resistance to physical separation or lack thereof. Final conclusions concerning physical separation must be based on evaluation of separation during actual processing, packaging and distribution. Nonetheless, based on the hypothesis that particles of similar size will not separate, and based on the mixing and shaking tests reported above, it appears reasonable to assume that MSG fortified with agglomerated white vitamin A will not separate physically.

5.0 COLOR AND APPEARANCE

MSG is a free flowing crystaline product with a lustrous, snow white appearance. Although the amount of vitamin A fortificant added is small (1.7%),

it can cause discoloration or change in appearance under certain circumstances. For example, when the fortificant is yellow, as in the case of uncoated vitamin A powder, it can cause the MSG to have a yellowish off-color. Or, if the fortificant is excessively large, as in the case of very large agglomerates, the fortificant might be visible to the user and might be considered to be a contaminant.

The colors of unfortified and fortified MSGs were measured using a Hunter color meter by measuring the lightness index, L, and yellowness index, b. The change in lightness index, [L] and the change in yellowness index, [b] are reported in Table 6. The values were computed using the respective unfortified MSG as zero. The [L] describes relative lightness (+ values) or darkness (- values) of fortified MSG compared to unfortified MSG. Likewise [b] describes the relative yellowness of fortified MSG compared to unfortified MSG (higher positive numbers indicate more yellowness).

As listed in Table 6, [L] values for all fortified MSGs indicated that those products are slightly darker than unfortified MSG. However the values were all less than 3 on a scale of 0 to 100 and are barely perceptible visually. The darkness values for MSG fortified with white and yellow vitamin A are essentially the same. The yellowness index values for agglomerate white A fortified MSG were less than 1% greater than the respective unfortified MSG. MSG fortified with yellow vitamin A was significantly more yellow than either MSG fortified with "white" vitamin A or unfortified MSG. MSG fortified with "white" vitamin A is not perceptibly yellow to an observer. The results of these tests indicate that MSG fortified with "white" vitamin is only very slightly darker and barely measurably yellower than unfortified MSG. It would not be expected that consumers could notice the difference except in a closely scrutinized, side-by-side comparison.

MSG fortified with agglomerated "white" vitamin A at a level of 3000 IU per gram f MSG retains its free flowing characteristics and is not perceptibly different in color or appearance.

Table 1. STABILITY OF VITAMIN A AT 25°C

	Half-life (Weeks) 1/		
	Dark		Light
Type of Vitamin A	Water Activity 0.11	Water Activity 0.75	Dry
175 White	90.4	34.0	49.2
250 CWS	78.8	34.4	21.2

1/ Half life is the storage time during which vitamin A loses one-half of its original potency. Loss of potency of vitamin A is a first order chemical reaction.

Table 2. STABILITY OF VITAMIN A IN DRY MSG

Lighting	Temperature	Half-life (weeks) 1/	
	°C	White	Yellow
Dark	25	116±107	257±455 3/
	35	108±53	168±89
	45	34±4	40±8
	55	25±2	31±2
Light 2/	25	2.8±0.2	1.7±0.2
	35	3.5±0.2	1.6±0.1
	45	9.8±1.2	3.6±0.6
	55	3.7±0.5	1.2±0.2

1/ Half-life is the storage time during which vitamin A loses one-half of its original potency. Loss of potency of vitamin A is a first order chemical reaction. Results reported are based on best estimate derived from regression analysis plus/minus the standard error of estimate.

2/ Light intensity was different at each temperature combination: the light intensity at 25°C was 310 foot candles, at 35° it was 230 ft.c., at 45° it was 85 ft. candles and at 55° it was 190 ft.candles.

3/ At low temperatures, the standard errors of the estimate were very large because only about 1% of the vitamin degraded during the 8-week storage period. Extrapolation from higher temperatures (using ln (shelf-life) versus 1/T°K) where the precision of half-lives was better and is the traditional approach in shelf-life studies, gave estimates of half-lives of 212 weeks and 355 weeks respectively for white and yellow vitamin A at 25°C.

Table 3. PARTICLE SIZE OF MSG AND VITAMIN A

Particle size		Percent					
		MSG 1/			Vitamin A		
Seive Size(mesh)	Particle Size(mm)	Sasa	Aji-no-moto	Miwon	Yellow 2/	White Not Agglomerated	White Agglomerated
Over 18	> 1.000			trace			trace
-18+20	0.841-1.000		trace	0.4	trace		2.5
-20+25	0.707-0.841	trace	1.5	9.4	0.5		6.8
-25+30	0.595-0.707	0.1	6.2	15.2	0.2	trace	9.9
-30+35	0.500-0595	8.0	18.9	28.7	1.1	2.5	16.1
-35+45	0.354-0.500	58.2	30.6	23.3	21.7	26.4	31.2
-45+60	0.250-0.354	31.1	27.0	11.1	47.1	45.8	26.3
-60+80	0.177-0.250	1.7	12.1	4.6	22.6	18.8	5.8
Thru 80	< 0.177	0.9	3.7	7.4	7.3	6.4	1.3

Mixing

Table 4. RESISTANCE TO SEPARATION DURING MIXING

MSG Product	Vitamin A Fortificant	Mixing Variability (%) 3/
SASA	Agglomerated White	12.27±9.96
Ajinomoto	Agglomerated White	5.21±2.01
Miwon	Agglomerated White	7.28±2.59
STD MSG 1/	Agglomerated White	6.65±3.97
STD MSG 1/	Yellow 2/	19.29±7.39
STD MSG 1/	Unagglomerate White	20.18±6.00
	22 Aug Sara (small packets) 2nd batch L# 880224-A	24.60± 12.70

1/ Miwon MSG, manufactured in Korea.
2/ Roche 250 CWS.
3/ See Appendix B.

Table 5. RESISTANCE TO SEGREGATION DURING SHAKING 2/

MSG Product	Vitamin A Fortificant	Location	Vitamin A Content 2/ Trial Means n=4 (IU/g) Trial 1	Trial 2
SASA	Agglomerated white	Top	2729	3001
		Middle	2717	2788
		Bottom	2691	2670
AJI-NO-MOTO	Agglomerated white	Top	2921	3013
		Middle	2853	2828
		Bottom	2689	2840
MI-WON	Agglomerated white	Top	2496	2695
		Middle	2622	2727
		Bottom	2476	2814
STD MSG	Agglomerated white	Top	2656	2751
		Middle	2605	2768
		Bottom	2301	2617
STD MSG	Yellow 3/	Top	2002 ab	1752 ab
		Middle	2712 a	2303 ac
		Bottom	3095 b	5106 bc
[illegible]	2nd Batch L# 820224-A	Top	2151	1826
		Middle	1383	1571
		Bottom	2356	3999

1/ Fortified with agglomerated "white" vitamin A unless otherwise noted

2/ Pairs of values in a column with the same letter (a, b, or c) are significantly different at the .05 level. Confidence interval for agglomerated vitamin A is 554 and for yellow viamin A is 442.

3/ Fortified with Roche 250 CWS.

Lines

Trial #1

Trial #2

78/89

90/101

102/113

114/125

2.1 b

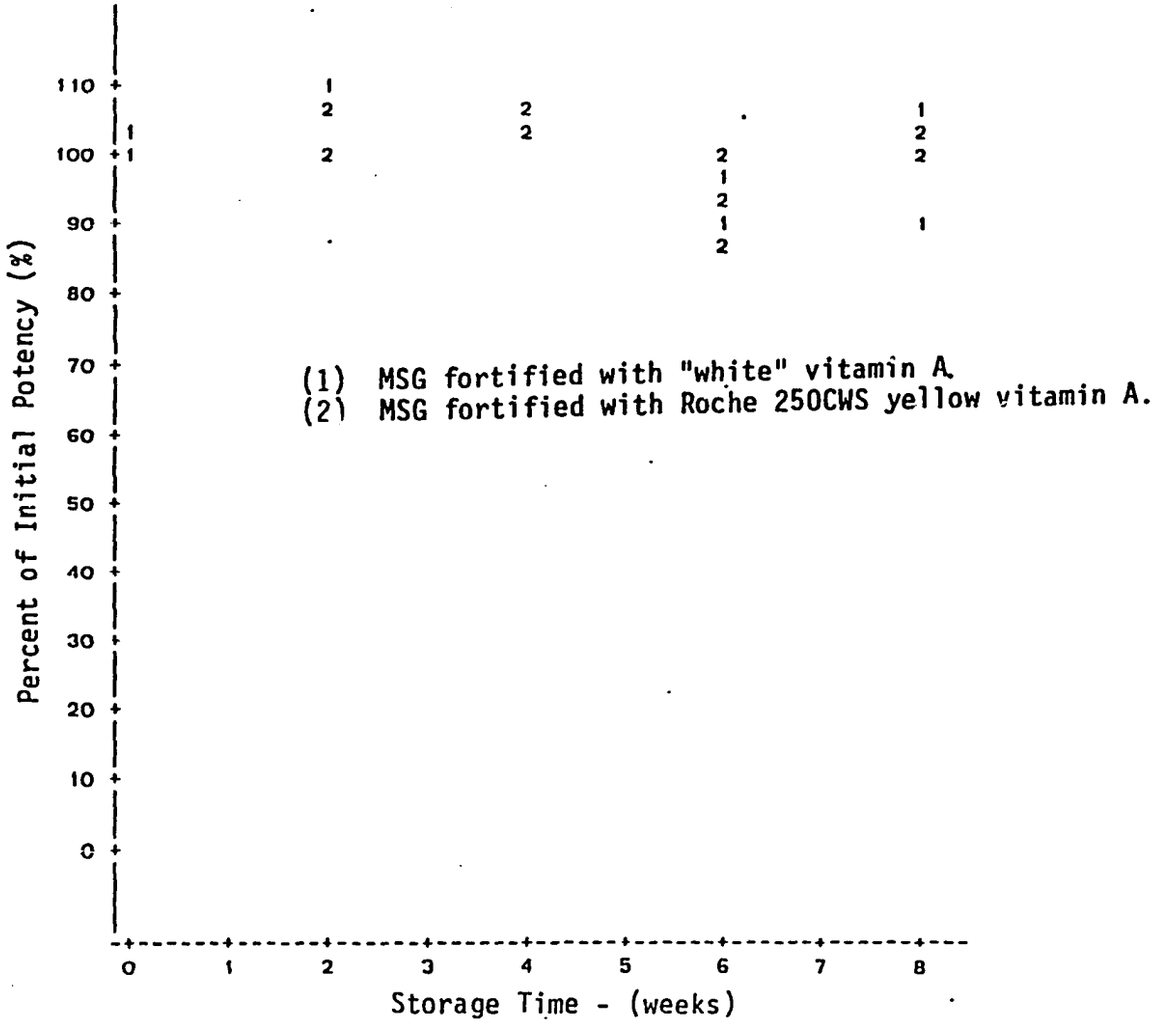
2.1 c

Table 6. HUNTER COLOR DETERMINATIONS OF FORTIFIED MSG

MSG	FORTIFICANT	[L]	[b]
AJI-NO-MOTO	White	-2.84±0.34	+0.77±0.11
	Yellow	-2.98±0.34	+3.44±0.79
MI-WON	White	-0.54±0.24	+0.50±0.21
	Yellow	-1.21±0.58	+1.43±0.55
SASA	White	-1.52±0.18	+0.52±0.16
	Yellow	-0.96±0.22	+2.05±0.63

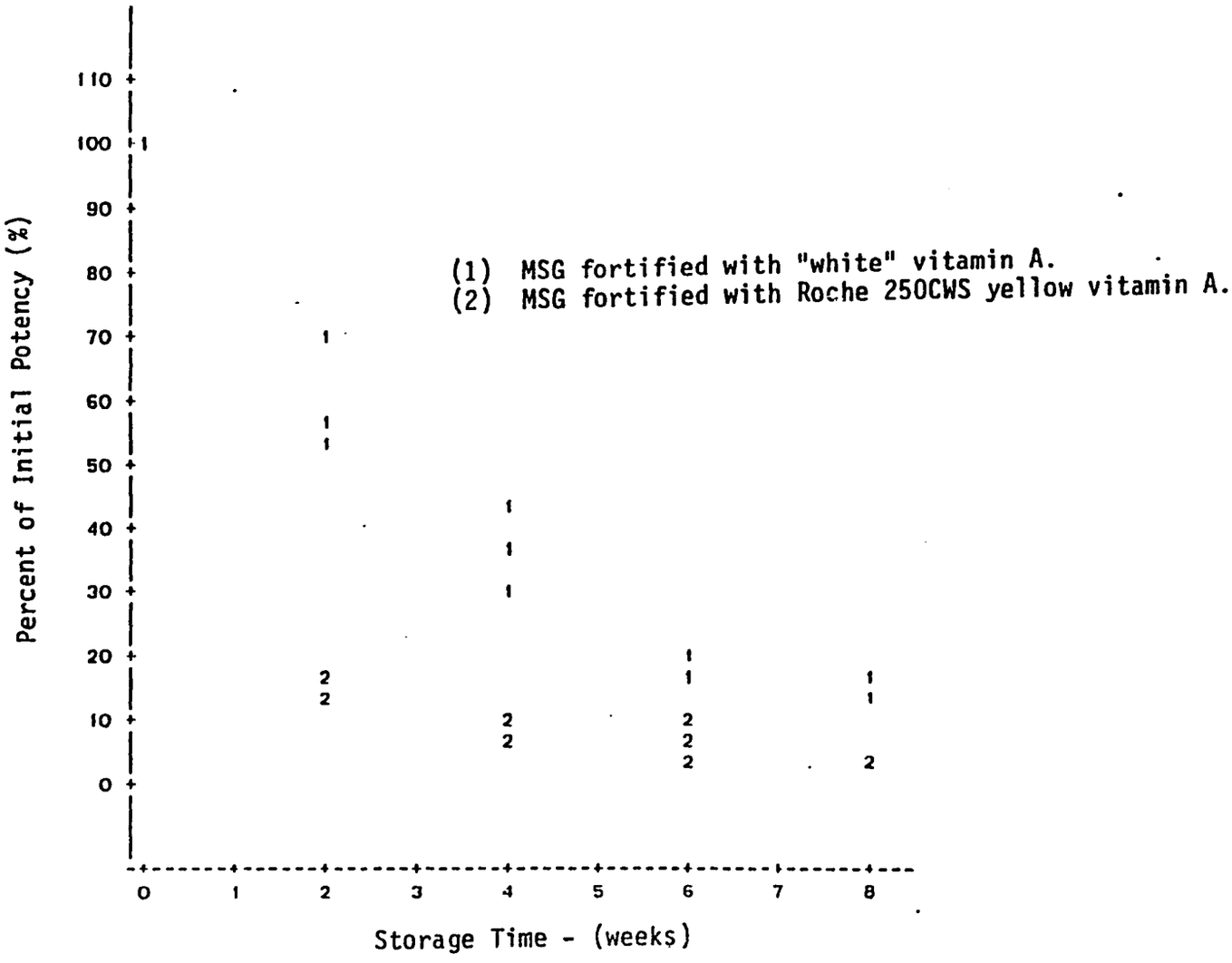
Note: [L] and [b] values are reported referenced to the respective unfortified MSG.

Figure 1A Storage Stability of MSG Fortified with Vitamin A (3000 IU/g; 25°C; Dark; Dry)



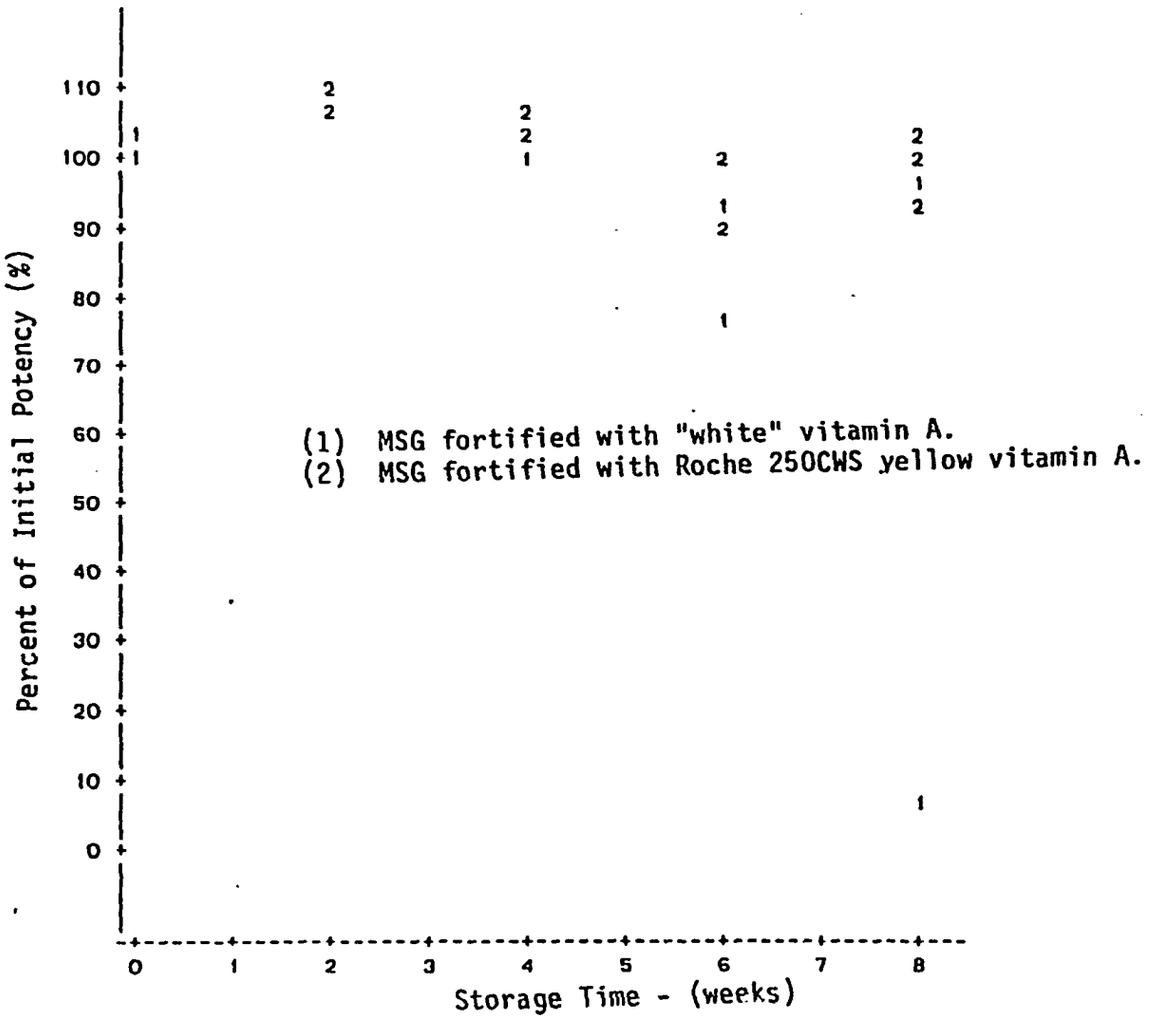
NOTE: 2 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 19 OBS HIDDEN

Figure 2B. Storage Stability of MSG Fortified with Vitamin A (3000 IU/G; 25°C; 310 foot candles; dry)



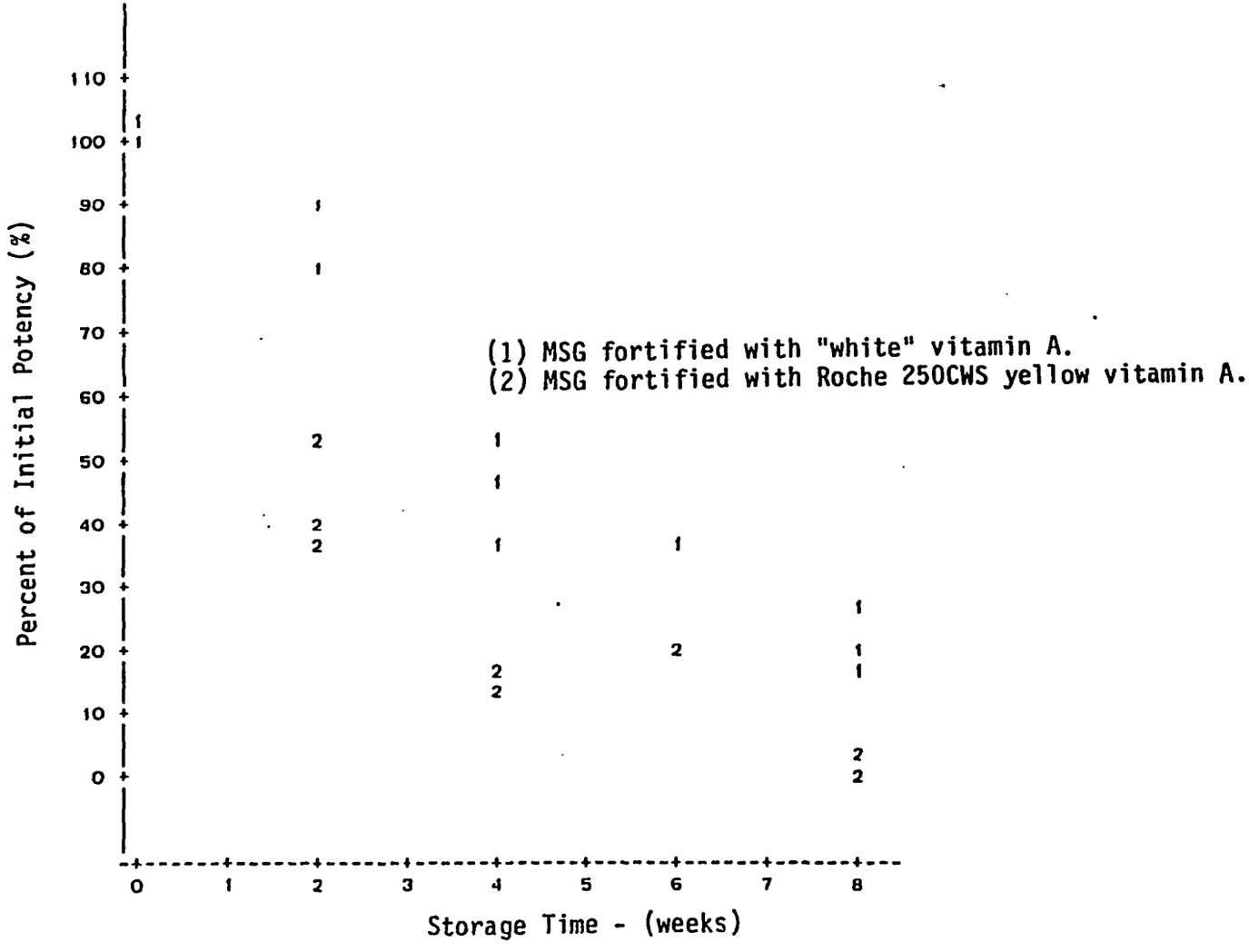
NOTE: 16 OBS HIDDEN

Figure 1C. Storage Stability of MSG Fortified with Vitamin A (3000 IU/g.; 35°C; dark; dry)



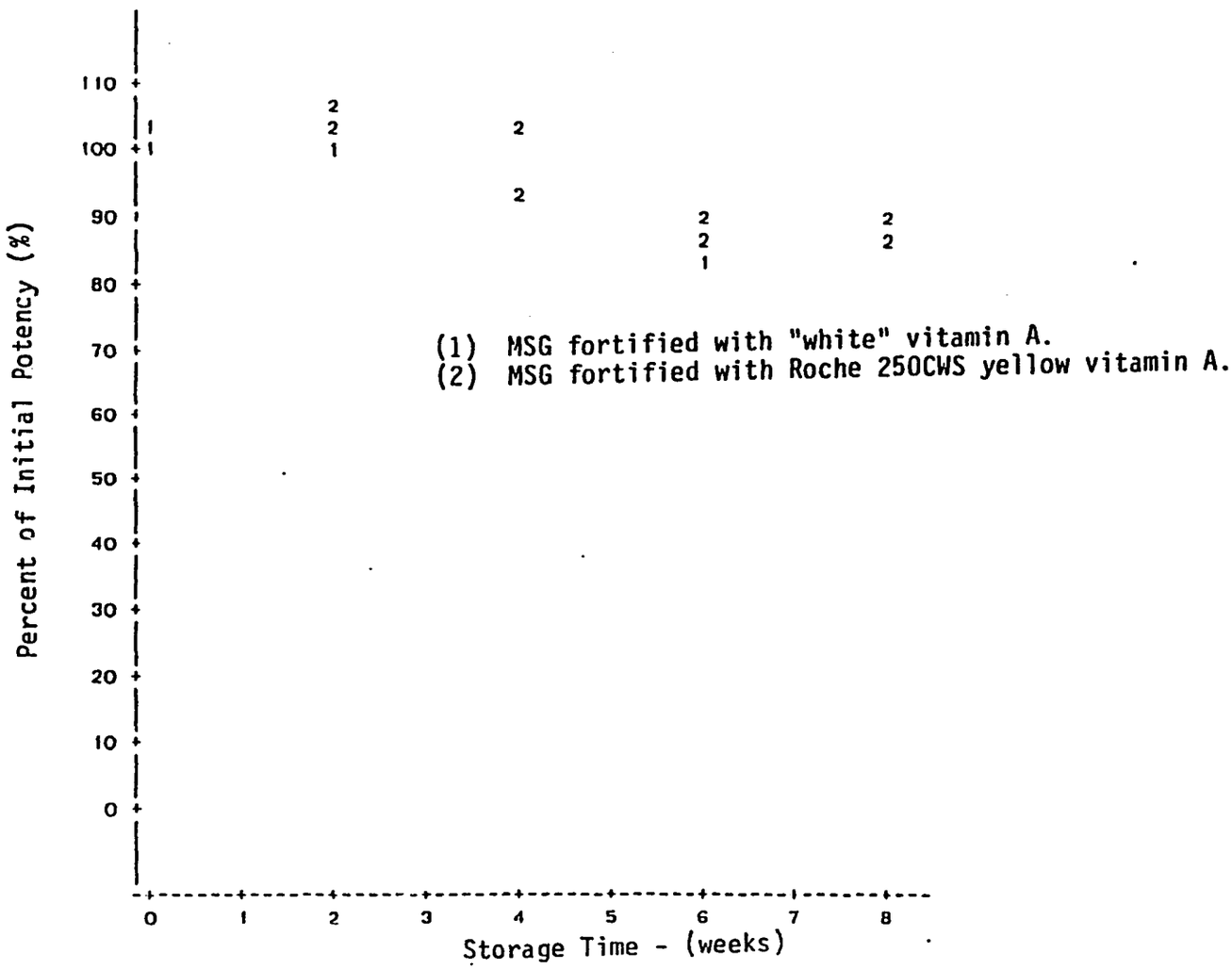
NOTE: 2 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 30 OBS HIDDEN

Figure 1D. Storage Stability of MSG Fortified with Vitamin A (3000 IU/g; 35°C; 230 ft. candles; dry)



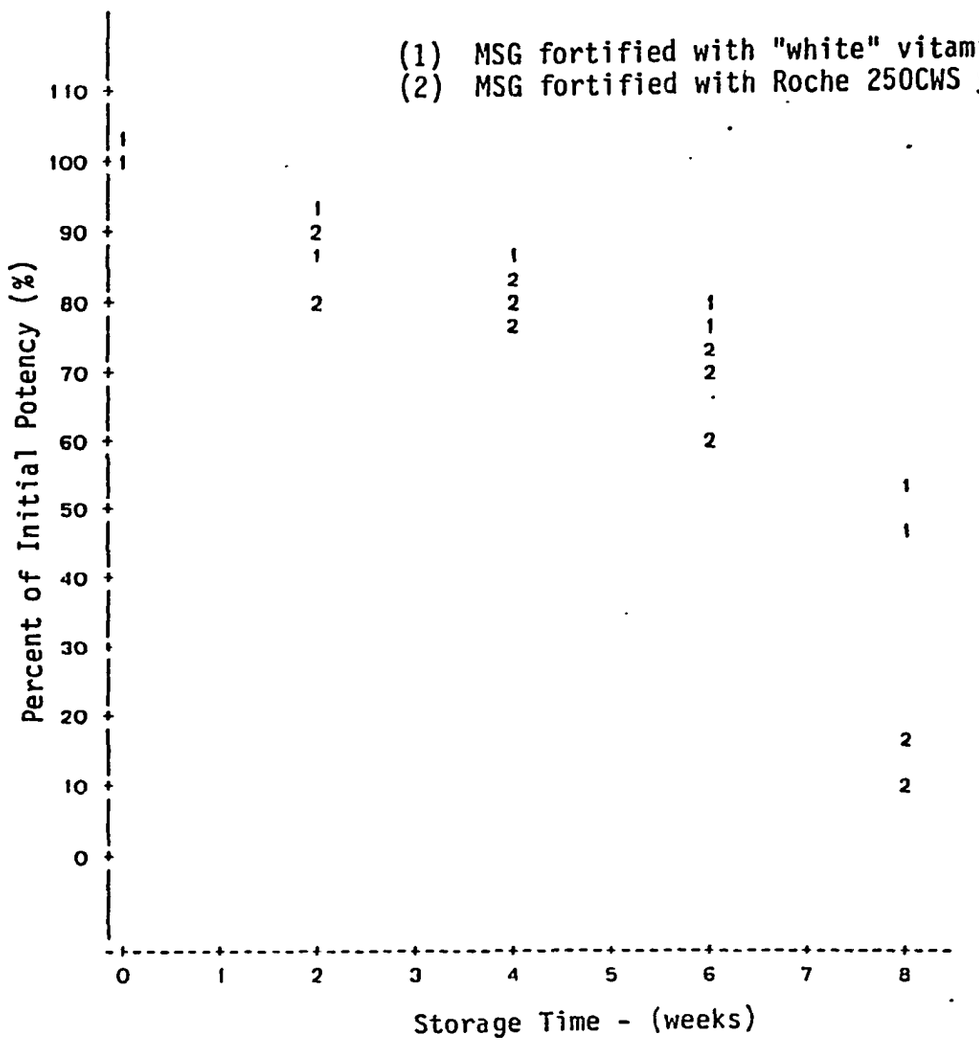
NOTE: 3 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 14 OBS HIDDEN

Figure 1E. Storage Stability of MSG Fortified with Vitamin A (3000 IU/g; 45°C; dark; dry)



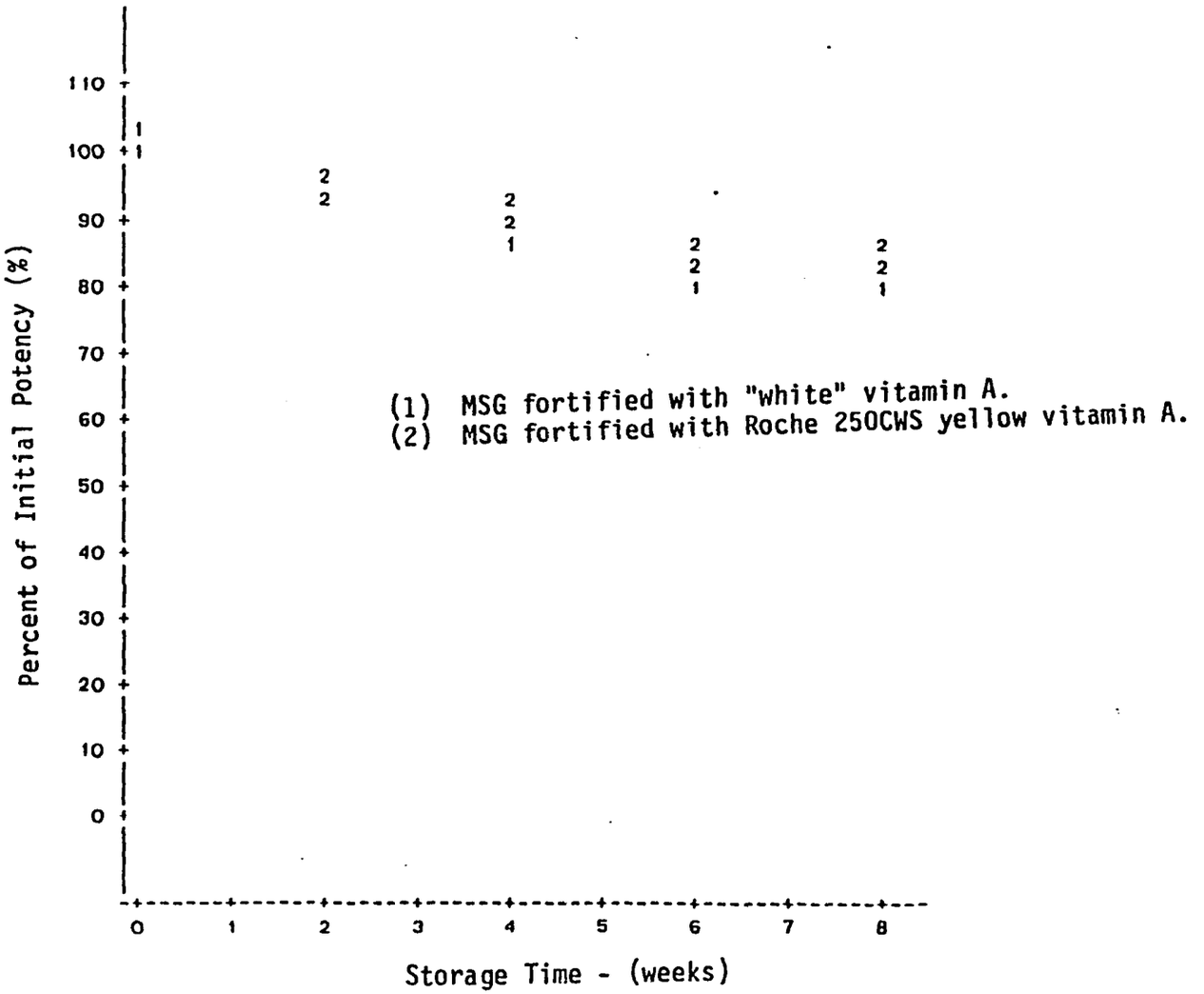
NOTE: 24 OBS HIDDEN

Figure 1F. Storage Stability of MSG Fortified with Vitamin A (3000 IU/g; 45°C; 85 ft. candles; dry)



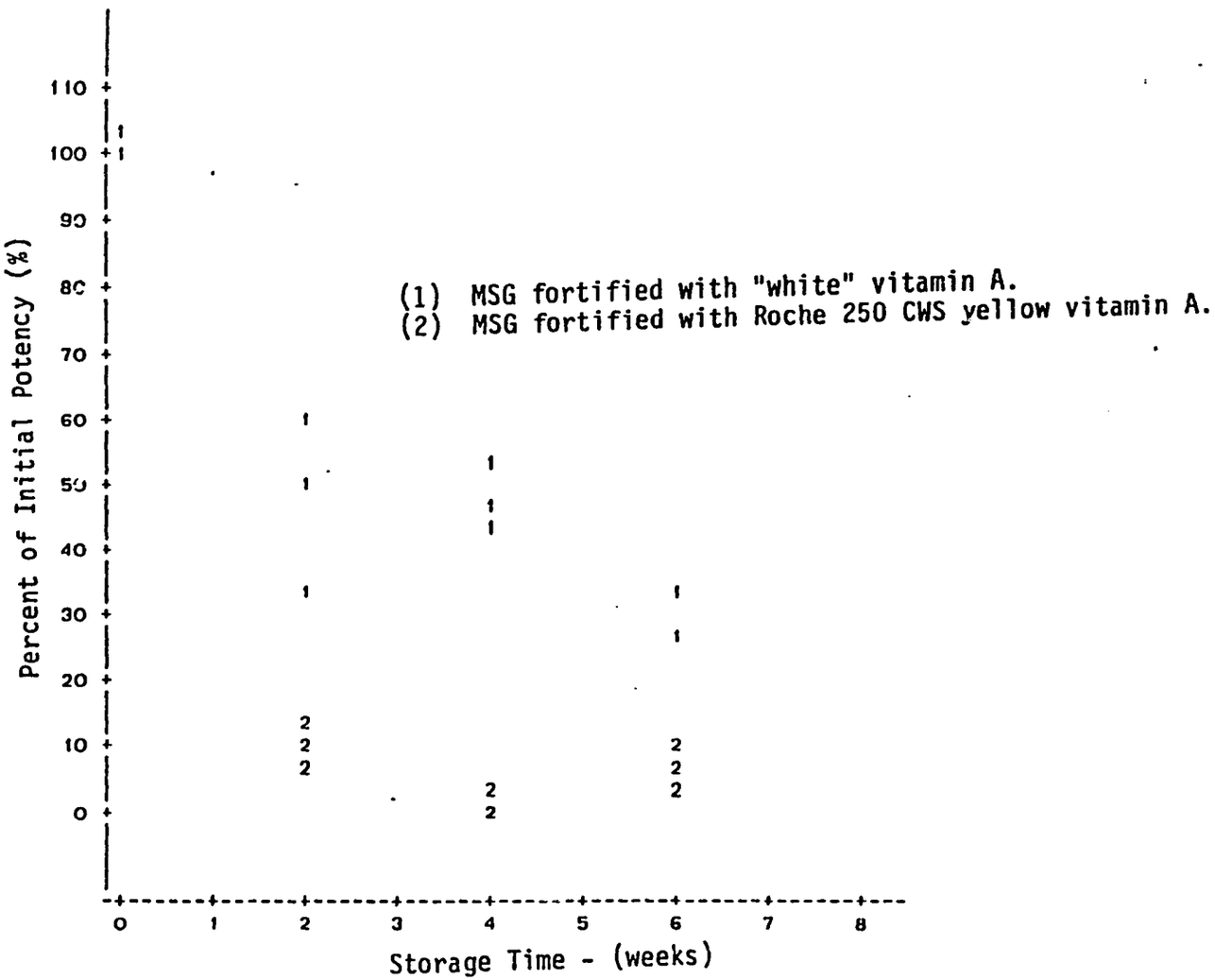
NOTE: 17 OBS HIDDEN

Figure 1G. Storage Stability of MSG Fortified with Vitamin A (3000 IU/g; 55°C; dark; dry)



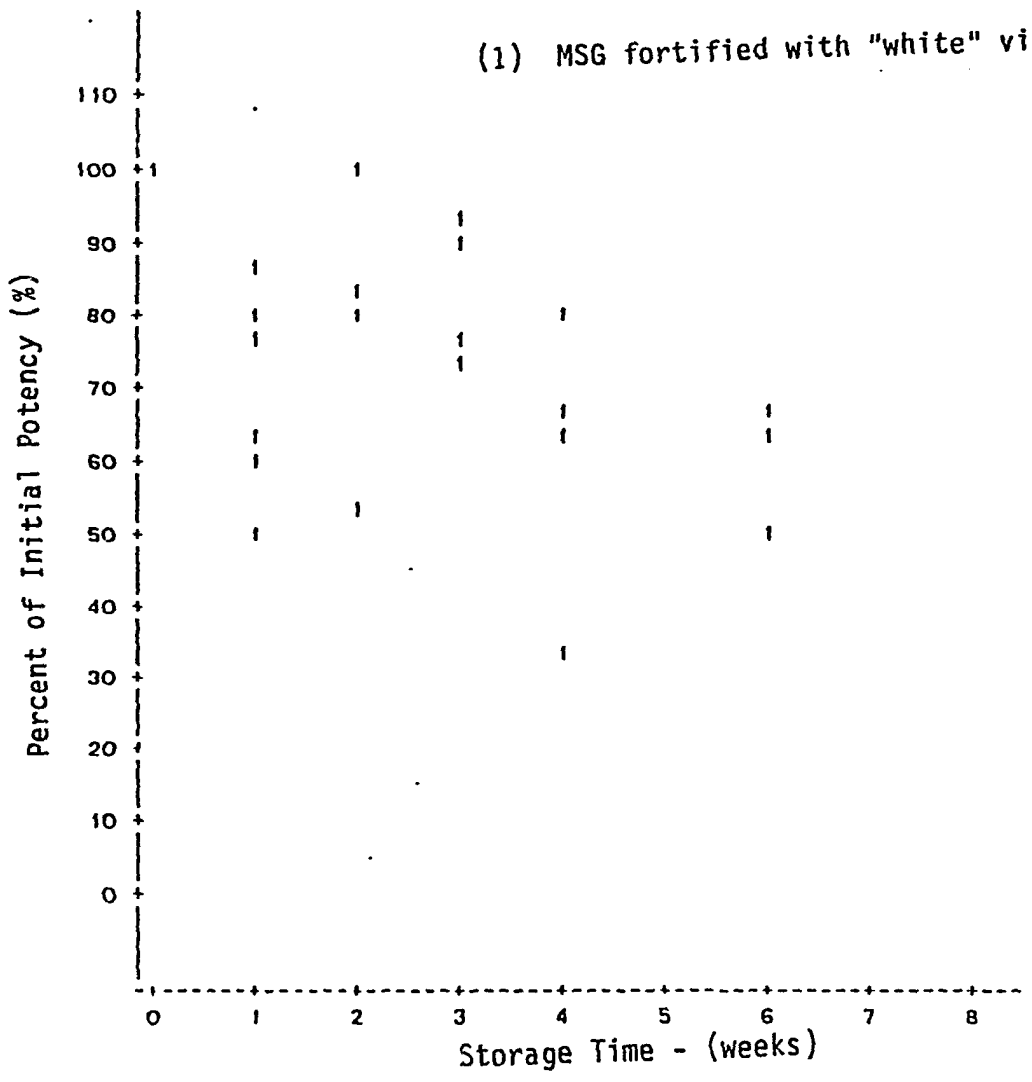
NOTE: 2 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 21 OBS HIDDEN

Figure 1H. Storage Stability of MSG Fortified with Vitamin A (3000 IU/g; 55°C; 190 ft. candles; dry)



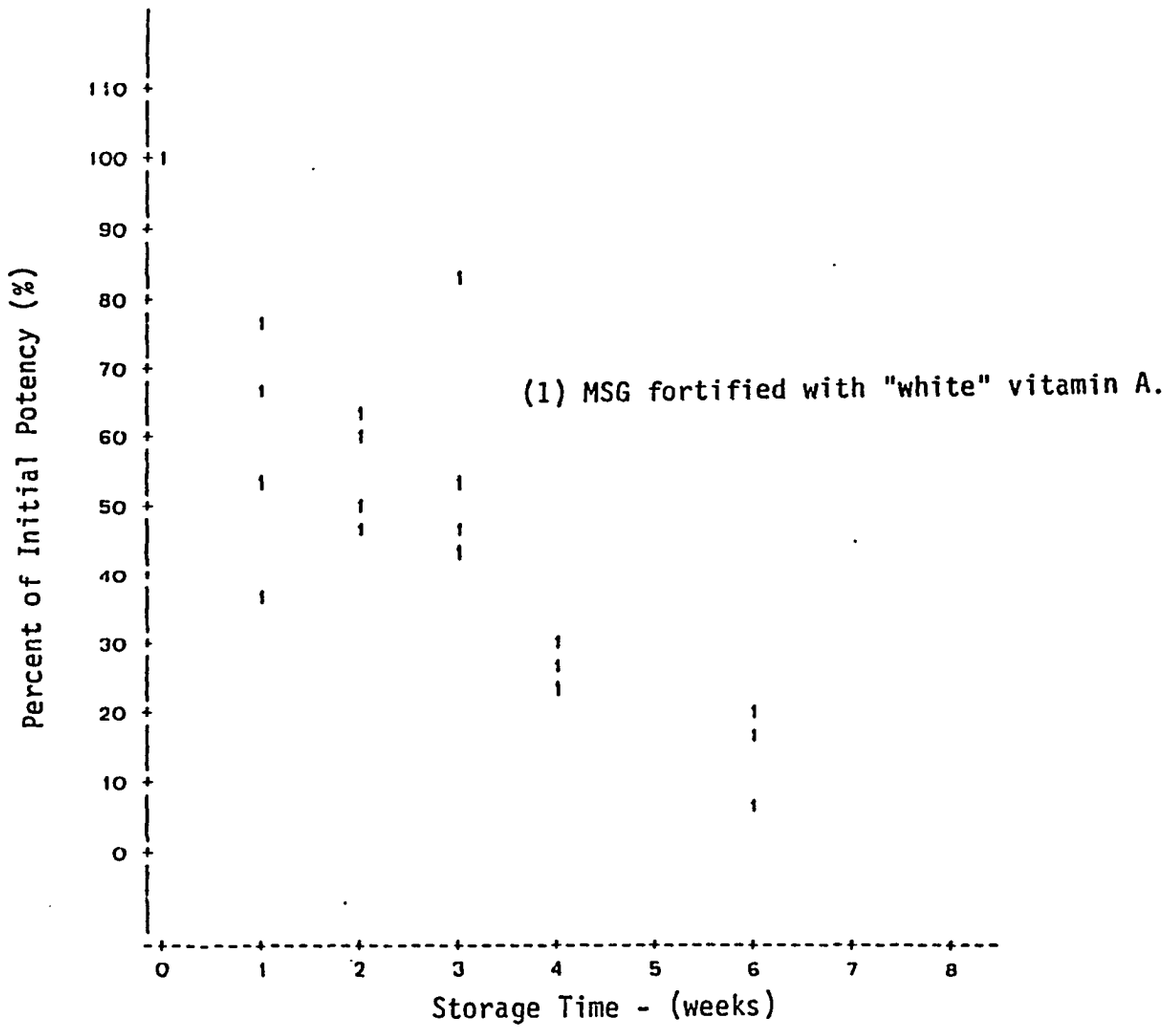
NOTE: 12 OBS HIDDEN

Figure 1I. Storage Stability of MSG Fortified with Vitamin A (3000 IU/g; 35°C; dark; wet, AW 0.75)



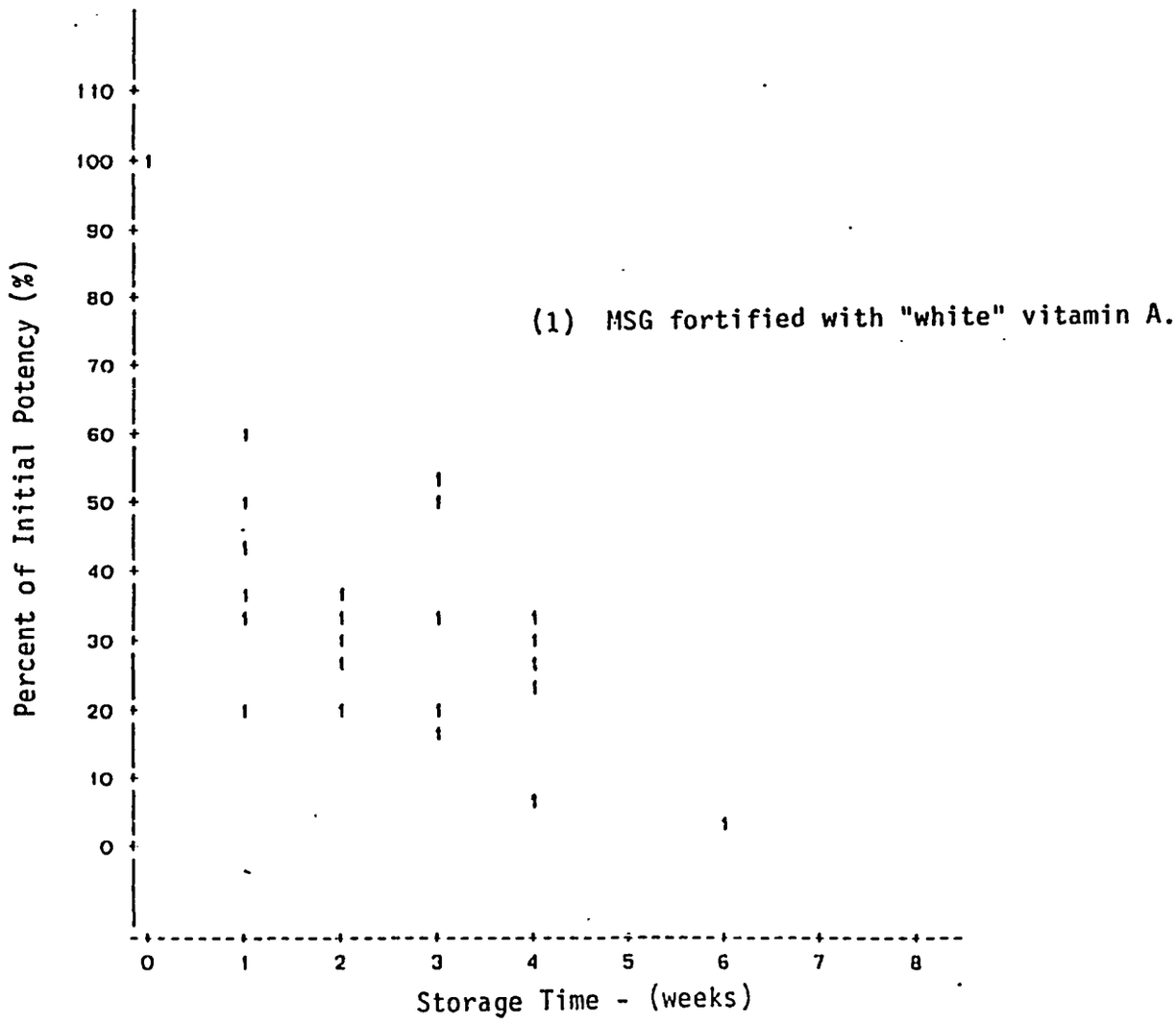
NOTE: 9 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 10 OBS HIDDEN

Figure 1J. Storage Stability of MSG Fortified with Vitamin A (3000 IU/g; 45°C; dark; wet, AW 0.75)



NOTE: 10 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 13 OBS HIDDEN

Figure 1K. Storage Stability of MSG Fortified with Vitamin A (3000 IU/g; 55°C; dark; wet, AW 0.75)



NOTE: 12 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 7 OBS HIDDEN

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No. ..... Bogor, December 11, 1985.

Mr. Paul R. Crowley
Director Nutrition & Agribusiness
Group USDA
Office of Int'l Cooperation & Dev.
Room 4300, Auditors Bldgs.
Washington, D.C. 20250.

Dear Rod,

When we were at Hyderabat I promised to carry out a quick test on the bio-availability of white vitamin A you gave to me.

I did the test using rats available in my laboratory. The indicator I used was the increase of serum vitamin A after being dosed. As a comparison I used vitamin A Roche type 500. The average weight of the rats was around 30 gm, and the vitamin A given was approximately 150 ug (500 I.U.) for each rat. Serum vitamin A analysis was using HPLC. Based on the increase of serum vitamin A levels at 3 hours after dosing indicated that the white vitamin A was absorbed very well. A slight difference observed between the serum vitamin A levels of rats given white vitamin A and those given vitamin A type 500 might be due to individual variation.

Enclosed please find the table of rat serum vitamin A of each group.

I hope this information is useful to you.

With best regards.

Sincerely,

Dr. Muhilal

cc.: Dr. Daniel Kraushaar - Jakarta.

Rat Serum Vitamin A

After Dosing With Vitamin A.

Groups	Type of Vitamin A given	Dose ug/rat	N	Individual level of serum vitamin A, 3 hours after dosing (ug/dl)	Average
A	(Control)	None	3	35.1 24.9 46.4	35.5
B	White vitamin A (Coating place ?)	150	3	120.0 98.4 97.2	105.2
C	Type 500 (Roche)	150	3	92.5 90.9 98.4	93.9

APPENDIX B

THE MIXING TEST

The MIXING TEST is used as a measure of the inherent resistance of two dry, particulate materials to separate during handling and shipping. Resistance to separation is tested by mixing the two materials thoroughly, and assaying the mixture to measure the variability in composition. If the variability is small (approximately equal to the standard error of analysis), the test indicates good resistance to separation. If the variability is large, the test indicates the material might tend to separate during handling and shipping.

The test is run in triplicate with each replicate comprised of four samples.

The procedure is as follows:

1. Weigh out a 20.0 g sample of MSG into a 100 ml beaker. The MSG to be used should be passed through the riffle box several times to facilitates taking representative samples. (Pick up the MSG for the sample using a teaspoon rather than by pouring to avoid selection of coarse surface particles.)

2. Weigh out 0.3429 g of white vitamin A or 0.2400 g of yellow vitamin A. This amount will result in nominal fortification of 3000 IU/g, although the actual fortification will depend on the potency of the particular vitamin A. (Use a teaspoon to remove the A from the batch container and take as much of the needed A at one time as possible to avoid selecting surface particles.)

3. Add the vitamin A to the MSG in 100 ml beaker.

4. Mix the MSG and vitamin A by passing the mixture through a riffle box into a metal tray. Repeat 12 times. Note: Make sure the riffle box and tray are completely dry.

5. After the final pass, dump (do not pour slowly) the contents of the tray back into the 100 ml beaker. Put a lid on the beaker and invert 2 or 3 times.

6. Dump the vitamin A/MSG onto the center of a metal sheet and section into 4 approximately equal-sized portions. Slide each portion onto a sheet of glassine weighing paper slid partially under the dividing sheet. Clean the dividing sheet with a damp paper towel after each sectioning.

7. Weigh the 4 portions to ± 0.01 grams, transfer to a tared weighing paper on the analytical balance, and then transfer the portion to a round bottom flask.

8. Repeat the procedure 3 times to obtain 12 portions.

9. Analyze the vitamin A content of each portion using the total quantity of each portion (roughly 5 grams). Report the results of the analysis as vitamin A content per gram of MSG.

10. Calculate the standard deviation of the 12 portions and report the MIXING VARIABILITY as the standard deviation divided by the mean vitamin A content, or: MIXING VARIABILITY = $\frac{S}{X} \times 100$.

APPENDIX C

THE SHAKING TEST

The SHAKING TEST is intended to provide information about segregation or settling of vitamin A from fortified MSG which is mechanically shaken in vertically suspended bags. After shaking, samples are taken from the top, middle and bottom segments of the bag in order to assess migration of vitamin A caused by the shaking action; four samples are taken from each of the three segments of the bags and the mean concentration of vitamin A compared using Scheffe's interval. Methods of sampling and analysis used in the SHAKING TEST are identified to those described in Appendix B for the MIXING TEST.

1. Weigh out 240±1g of riffle mixed MSG. (see Appendix B).
2. Weigh out 4.11 g of agglomerated white vitamin A or 2.88 g of yellow vitamin A. Use a teaspoon to withdraw the vitamin A to avoid selecting for surface particles. This should result in fortified MSG containing about 3000 IU vitamin A per MSG.
3. Mix the vitamin A with the MSG by passing through a riffle box twelve times.
4. After the final pass, rapidly pour the contents of each tray into a Whirl-Pak bag (4" x 9").
5. After pressing out the air, fold the top of the bag over once. Fold the corners of the bag to form thirds and tie the bag closed with an 18 gauge wire as close as possible to the top of the bag.
6. Attach the bag via the wire to the thumping arm of a Ro-Tap shaker in the middle of the arm. The bag should hang about 5-6" below the arm. Prevent swinging of the bag during shaking by using a three-finger clamp or other suitable guide. Run the Ro-Tap for 10 minutes.
7. Remove the bag, flatten slightly by pushing together with the palms of the hand and lay it flat on a cutting board. Be careful to not allow gross shifting of the MSG.

8. Cut the bag laterally 1/3 from the bottom and insert a piece of cardboard through the cut to maintain separation of the contents. Remove the contents of bottom third of the bag and place in a sample cup. Repeat the procedure for each of the other thirds of the bag.

9. Dump the top one-third portion onto the center of a dividing sheet after inverting sample cup 2 to 3 times. Center each portion about the four quadrant marks and divide in four equal portions with a plastic ruler. Further divide each quadrant into four to make 16 roughly equal portions.

10. Choose one sample at random from each of the main four quadrants. Scrape the sample to the edge of the dividing sheet, weigh the portion and transfer to a round bottom flask for vitamin A analysis.

11. Repeat the process for the middle and bottom sections of the bag. Clean the dividing sheet with a damp paper towel between sectionings.

12. Analyze the 12 samples for vitamin A within 24 hours.

13. Determined the mean vitamin A content for each section of the bag. The difference in vitamin A contents of the MSG fractions should be assessed by Comparing the means using Scheffe's interval test (1,2). The confidence interval is calculated according to:

$$\bar{Y}_1 - \bar{Y}_2 = \pm\sqrt{f_{a,1,n-t} \times MSE \times (1/r_1 + 1/r_2)}$$

14. Means that fall outside the confidence interval are considered significantly different at the .05 level and indicate that segregation occurred.

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(1) Gill, J.T. 1978. Design and analysis of experiments in the animal and medical sciences. Iowa State Press, Ames, IA, USA.

(2) SAS Institute Inc., SAS Circle, P.O. Box 8000, Cary, NC.

EVALUATION OF VITAMIN A FORTIFIED MSG

MIWON & AJINOMOTO

Samples of MSG fortified with white vitamin A were received the beginning of April, 1989. Miwon sent one sample of unfortified MSG. Miwon and Ajinomoto sent fortified MSG samples from the top (T), middle (M) and bottom (B) of the V-mixer. Each sample was mixed thoroughly by passing 12 times through the riffle box. Each sample was subdivided by pouring the riffle box-mixed MSG onto graphical coordinate paper and divided into quarters with a plastic ruler. One quarter was taken to measure sieve size of the MSG and to evalute the vitamin A content on each sieve. The remaining 3 quarters were further subdivided into quarters thus yielding 12 subsamples from each sample. The procedures for subdividing the MSG have been described in detail in the December 1988 report on fortification of MSG for Indonesia. Nine of the subsamples were assayed for vitamin A content. One subsample was sent to Harlan Hall at the Coating Place, Inc. for his comments. Another subsample was given to Dr. Harold Furr in Dr. James Olson's laboratory for vitamin A analysis. The final subsample was saved.

The content of vitamin A in the each of the MSG fractions are reported in Table 1. The data indicate that based on vitamin A content, there was no significant segregation of the white vitamin A into any layer of the mixer in either Miwon or Ajinomoto MSG. The mixing variability [% = (standard deviation / average) x 100] were larger than we observed in mixing experiments in our lab and reported in the Indonesia report. Because we really only had one sample to work with for each layer, the results are not unexpected. The results reported in the Indonesia report are from several experiments and the % variability is the average of the mixing variability for several experiments.

The sieve size analysis of the Miwon and Ajinomoto MSGs are reported in Tables 2 and 3, respectively. The size distributionas were quite similar to what we have observed before with these types of MSG. No unsual characteristics were observed.

The percent vitamin A on each sieve size for each T, M, and B for both MSGs are reported in Table 4. The percent vitamin A in reference MSG refers to data reported earlier in the Indonesia report. This was the trend we expected for the T, M, and B fractions given that the average vitamin A content was similar for each fraction within an MSG brand. For Miwon MSG, the middle fraction appeared to have a size distribution of white vitamin A similar to the parent material. The top and bottom seemed to have more smaller particles than the parent material but acceptable values for the larger agglomerates. There appeared to be a possible breakdown of the 45 mesh size agglomerates. This may not be a correct conclusion because we do not know how the samples were taken and we only had one sample to run the analysis on. If the Indonesian MSG manufacturers had sent several samples from each T, M and B layer, we could make a more reasonable interpretation. A similar trend was observed for the T layer of Ajinomoto MSG. Again there appears to be a breakdown of the intermediate size agglomerates

to smaller sizes. The amount of largest agglomerates appears normal. This also may be the result of how the sample was taken initially. Because there was no gross segregation observed in the average vitamin A content reported in Table 1, we are not too woried about this apparent discrepancy. However, it deserves more study with additional samples of fortified MSG to make a more reasonable conclusion.

In summary, it appears that the fortification of MSG by Miwon and by Ajinomoto gave vitamin A concentrations in the acceptable range. There appeared to be no gross segregation of the agglomerated white vitamin A. The size distribution of the MSG was normal. There were concerns regarding the apparent breakdown of intermediate size agglomerates of vitamin A in the different fractions of MSG but more samples should be analyszed before we reach a final conclusion.

TABLE 1

MSG MIXING VARIABILTY
FOR MIWON AND AJINOMOTO VITAMIN A FORTIFICATION
IN INDONESIA

MIWON

VITAMIN A CONTENT (IU/g)		
TOP	MIDDLE	BOTTOM
3404±420	3331±431	3169±507
12%	13%	16%

n=9 for each fraction

AJINOMOTO

VITAMIN A CONTENT (IU/g)		
TOP	MIDDLE	BOTTOM
2379±320	2124±538	2504±300
13%	25%	12%
n=9	n=9	n=6

TABLE 2

SIZE DISTRIBUTION OF MIWON AND AJINOMOTO MSG

AJINOMOTO

SIEVE	% DISTRIBUTION (BY WEIGHT)		
	BOTTOM	MIDDLE	TOP
20	0.1	0.1	0.0
25	1.3	0.8	1.2
30	6.3	6.3	5.6
35	26.1	23.2	24.0
45	42.8	36.0	34.9
60	13.0	22.4	21.1
80	8.2	8.7	9.8
>80	2.2	2.5	3.5

TABLE 3

MIWON

SIEVE	% DISTRIBUTION (BY WEIGHT)			
	UNDIVIDED	TOP	MIDDLE	BOTTOM
25	1.3	0.1	0.3	0.2
30	1.6	1.4	2.7	1.9
35	13.3	16.2	19.8	19.3
45	44.0	42.4	40.7	46.2
60	29.1	28.2	26.1	23.1
80	6.3	7.4	6.8	6.5
>80	4.3	4.4	3.6	2.9

TABLE 4

VITAMIN A CONTENT IN FORTIFIED MSG BY SIEVE ANALYSIS

	% VITAMIN A (by IU)						
Sieve	Reference	Miwon			Ajinomoto		
		Top	Mid	Bottom	Top	Mid	Bottom
20	-	-	-	-	0	4	5
25	11	2	2	4	27	7	12
30	9	24	17	34	31	15	18
35	15	22	19	23	5	18	18
45	27	8	18	6	8	22	27
60	28	12	22	18	13	26	14
80	8	28	17	13	13	7	6
>80	1	6	4	1	3	1	1

1. Reference is white vitamin A (L880224-A)

SIZE DISTRIBUTION OF MIWON AND AJINOMOTO MSG

AJINOMOTO

SIEVE	% DISTRIBUTION (BY WEIGHT)		
	BOTTOM	MIDDLE	TOP
20	0.1	0.1	0.0
25	1.3	0.8	1.2
30	6.3	6.3	5.6
35	26.1	23.2	24.0
45	42.8	36.0	34.9
60	13.0	22.4	21.1
80	8.2	8.7	9.8
>80	2.2	2.5	3.5

MIWON

SIEVE	% DISTRIBUTION (BY WEIGHT)			
	UNDIVIDED	TOP	MIDDLE	BOTTOM
25	1.3	0.1	0.3	0.2
30	1.6	1.4	2.7	1.9
35	13.3	16.2	19.8	19.3
45	44.0	42.4	40.7	46.2
60	29.1	28.2	26.1	23.1
80	6.3	7.4	6.8	6.5
>80	4.3	4.4	3.6	2.9

April 3, 1989

P. Rod Crowley
USDA/OICD
TA-Nutrition Group
Washington, D.C.

Dear Rod,

Enclosed are the results so far for the size and vitamin A distribution of the white vitamin A in Miwon and Ajinomoto MSG. What is not included is the vitamin A content of the MSG on each sieve. We are having big troubles with our HPLC but we should have the last numbers ready by the middle of the week.

The MSG samples arrived from Miwon and Ajinomoto. Miwon sent top, middle and bottom fractions as well as an unfortified sample of MSG. Ajinomoto sent just top, middle and bottom fractions of fortified MSG. Each sample was mixed by passing through the riffle box 12 times. To subdivide the fractions, the riffle box-mixed MSG was dumped on graph-coordinate paper and divided in quarters with a plastic ruler (as Al has done previously). One quarter was used for sieve size analysis and the vitamin A content on each sieve will be measured. The other three quarters were subdivided again into quarters to yield 12 subsamples. One of these subsamples was sent to Harlan Hall for him to examine. Nine of the subsamples were assayed for vitamin A to give a measure of the average amount of vitamin A in each top, middle and bottom fraction. The last two fractions were saved in case we need an additional analysis.

Based on what we can see now, it looks to me that we do not see size segregation in the samples of top, middle and bottom sent from either of the companies. The vitamin A content for the Ajinomoto samples was lower than we expected. The % coefficient of variation (standard deviation/ average x 100) are slightly larger than we saw in our smaller experiments in Ames, but they look like managable values.

I will fax the % distribution of vitamin A to you as soon as it gets done.

Sincerely,
Pat
Pat

cc. Harlan Hall - V sent the samples UPS to you this morning.

April 7, 1989

P. Rod Crowley
USDA/OICD
TA-Nutrition Group
Washington, D.C.

Dear Rod,

Attached are the sieve analysis of vitamin A content in the Ajinomoto and Miwon MSG. The values are expressed as a % of the total vitamin A content in the fraction (top, middle or bottom). The reference white vitamin A is the material prepared early in 1988 by Harlan for testing and preliminary information for Indonesia. I have included the size distribution of the white vitamin A prepared late in 1988 for Indonesia that we ran sieve analysis on. You already have these data but I include them again for comparison. I am not sure which of these later batches of white A went to Indonesia or whether Harlan remixed these lots. These data are % by weight vitamin A on a sieve without MSG present.

My interpretation of these size/% vitamin distributions is that some of the agglomerated particles may have broken in the 45 mesh range accounting for the low %s on that sieve. The top fraction of Miwon has more small particles. The top fraction of Ajinomoto has more large agglomerates. Both middle fractions and the Ajinomoto bottom fraction look like what I expected from our earlier data. The bottom Miwon fraction appears to show some size segregation too. Since the average vitamin A content in each fraction were not significantly different, I do not think this minor segregation is a problem at this point. I would like to think about it some more, though. These data are from only one sieve analysis of each top, middle and bottom fraction.

Sincerely,

Pat

Pat

cc. Harlan Hall

Trip Report

Investigation of Vitamin A-Fortified Monosodium Glutamate Discoloration in Indonesia
August 29 - September 2, 1989

Dr. Patricia A. Murphy
Iowa State University

Harlan S. Hall
The Coating Place, Inc.

Dr. Benjamin Borenstein
B & B Consultants

Introduction

In mid-July, 1989, it was discovered that MSG fortified with white vitamin A (MSG-F) in Indonesia begins to become clumpy and discolored approximately 2+ months after blending/packaging. In order to evaluate and solve this problem, a technical team assembled in Indonesia the week of August 28 to observe the situation first-hand and gain an in-depth understanding of the nature of the problem.

The main objectives of the team were: 1) to determine ways to make future batches of white vitamin A (WVA) whiter, 2) to assess the hydroscopic tendencies of MSG-F which result in yellowing, clumping and loss of potency as they relate to local climatic exposure and shelf-life, 3) to design alternative production and material parameters which might make MSG-F less hydroscopic, and 4) to devise a research plan and budget for testing the viability of resultant new prototypes of MSG-F. A more detailed description of visit objectives appears in Appendix 1, "Scope of Work".

Team members included Dr. Benjamin Borenstein, formerly of Hoffman LaRoche and now an independent food technology consultant; Dr. Patricia Murphy, long-time project technical consultant with Iowa State University; and Mr. Harlan Hall, President of the Coating Place (the company that coats the vitamin A white).

Historical Perspective

Development of the current form of WVA has been an incremental process. The Coating Place, Inc. (CPI) first became involved in 1983 when approached to develop an improved binder to adhere vitamin A(VA) beads (Roche) and MSG crystals to each other. Previous work using petroleum jelly and other agents had been less than satisfactory due to melting of the binder, resulting in poor flow of fortified product. (Solon et al., Food Tech 39(11) 71, 1985; R. Tilden to R. Crowley, 4/19/83). CPI developed a binder system based on hydroxypropylcellulose (HPC) which could be applied with alcohol and dried. Upon removal of the alcohol, HPC forms a dry film which dissolves completely in water, leaving no residue. A secondary objective to mask the bright yellow color of VA was achieved by adding white pigment (TiO_2) to the HPC/alcohol binder system (Figure 1). The resulting product was then dusted with powdered MSG to further mask the color and prevent caking of the product during tray drying.

In 1984, Harlan Hall and Rod Crowley traveled to Indonesia to demonstrate preparation of this product. Under the guidance of Dr. Muhilal of the National Center for Nutrition Research, the formulation was modified and TiO_2 eliminated (Muhilal, Am. J. Clin. Nutri. 48:1271, 1988). This product was used for phase I, the Bogor field trial of fortified MSG in 1985. The product was deemed to be a technical success (Muhilal, 1988) with no solubility problems noted. The only reported concern was a desire for improved whiteness of the MSG.

In 1987, a sample of WVA was prepared using fluid bed coating technology. This sample was whiter than previous samples and was judged acceptable in color by the MSG manufacturers. Tests of product at Iowa State University (ISU) revealed serious segregation of the WVA beads from the MSG crystals. Additional work by CPI and ISU and agreement by manufacturers to use a single size MSG crystal for the small packets (5, 10, 25 Rp) established

size specification for WVA to prevent segregation (Murphy et al., 1987). (Samples 871020-A and 880224-A)[1].

In September, 1988, Ajinomoto reported that under the combined conditions of 50°C, 75% RH and direct sunlight, samples of WVA were turning light tan (probably samples 8904XX which were sent in large quantities). These conditions are extreme (HSH to PRC, September 8, 1988) because even very humid air has a low RH when heated up and the highest temperatures will be encountered in the dark (a closed van or storage building) conditions (Table I). ISU reported (RC to JP, September 27, 1988) that exposure to temperatures as high as 65°C at low humidities resulted in little or no color development. On September 30, 1988 (JP to SE), it was reported that Ajinomoto was satisfied.

Initiation of fortification (Phase II) began with scale-up of the agglomeration/coating of WVA by CPI in November, 1988. Some difficulty in sealing up the process from research to production scale was anticipated, however, achieving particle size specifications proved more difficult than anticipated. During the processing of this first commercial-scale quantity, the process was adjusted until the product conformed to product size limits determined above.

The resulting product (8811XX) was not as white as the small scale samples produced earlier (880224-A) but was deemed acceptable by CPI and later by USDA. Due to delays in shipping and utilization, the WVA (8811XX) was first opened in Indonesia in February, 1989 (JP to SE, February 10, 1989) and it was noted that the color was not white but off-white. This was perceived in the United States as being the off-white color noted by CPI above.

In August, 1989, a sample of discolored WVA (8811XX) was carried by Jim Olson from Indonesia to ISU as an example of discolored material. This sample was much darker than retained samples held by CPI, indicating darkening occurring in the months of shipping and storage. This has not been previously observed.

At the same time, samples of discolored MSG-F were received. Microscopic examination of these revealed that the beads were not discolored; they were gone! Only a stain remains.

ISU did a series of tests (WVA alone and with MSG) at extreme T and RH which resulted in a damp appearance within 48 hrs along with discoloration of the product. <u>All</u> samples, including uncoated 250 CWS and earlier "good" batches of WVA absorbed water and began to discolor. The production materials (8811XX and 8904XX) showed the effect more rapidly (<24 hr) indicating some difference from earlier samples, probably related to scale-up conditions. At this time, Hoffman-LaRoche (Nutley) (HLR-N) has not indicated anything different about the starting 250 CWS. MSG alone picks up water (Figure 2; Sasa) and discolors, but at a slower rate.

ISU repeated the tests using packaged MSG-F from Ajinomoto and Miwon with similar results. The packaging materials are moderately good water vapor barriers, but the duration in the marketplace (Table 1) and surface area to mass (SA/M) of MSG ratio indicated that marked improvement in packaging is not likely. The significance of film permeability was confirmed by field observations during our visit where the smallest packets (greater SA/M) show caking and discoloration first (Table 2). For example, Ajinomoto packaged

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[1]CPI numbering code = AABBCC-XX, in which AA = year and BB = month. Thus, 8802 was processed in February, 1988.

March 8 showed the 25 Rp is still good and the 5 Rp is in stage 3. The stages of MSG-F deterioration are ranked on the Penberthy scale (JP to file, August 14, 1989) as follows:

Stage 1: Normal. WVA and MSG particles are completely separate and integral, in their original colors and free-flowing. WVA looks like popcorn under microscope.

Stage 2: MSG particles stick to WVA in small clumps. Some clumps may stick to upper interior of sachet above the bulk of MSG-F. Slight yellowing concentrated in clumps, with overall package appearing off-white at arms length. WVA looks like popcorn with MSG stuck to it under microscope.

Stage 3: Clumps much larger (most MSG involved in clumps), with more widespread discoloration, causing contents to appear slightly yellowish at arms length. MSG-F appears damp and does not flow normally. WVA looks like smashed popcorn with MSG stuck to it under microscope.

Stage 4: Entire contents involved in clumping and thoroughly discolored. MSG-F appears wet and caky and has poor pouring characteristics. No WVA agglomerates present, just stains, under microscope.

Activities

All the visiting consultants have extensive technology background in fortification with VA and/or VA market form development. Dr. Pat Murphy, Professor, ISU, has been associated with monitoring VA stability and in fortification feasibility in several international programs, including the Philippines, Bangladesh, Nepal and Malawi as well as the Indonesian project. Mr. Harlan Hall, President, CPI, an expert in fluid bed coating has been involved in this project since 1983 in developing and commercializing the process for color and product-size modification of the VA beadlets. Dr. Ben Borenstein, President, B & B Consultants, was Director of Product Development, Roche Chemical Division, Nutley, NJ, and has extensive experience and publications in vitamin stabilization, bioavailability and fortification.

During the six-day visit (August 28 through September 2), the team was briefed and dialogued continuously with Mr. John Penberthy, HRI project manager, met Dr. Karyadi, Director, Research and Development, NIHRD, interacted with Pak Benny, Head, Directorate Community Nutrition, and Dr. Muhilal, NRDC. Extensive meetings were held with Ajinomoto management, technical and production experts in Jakarta and Surabaya, and similarly with Sasa. The plant, packaging and warehouse visits were valuable. The open and detailed discussions were excellent. The collaboration of the manufacturers and Mr. Penberthy were essential to the success of the mission. Individuals visited are listed in Appendix 1; the itinerary is Appendix 2.

Current Status

The fortification project has been suspended pending resolution of the darkening problems. The darkening of WVA derives from two different sources: A) darkening of WVA between time of manufacture and use in blending and B) darkening of blended MSG-F.

A. Darkening before blending: There is significant variability in the rate of darkening between individual drums of WVA. Some of the material (Ajinomoto, August 31, 1989) looks quite good after 10 months of storage in original containers at ≤29°C. Other containers were noticeably yellow at 4 months (JP to SE, February 10, 1989) and at least certain samples have continued to darken under variable conditions.

Factors thought to contribute to this early darkening reaction relate to probable damage to the beads during processing (Figure 1).

1. In the large-scale processing, the modest amount of water used in the process seems to have a greater effect on the beads than was noted in the small-scale trials.

2. Some beads appear to be physically damaged (broken). This may occur during sizing resulting in exposure to air and degradation of VA.

3. Factors 1 and 2 can be better controlled in the manufacturing process. This will be addressed in the work planned for September through December, 1989.

4. All drums sampled have not been resealed since date of sampling allowing moisture uptake before indicated date of blending. This probably has more effect on later darkening.

5. Various delays have resulted in WVA still being unused after 10 months, much longer than the projected 3 months. This has resulted in further degradation of the product. Although WVA has been kept below 30°C during this time, the storage area is not dehumidified. No color change has been observed in samples stored ≤22°C and dew points ≤14°C.

B. Darkening of blended MSG-F

1. After blending with MSG, the resulting MSG-F is packaged in 5, 10 and 25 Rp packs. From hereon, MSG-F is handled like unfortified MSG. Storage at the factory is variable. Ajinomoto has records indicating a maximum storage temperature of 29°C.

2. Test by ISU, field observations and data from the manufacturers confirm that moisture vapor will permeate the sealed packages. This is expected to be dependent upon time and conditions of storage, i.e., humidity. Pure MSG will cake and discolor under severe conditions, but the WVA dissolves in free water. This releases the VA which is unstable and quickly degrades.

3. This problem was not observed in Phase I, the Bogor trials. This may be due to a shorter time of exposure after packaging, i.e., higher turnover on Java. Slightly lower humidity at the higher elevation may have further extended shelf-life.

Product Goals

MSG-F should have a similar shelf-life profile to the current unfortified commercial Indonesian MSG, with respect to color and flow properties. This means that the product must survive, at least, six months in the warung (outside stall). MSG-F should not segregate physically and packaging rates should be similar to current practice. Attainment of these goals requires that the vitamin A be white in color, have a majority of the particle sizes in the range from .25 to .71 mm, be sufficiently nonhydroscopic to prevent discoloration, clumping and loss of product integrity. Attainment of these goals will also optimize the chemical stability of VA. Specifications must be developed for WVA which insure achievement of these goals. Color will be measured by Hunter instrumentation on experimental batches in September/October, 1989, and tentative specifications established when performance data are available. Accelerated tests will be developed to predict rate of field discoloration and clumping.

Tentative Specifications for WVA

Size Distribution - same as earlier agglomerated WVA.

Color - to be established.

A. Hunter Color Difference Meter
B. Color Chips Meeting Color Test

Potency - ≥165,000 IU/g at CPI manufacture
≥120,000 IU/g at 6-month storage at 45°C, 40% RH in dark

These specifications may be modified depending on data obtained in testing prototypes.

Recommendations

1. Current WVA is not water/humidity resistant; an improved product is needed. Although 250-CWS is hydroscopic, it is the only VA fortificant that conforms with religious dietary laws.

2. CPI will modify process to minimize darkening resulting from processing.

3. CPI will develop prototype samples for water resistance and submit samples to ISU for evaluation.

4. Samples will be tested as follows:

   a. whiteness,
   b. high stress test, and
   c. hot water dissolution of coated beads.

5. Those prototypes passing above tests will be placed by ISU in long-term test for functional and cosmetic stability. Simultaneously, CPI will scale-up to commercial-size operation with up to 4 of the same samples to determine any problems in large scale processing. Samples will be sent to ISU for testing.

6. Samples that appear promising will be sent to Indonesia via HKI for evaluation in field conditions.

7. A member of this technical evaluation team should meet with the manufacturers in Indonesia to discuss and view results of their evaluation. Completion of the circle of communication is vital for final success. Too much useful information is lost via third-party messages.

8. Re-establishment of fortification may be reconsidered in high turnover areas based on 6-month stability data. Other areas in Indonesia should wait for longer term data.

Testing Parameters for New White Vitamin A Prototypes

The protocol for testing new white vitamin A prototypes will consist of two phases. Initially, each new prototype will be subjected to a rapid stress test. This will involve subjecting the prototype, alone and with MSG, to high relative humidity (75% and 90%) and high temperature (55°C) for several hours to several days. Visual inspection of the material will be used to evaluate stability using the stage scale of Penberthy (August 14 memo) in comparison with 88/89 white vitamin A materials. Resistance to moisture uptake (longer than 88 white vitamin A) will be considered evidence of increased stability. These prototypes will be used in the second phase.

Survivors of Phase I will be subjected to more critical evaluation with respect to vitamin A stability, size of agglomerates, mixing and size segregation stability, warm water (85°C) solubility and color as well as resistance to moisture uptake. The first six parameters will be evaluated by established procedures. Resistance to moisture uptake testing will be designed to try to replicate conditions that MSG-F will experience in Indonesia. Efforts to predict stability to water uptake will be accomplished by measuring rates of H_2O uptake at elevated temperatures and humidities

(controlling for MSG moisture uptake). These data will give us an estimate of stability of the new white vitamin A's. To obtain a realistic estimate of stability, samples of MSG and new prototypes will be packaged in 5 Rp sachets (at ISU and in Indonesia) and subjected to cycling changes in temperature and humidity (constant absolute humidity) for 6 months. (If A_w chambers cannot be set to cycles, stability will be evaluated at 35°C, 75% RH and at 50°C, 42% RH. Several high temperatures at 75% RH will also be employed. Successful prototypes will show no color change nor observable moisture uptake as well as reasonable vitamin A levels.

Transfer of Coating Technology

Assuming eventual project success and expansion, it is desirable to coat the vitamin A white in Indonesia. As no fluid bed technology now exists in Indonesia for such activities, transfer of such technology will eventually be warranted. According to Dr. Muhilal, it is the intention of the Indonesian Department of Health that a local pharmaceutical company incorporate coating operations into their activities. This is a theoretical plan only, no specific firms have yet been identified or solicited.

Mr. Hall estimates that required capital equipment costs will range from $250,000 to $500,000. In addition, there will be plant, training and other start-up costs. It is assumed that this would be a privately funded operation based upon normal proforma financial prospects. Thus, before Indonesian coating activities can begin 1) commercial and technical feasibility of MSG fortification must be clearly established and 2) the project will need to be scaled up to a high semi-national level so as to maximize economies of scale for the coating operation. Given the current outlook, this appears to be at least three years in the future. Mr. Hall has generously offered to share technological assistance and know-how when the time comes.

Appendix I
Individuals Contacted
September 1, 1989

HKI/Jakarta
John Penberthy, Fortification Project Manager

Nutrition Research and Development Center, Bogor
Prof. Darwin Karyadi, Director
Dr. Muhilal, Chairman, Main Nutritional Problems

Department of Health, Government of Indonesia, Jakarta
Dr. Benny A. Kodyat, MPA, Head of Directorate of Community Nutrition

P. T. Ajinomoto, Indonesia
Isao Miyaaki, President Director, Jakarta
T. Motooka, Director of Marketing, Jakarta
Isao Takemura, Vice President Director, Mojokerto
M. Huiata, Assistant Plant Manager, Mojokerto

P. T. Sasa
Y. Kobayashi, Executive Vice President of Marketing, Jakarta
Margono, Director and President, Jakarta
I. Liang, Product and Development, Jakarta
Hartono, Plant Manager, Surakaya

Appendix II

Itinerary for Technical Team Evaluation of MSG Fortification

August 27 - September 2, 1989

Team Members

Dr. Patricia Murphy, Iowa State University
Mr. Harlan Hall, The Coating Place
Dr. Ben Borenstein, Hoffman LaRoche

Sunday, August 27

Arrival in Jakarta. Pick up at airport. Reservations at Hotel Kemang.

Monday, August 28

8:30 a.m. All-day briefing at HKI offices.

Tuesday, August 29

9:00 a.m. Meeting with Prof. D. Karyadi and Dr. Muhilal in Bogor.
2:00 p.m. Meeting with Mr. Motooka, Marketing Director for Ajinomoto.
7:00 p.m. Dinner at Penberthy's.

Wednesday, August 30

9:00 a.m. Meeting with Pak Margono, President of Sasa.
3:30 p.m. Flight to Surabaya. Reservations at Hotel Elmi.

Thursday, August 31

9:30 a.m. Visit Ajinomoto factory.
1:00 p.m. Visit Sasa factory.
4:00 p.m. Return flight to Jakarta.

Friday, September 1

9:00 a.m. Meeting with Prof. D. Karyadi and Dr. Muhilal in Bogor.
7:00 p.m. Dinner at Penberthy's.

Saturday, September 2

9:00 a.m. Meeting with Pak Benny, MPA, Head of Directorate of Community Nutrition.
Afternoon Wrap-up, reporting and evaluation.
Team departs Jakarta.

Table 1

Preliminary Estimates - (Off) Not on Java

Time & Temperature Exposure of MSG-F
at Various Stages of Marketing Distribution

Manufacturer _____

Destination Kabupaten _____

	1. Factory Storage bulk & boxed	2. Truck Shipment	3. Warehouse Storage	4. Ship Shipment	5. Agent Storage	6. Local Truck Delivery	7. Warung Display	Total Total
Time (Days)								
Average	20	2	30	7	30	2	45	4.5 mo
Extreme (longest)	30	3	90	10	60	3	180	± 1 yr
Temperature								
Average (24 hr.) (Range)	29 (24-34)	30 (24-37)	29 (24-34)	29? (24-34)	29 (24-34)	40 (24-55)	29 (24-34)	
Extreme (highest)	34	40	50	?	50	60	34	

Table 2

Effect of Temperature on Relative Humidity
at Constant Absolute Humidity

Absolute Humidity (Grains)	Dew Point (°F)	Temperature °F	Temperature °F	RH At Temp.
236 grains	75°	100°	100°	80%
236 grains	75°	122°	50°	42%
430 grains	110°	122°	50°	85%

Table 3

Surface to Mass Ratio for MSG Packets

Sachet Rp	Surface (mm^2)	MSG Mass (g)	Surface/Mass (mm^2/g)	Moisture* Uptake (mg/24 hr)
5	21 mm	0.6	35	0.105
10	31.5 mm	1.2	26	0.168
25	55 mm	5.5	10	0.275

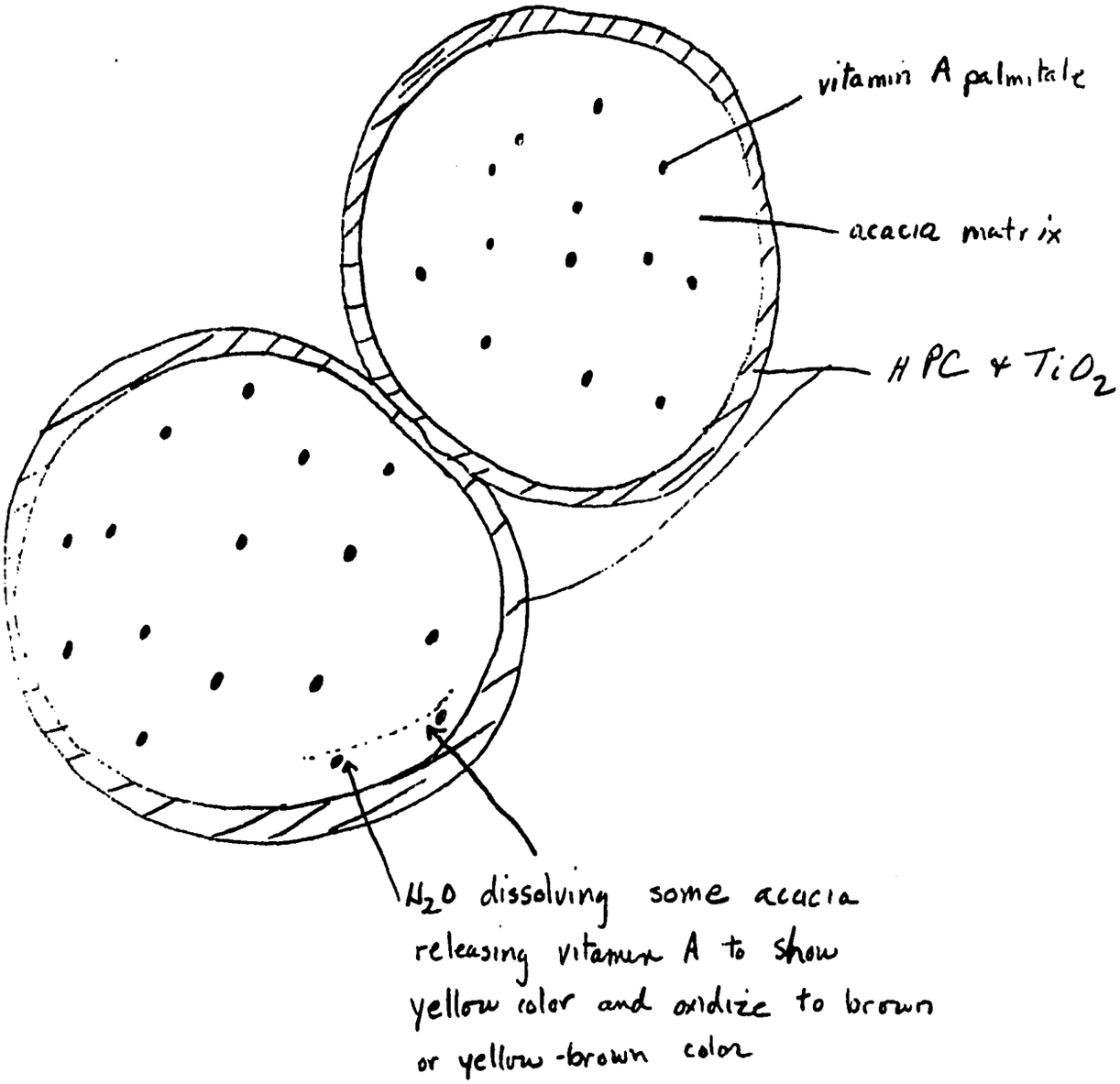
*40°C/90% RH

BUDGET (for 12 months)

Salaries (Technician) + Benefits	$20,000
Supplies	8,000
Travel (Murphy)	500
Communications	500
Computer Time	1,000
Contracts and Consultants[A]	3,000
Total Direct Cost	$33,000
Indirect Cost[B]	11,880
Total Cost	$44,880

[A]Contracts and Consultants includes consulting costs and travel expenses for Borenstein and Hall for, at least, two meetings with Murphy.

[B]ISU charges 36% indirect cost subject to negotiation between ISU Contracts and Grants Officer and HKI.



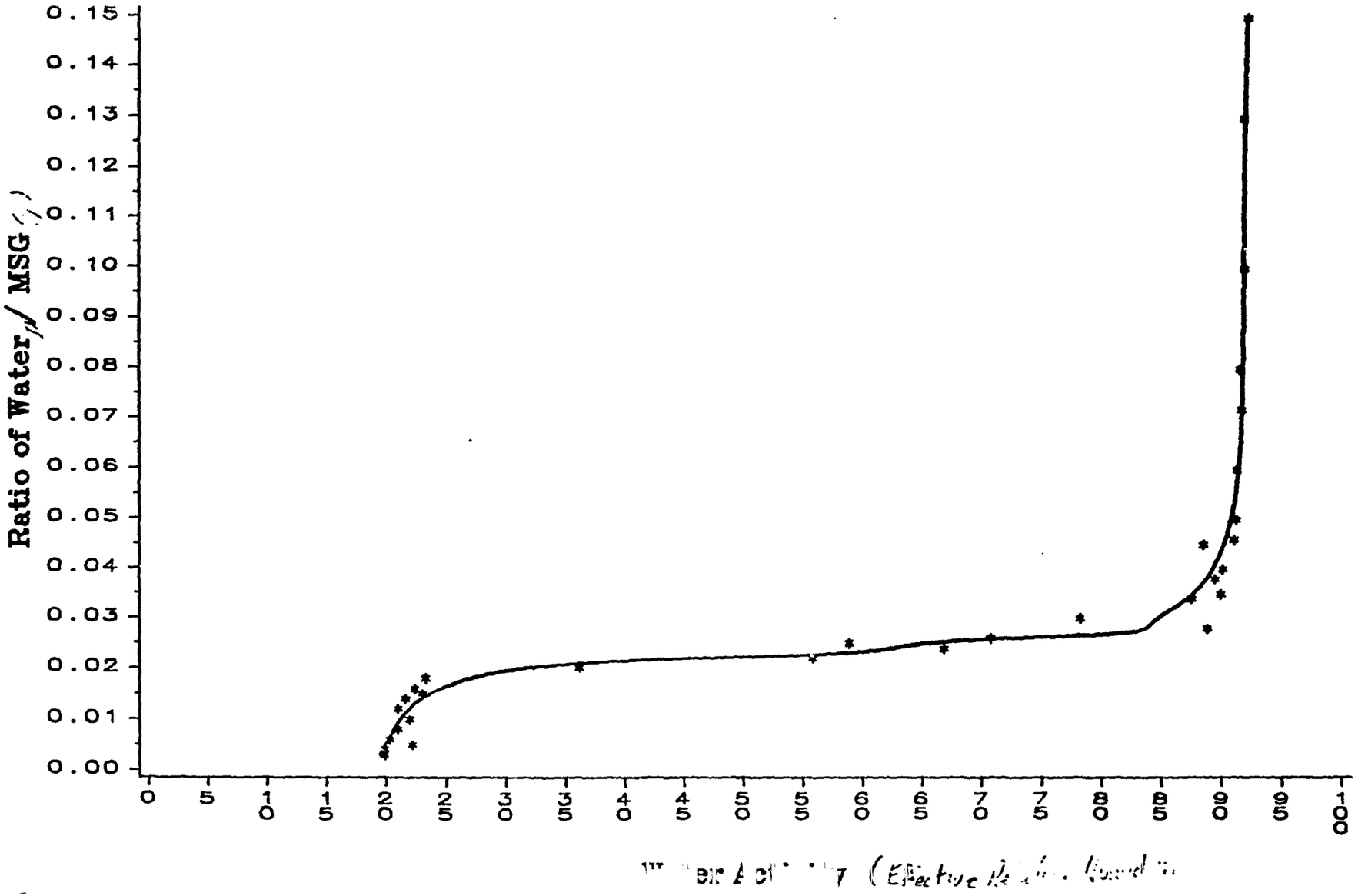
White Vitamin Agglomerate

figure 1

EFFECTS OF CLOFIBRIC ACID ON PORCINE HEPATOCYTES: A NEW MODEL FOR THE STUDY OF PEROXISOMAL METABOLISM.Kenneth W. Turteltaub and Patricia A. Murphy, Department of Food Technology, Iowa State University, Ames, IA 50011.

MSG ISOTHERM

Water / MSG vs. Water Activity



VITAMIN A FORTIFICATION FOR DEVELOPING COUNTRIES

REPORT OF ACTIVITIES JANUARY 1, 1988 TO JUNE 30, 1988

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Physical and chemical stability were evaluated for six different food items intended for fortification in developing countries. These foods were wheat, monosodium glutamate (MSG), rice, salt, synthetic wheat (Ultrawheat) and synthetic rice (Ultrarice). The report will be divided into presentation and discussion of the results obtained through study of these materials.

Wheat

Three batches of wheat premix were prepared by Wright Enrichment of Crowley, LA, in late 1987 and evaluated for their stability and resistance to washing. The three batches, identified as B13, B14, and B15, were all prepared in the same manner, according to Wright, except for three different food colors used to tint the premix. Stability of vitamin A in the wheats was determined by storing the premix at three different temperatures and at three different water activities (Aw) or relative humidities in accelerated stability studies. The stabilities of the vitamin A were determined by measuring the vitamin A content of the wheat over an eight to twelve week sampling period. The rate of vitamin loss was an apparent first order rate loss. Therefore, half-lives could be determined from rate constants derived from first order rate plots. The first order rate constants were derived using linear regression plots of ln (% vitamin A remaining) versus time. Half-life ($t_{1/2}$) was calculated using the relationship, $t_{1/2}=-k/0.693$.

The $t_{1/2}$ are presented in table 1. The estimated half-lives at 25°C are calculated by extrapolation from higher temperatures. In comparing the half-lives between batches, it can be seen that although the reported method of fortification was the same, something is affecting the stability of the vitamin A in these premixes. B14 was almost twice as stable at low Aw as the other two batches. At higher Aw, the differences become less as overall stability declines for all three batches. At Aw=0.90, the stability of the wheat premixes is about the same. The variation in the half-lives with Aw suggests a different mechanism for degradation of vitamin A depending on the preparation of premix. At this point, we can only say that the type of food color additive may be having an effect on the stability of the vitamin A in the wheat premix. For comparison, the stability data for B10 completed earlier are included in table 1.1. B10 gave vitamin A stabilities of almost one year. Only B14 has come close to replicating these earlier results.

A batch of premix (B16) was prepared by Wright Enrichment using vitamin A palmitate oil. The stability of this premix was evaluated and the results are presented in table 2. The results show the unacceptability of a premix made with oil with insufficient protection from oxidation. The $t_{1/2}$s are in hours rather than weeks as reported for the other wheat premixes.

Washing tests, designed by Fred Barrett (USDA), were performed to evaluate the physical stability of the vitamin A on the premixes B13, B14 and B15. The results are presented in Table 3. Significant losses of vitamin A will occur if a washing step occurs during the use of this premix or wheat fortified from it.

In an effort to explain some of the variability observed in the vitamin A level in the wheat premixes, an assay of individual wheat kernels was undertaken for B11, B14 and B15. The results are presented in Table 4. By examining the coefficient of variation (standard deviation/mean x 100%), it is apparent that the kernel size and amount of fortificant per kernel contributes to the variability of the vitamin A content in these premixes.

The stability of the fortified wheat to maintain an adequate level of vitamin through the chapatti making process was evaluated using wheat premixes B11 and B14. The data have already been reported to the Bangladeshi government. Wheat was fortified with premix to yield a 10 IU/g level. Grinding conditions were set up to give a meal particle size similar to the conditions in Bangladesh. Chapattis were made and evaluated for vitamin A content in flour,

raw chapatti and cooked chapatti. The effect of storage time of the flour and dry chapatti on vitamin A content was evaluated.

In the first series of experiments, fortified wheat was prepared in lab and ground to simulate grinding conditions in Bangladesh. Chapattis were made according to directions given by Indian national in Ames. The results of this trial are presented in Table 5. The unground wheat was highly variable in vitamin A content, partially due to the variability observed in the kernel fortification level already presented in Table 4. Table 6 presents additional data to show the variability if fortificant level in the unground wheat samples. Grinding the kernels markedly reduced the sample variation. The flours were at the target level of fortification of 10 IU/g. The chapatti produced from flour resulted in vitamin A contents that were not significantly different from the flour levels.

In a second trial run, the milling conditions were adjusted to produce a 120°C exit temperature for the meal to more closely simulate the conditions in Bangladesh. The results of this trial are presented in Table 7. A loss of about 14% from whole wheat to cooked chapatti. There was a significant loss between flour and cooked chapatti. In order to examine the possibility that increased heat would contribute to the loss, wheat premix and vitamin A (250SD) were heated in a convection oven and sampled at intervals to determine if any loss occurred. The results are presented in Tables 8 and 9. When linear regression of vitamin A content was plotted versus time for both data sets, the slopes were not significantly different from zero. No significant loss in vitamin A content was observed in either trial. The loss of vitamin A during the grinding process may be physical loss rather than chemical degradation.

To determine if time after grinding and time after cooking chapattis would have an adverse effect on vitamin A content, experiments were performed to evaluate vitamin A loss in the light and dark over several days. These data are presented in Tables 10 and 11. These trials were done using Batch 14 wheat premix. There was little loss of vitamin A in ground wheat stored in the light or dark. When chapattis were stored after production, there was a significant loss of vitamin A over time. Since the chapattis are probably consumed as soon as they are prepared, this observed loss is probably not a real problem.

Chapattis were made from flours that were ground and then stored for several days prior to chapatti making. These data are presented in Table 12. There does not appear to be a significant loss of vitamin A in the chapattis made from stored flour.

Finally, a collaborative study was conducted on vitamin A content of materials made in Bangladesh and assayed by ISU and Institute of Nutrition and Food Science (INFS), University of Dhaka. The samples were made in Bangladesh and samples for ISU were airshipped to the US arriving in 6 days. Samples remaining on Bangladesh were held for 7 days. All samples were assayed on the same day. The results of the interlaboratory comparison are presented in Table 13. There were no significant differences between the labs nor were there significant differences between the products at the 5% level.

A water adsorption isotherm was determined for the wheat used in chapatti making trials. The isotherm is presented in figure 1. These data can be used to compare the Aw to % moisture.

MSG

Fortification of MSG with white agglomerated vitamin A has been recommended to the Indonesian government. Efforts these past two quarters have concentrated on assaying new batches of agglomerated white vitamin A (table 14), measurement of the size distribution of MSG from the three manufacturers for the crystal size proposed for fortification (table 15), size distribution of agglomerated white vitamin A alone and mixed with MSG (table 16), and weight/size

distribution of agglomerated white vitamin A (figure 2). The size distribution of the new white vitamin A and Indonesian MSG indicate that the sizes match to allow direct fortification except for the Rp 25 Sasa brand. This size/manufacturer may need reworking or a different and smaller size MSG could be used such as Rp 10.

RICE

Wright Enrichment prepared three forms of vitamin A fortified rice for potential use in the Philippines. These were identified as long grain/vitamin A with iron (LGA Fe), long grain/vitamin A/SD 250 (LGA SD), and long grain/vitamin A oil (LGO). All were prepared to yield 2 million IU/lb (4,405 IU/g). The resultant products gave vitamin A contents of LGA Fe=1924±125, LGA SD=2678±176, and LGO=3411±95. Washing tests were performed on the LGA SD and the LGA Fe to simulate the precooking washing that are of concern to the fortification collaborators in the Philippines. The results indicated that the rice fortified without iron lost 58% of its vitamin A upon washing while the rice fortified with iron lost no vitamin A (table 17). Stability trials are currently underway. The accumulated results are presented in table 18. The rice fortified with 250 SD (LGA SD and LGA Fe) are significantly more stable than the oil fortified rice. The vitamin A in rice without iron appears to be slightly more stable than in the rice with iron. Although Wright was able to achieve higher vitamin A fortification with vitamin A oil, the oil product was about 3 times less stable than the rice fortified with 250 SD.

SALT

The Coating Place prepared a new formulation of vitamin A (lot # 861029-A1) in an effort to make it more stable to the oxidizing conditions found in NaCl. The parent compound was 500 Palmabead. The new formulation was evaluated for stability alone, with NaCl and with a mixture of salts to simulate dried sea water or 'sea salt' (NaCl-98%, KCl-1%, $MgCl_2$-0.5%, $CaCl_2$-0.5%). Stability studies for vitamin A in salts were run at 0, 0.1 and 3.5% moisture to simulate dry, caked and saturated salt, respectively.

The results of storage of the vitamin A preparation alone at two temperatures and two Aw are presented in table 19. The vitamin preparation alone is so stable that the error in the half-life of the vitamin A is as large or larger than the estimated half-life. This means the vitamin A is stable in these conditions and no difference between the parent and protected vitamin A preparations could be detected.

The stability of vitamin A in NaCl was measured at one temperature and three moisture conditions. The half-lives calculated for the parent and protected vitamin A are presented in table 20. The stability of vitamin A at no and low moisture was such that no difference in the stability of vitamin A could be detected between the two preparations. At saturating conditions, the stability of the two preparations was significantly lower than at lower moisture levels. The error in the half-life estimates at 3.5% moisture was large so that it is not possible to predict if the parent or protected vitamin A are more stable. The estimated half-lives at high moisture are approximately the same as half-lives determined for an earlier protected vitamin A preparation.

Similar results were obtained for prediction of stability in 'sea salt'. The results of the stability study are presented in table 21. The protected vitamin A (called MIVA in the tables) did not appear to provide increased protection for vitamin A compared to the parent compound at 25° due to the large error in the half-life estimates. It appears that further explorations must be done to find a formulation that will significantly increase the stability of vitamin A in salt.

ULTRA-RICE AND ULTRA-WHEAT

The Bon Dente Company prepared a simulated rice and wheat product that was fortified with vitamin A and sent to us for evaluation. The first batches (called Ultra-Rice I) were formulated with retinol palmitate oil. These products were evaluated for their vitamin A content, the stability to washing in water and to cooking. The Ultra-Rice I was subjected to a quick stability estimate.

The vitamin A content was measured in four packages of premix and one fortified mixture in the Ultra-Rice I and the results are presented in table 22. The Ultra-Rice I had much lower levels of vitamin A than the company had predicted. This was eventually traced to the fact that they used less vitamin A than they thought and because the vitamin A oil was so much less stable than other preparations that it was degrading very rapidly. To assay the Ultra-Rices for vitamin A, we determined that the product must first be milled, if dry, or homogenized, if wet, to adequately extract the vitamin A from the particles. When the Ultra-Rice I was washed with water, no losses in vitamin A could be detected.

The Ultra-Rice I was subjected to a stability study at one temperature to rapidly get an idea of its half-life. We suspected that the oil fortification would give a pretty unstable product. The results of the stability measurement are given in table 23. Note that the half-life are calculated in days instead of the usual weeks. This form of rice premix would be unsuitable for fortification programs because it is not stable for much more than a week or two. The resistance to washing, however, still makes it an attractive idea.

The effect of cooking the premix for 5 minutes on vitamin A content are reported in table 24. The oil formulation is also quite unstable to cooking. The dry premix vitamin A content also indicated how much of the vitamin has degraded over a few weeks at refrigerator temperatures in our lab.

A stabilized vitamin A preparation, 250 SD, was recommended to the Ultra-Rice manufacturers and a second set of Ultra-Rice was prepared. The vitamin A content of this preparation was determined and the results are presented in table 22 under the heading of Ultra-Rice II. This run gave much better vitamin A levels. We believe this formulation will be much more stable to storage and cooking. Those experiments are now underway.

A simulated wheat product has also be prepared called Ultra-Wheat. This product was made just after Ultra-Rice I was made. The vitamin A form used was oil. The fortification levels are presented in table 25. We have recommended that the company try again with 250 SD to obtain a more stable product.

Table 1
Stability of Vitamin A in Wheat Premixes

Aw	T*	$t_{1/2}$ (wk) B13	B14	B15
0.11	35	16.6±5.4	42.2±5.3	28.1
	45	12.6±2.9	15.0±0.8	16.8
	55	8.0±0.8	7.3±0.3	7.1
	65			2.6
0.75	35	29.1±5.5	22.6±2.2	12.7
	45	6.5±0.7	6.5±0.2	7.2
	55	2.1±0.1	1.9±0.0	1.8
	65			0.5
0.90	35	11.5±1.3	9.8±0.8	20.1
	45	6.2±0.3	5.4±0.1	4.9
	55	1.6±0.0	1.5±0.0	1.4
	65			0.3
0.11	25**	21.0	56.4	35.3
0.75		39.6	31.1	13.7
0.90		16.3	13.8	22.4

-40

TABLE 1.1

SHELF-LIFE OF FORTIFIED WHEAT- Batch 10

TEMPERATURE(C)	Aw	HALF-LIFE(WEEKS)
35	.11	35.9+12.6
	.75	36.1+12.4
	.90	19.7+3.2
45	.11	12.7+1.0
	.75	7.4+0.4
	.90	6.9+0.2
55	.11	9.3+4.3
	.75	3.0+0.1
	.90	2.5+0.1
*25	.11	46.7
	.75	50.7
	.90	27.9

*= extrapolated values from higher temperatures

Table 2

WHEAT/VITAMIN A DEGRADATION BATCH 16--OIL BASED June 29, 1988

Temperature	Aw	Half-life (hours)
45	0.11	3.12±0.31
55		2.74±0.41
65		1.73±0.29
45	0.75	3.07±0.34
55		2.83±0.48
65		1.87±0.36
**25	0.11	4.62
	0.75	4.39

** extrapolated estimates

Table 3
PHYSICAL STABILITY OF WHEAT PREMIX TO WASHING

Premix	% Loss Vitamin A
B13	14.7±5.5
B14	30.8±5.5
B15	20.7±5.5

Table 4
VITAMIN A VARIABILITY ON WHEAT PREMIXES

Variable	N	Mean	Std. Dev.	C.V.
		B11		
Kernel Wt	22	30.1	8.0	26.5
IU/Kernel	22	197.7	71.4	38.1
IU/g	22	6794	2653	39.1
		B14		
Kernel Wt	24	31.1	6.5	21.1
IU/Kernel	24	170.7	106.2	62.2
IU/g	24	5396	3258	60.4
		B15		
Kernel Wt.	24	30.3	8.6	28.3
IU/Kernel	24	181.4	134.6	74.2
IU/g	24	5732	3271	57.1

TABLE 5

VITAMIN A CONTENT OF FORTIFIED WHEAT PRODUCTS TRIAL 1

PRODUCT	n	SAMPLE SIZE(g)	VITAMIN A(IU/g)
Wheat	3	100	8.15+2.26
Flour	6	12	10.49+1.49
Chappati	8	13	9.77+0.95
Target Level			10.50

TABLE 6

VARIABILITY OF VITAMIN A CONTENT WITH SAMPLE SIZE

PRODUCT	SAMPLE SIZE(g)	VITAMIN A(IU/g)
Wheat	10-15	5.80+4.00
Wheat	100	8.15+2.26

TABLE 7

VITAMIN A CONTENT OF FORTIFIED WHEAT PRODUCTS TRIAL 2

PRODUCT	n	VITAMIN A(IU/g)
Wheat	-	10.0
Meal	6	9.64±0.74
Chapatti	6	8.28±0.38

Table 8
Effect of Heat Treatment on Vitamin A Wheat Premix

Time of heating	Vitamin A (IU/g)
0 minutes	3439±331
5	2988±259
10	3288±295
15	2659±261

Temperature=110°C, n=3

Table 9
Effect of Heat Treatment on Vitamin A (250SD)

Time	Vitamin A (IU/g)
0 minutes	190,364±12,752
2	183,075±14,66[illegible]
4	177,327±12,948
6	181,738± 6,555
8	169,892±19,348
10	180,714±11,229

Temperature=110°C, n=3

Table 10
Effect of Storage on Vitamin A Content in Wheat Flour

Time (d)	Dark		Light	
	Vitamin A (IU/g)	%*	Vitamin A (IU/g)	%
0	9.64±0.74	100	8.07±0.09	100
1	9.17±0.60	95	8.77±0.30	109
2	8.88±0.54	92	8.37±1.15	104
3	8.89±0.16	92	7.95±1.67	99
4	8.86±0.26	92	8.35±0.26	104

* Zero time mean dark represents 96% target fortification level. Zero time mean light represents 81% target. n=6/d.

Table 11
Effect of Light and Dark Storage on Vitamin A in Cooked Chapattis

Time (d)	Dark		Light	
	Vitamin A (IU/g)	%	Vitamin A (IU/g)	%
0	8.28±0.38	100	8.42±0.26	100
1	8.47±0.65	102	7.72±0.41	92
2	8.03±0.56	97	6.91±0.44	82
3	7.71±0.23	93	6.15±0.26	73
4	7.29±0.52	88	6.16±0.22	73
7	6.60±0.29	80	-	

Zero time mean represents 83% for dark storage and 84% for light storage of target level fortification. n=6/d.

Table 12
STORED FORTIFIED FLOUR

Time after grinding(days)	In Chappatti	
	Vitamin A (IU/g)	% Remaining
0	7.81±0.50	100
1	7.63±0.31	98
2	6.86±1.03	88
3	7.60±0.28	97
6	8.21±0.41	105

Zero-time mean represents 78% of targetted fortification. n=6 for each mean.

Table 13
Bangladesh Chapatti trial

SAMPLE	VITAMIN A (IU/G)	
	INFS	ISU
Chapatti	111.13±0.49 (2)	11.93±1.28 (5)
Dry, ground Chapatti	11.23±0.18 (2)	10.96±1.14 (5)
Wheat flour	12.02±0.05 (4)	11.12±1.33 (6)

(n) represents number of replications.

Table 14
VITAMIN A CONTENT OF WHITE AGGLOMERATED VITAMIN A

Lot #	Vitamin A (IU/g)
880223-A1	187,538± 6,260
880223-A2	183,805± 2,260
880224-A1	161,788±16,500
880223-A2	181,043±11,420
880224-A	186,299± 3,716

n=6 Prepared by the Coating Place, Inc.

Table 15
MSG SIZE DISTRIBUTION

MESH	% DISTRIBUTION					
	Ajinomoto			Miwon	Sasa	
	Rp5	Rp10	Rp25		Rp5/10	Rp25
+18	0.01	0.01	0	0	0	0.03
18/20	0.07	0.03	0.07	0.01	0	0.35
20/25	1.98	0.82	3.35	1.85	0.07	15.00
25/30	7.13	2.64	7.16	9.91	2.00	26.12
30/35	18.88	9.76	19.81	29.54	31.47	40.15
35/45	36.01	32.96	30.62	33.21	50.16	15.53
45/60	27.44	37.21	22.27	20.10	13.30	2.28
60/80	7.43	14.05	10.74	3.13	1.35	0.26
-80	1.04	2.52	5.97	2.25	1.65	0.29

Table 16

Vitamin A Distribution

Mesh	% Vitamin A		
	Indonesia Report	New Vitamin A (by wt)	MSG/Vitamin A (by vit A content)
+25	9.3	8.99±1.54	11.05±2.05
25/30	9.9	7.29±0.99	9.05±0.35
30/35	16.1	11.31±1.42	15.10±0.42
35/45	31.2	27.10±1.13	27.30±0.71
45/60	26.3	28.85±2.90	28.15±0.07
60/80	5.8	12.58±1.16	8.30±2.12
-80	1.3	3.94±1.19	1.10±0.71

Indonesia report are data presented in report on vitamin A fortification of MSG report prepared for the Indonesian government in late 1987. The new vitamin A is material recently prepared by the Coating Place to send to Indonesia for trials (L880224-A).

Table 17

WASHING TEST FOR FORTIFIED RICE AT 0.5% PREMIX

Rice was fortified to 13.3 IU/g.

Total lost from w/o iron rice	57.9%.
Total lost from w/ iron rice	0%

Table 18
STABILITY OF FORTIFIED RICE

TEMP	Aw	HALF-LIFE (WEEKS)		
		w/ iron	w/o iron	oil
35	0.11	6.30	-	2.17
	0.75	7.26	-	1.56
	0.90			1.41
45	0.11	3.18	8.80	0.83
	0.75	3.88	5.94	0.80
	0.90			0.71
55	0.11	2.17	6.65	-
	0.75	1.26	1.90	-
* 25	0.11	8.01	13.10	3.51
	0.75	10.13	14.02	2.32
	0.90			2.12

* Extrapolated to 25°C. The w/o iron rice and oil fortified rice values are from a two point line. - indicates not complete.

Table 19
Stability of Vitamin A Formulated for NaCl

T	RH	HALF-LIFE (WEEKS)	
		250 PARENT	170 PROTECTED
35	0	25.8±35.0	26.2±28.9
	75		11.1±2.6
45	0	121±840	9.1±2.7
	75		7.3±1.4

Table 20
STABILITY OF VITAMIN A IN NaCl

Temp=35

% Moisture	HALF-LIFE (WEEKS)	
	250 PARENT	170 PROTECTED
0	33.6±20.1	27.6±3.8
0.1	27.5±13.4	29.4±6.3
3.5	9.7±10.2	12.7±3.5

Table 21

STABILITY OF VITAMIN A IN 'SEA SALT'--NaCl, $CaCl_2$, $MgCl_2$

% MOISTURE	TEMP	HALF-LIFE (WEEKS) PARENT	MIVA
0	35	47.2±32.7	49.3±69.1
	45	19.2± 9.1	23.6± 5.8
	55	21.1± 8.6	19.6± 2.2
0.1	35	86.6±79.4	38.3±20.8
	45	43.5±27.8	21.7± 3.2
	55	12.3± 1.4	18.1± 2.7
3.5	35	5.7±0.8	9.7±0.9
	45	2.4±0.5	12.4±3.0
	55	0.6±0.1	11.9±4.7

PREDICTED HALF-LIFE AT 25°

% MOISTURE	PARENT	MIVA
0	55.3	60.5
0.1	121.8	46.2
3.5	8.0	9.1

Table 22
Vitamin A Content of Ultra-Rice

Bag		Vitamin A (IU/g)	
		Ultra-Rice I	Ultra-Rice II
1	premix	737±14	2307±60
2	"	733±15	2467±56
3	"	737± 3	2406±47
4	"	742± 5	-
5	fort.rice	5± 1	

6 replicates for each mean

Table 23
Stability of Vitamin A in Ultra-Rice I

Aw	Half-life (days)
0.11	13.7±2.2
0.75	5.2±0.3

Stability determined at 55°C.

Table 24
Cooking Test for Ultra-Rice I

Sample	Vitamin A (IU/g)
Cooked	22.2±8.9
Uncooked	378.3±6.1

Cooked n=4; uncooked n=2

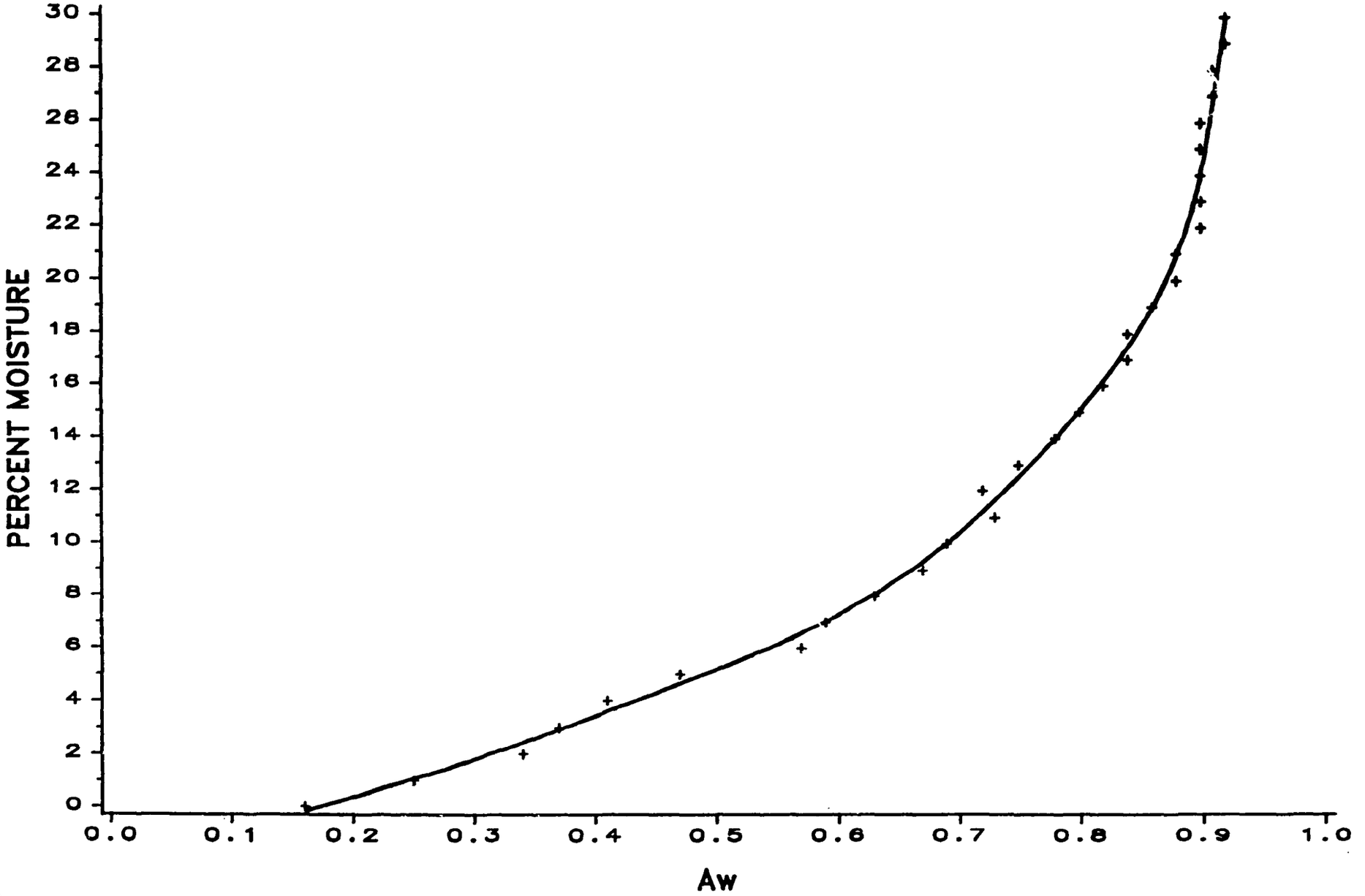
a8

Table 25
Vitamin A Content of Ultra-Wheat I

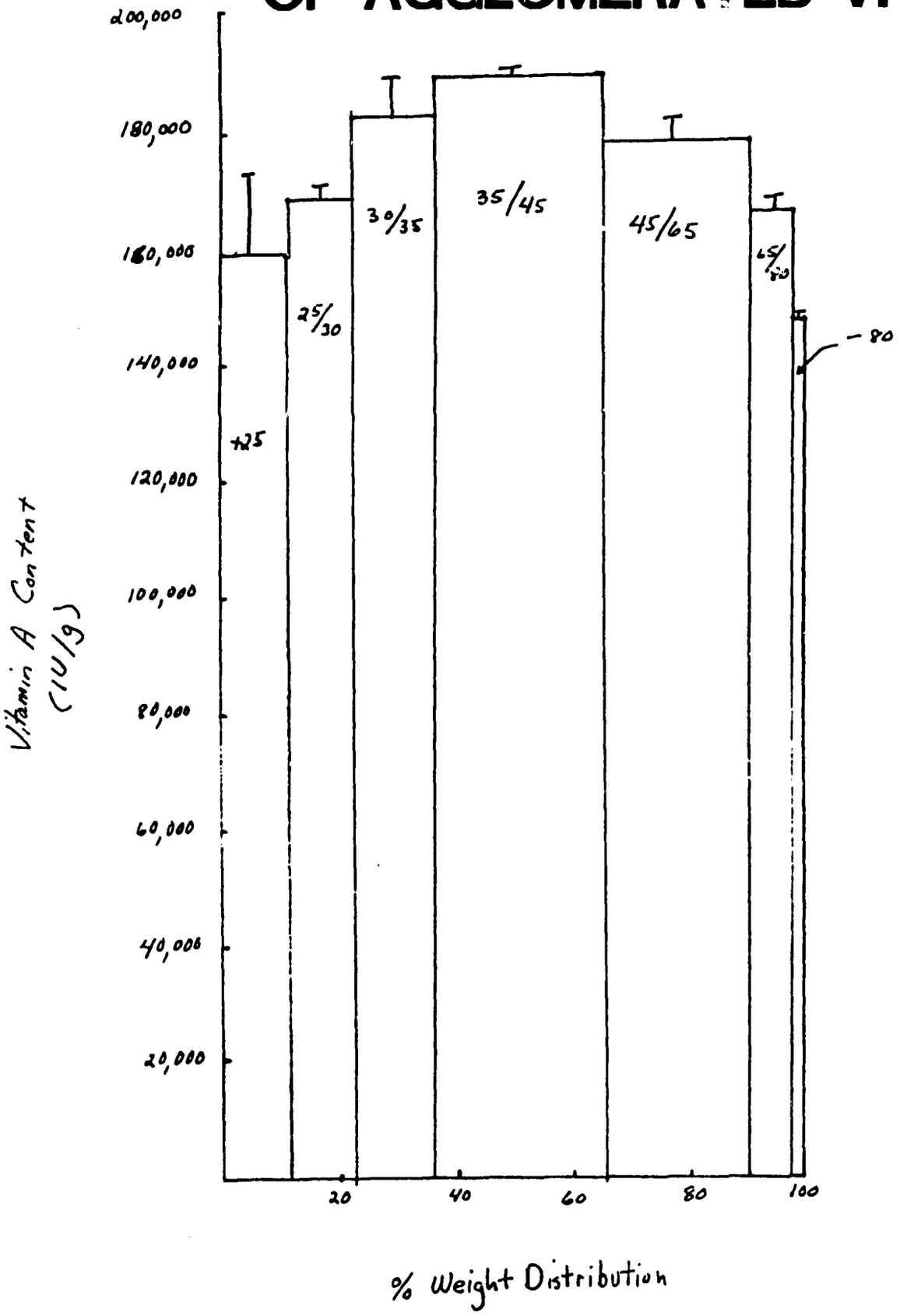
Bag	Vitamin A (IU/g)
1	1015 ± 61
2	753 ± 113
3	958 ± 75
Target	4405

n=6 per mean.

WHEAT ISOTHERM



SIZE DISTRIBUTION OF AGGLOMERATED VITAMIN A



Appendix 5

FORTIFICATION OF ULTRARICE WITH VITAMIN A [1]

by

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Iowa State University
Ames, IA

January 16, 1989

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1. INTRODUCTION

Vitamin A fortification of simulated rice grains, hereafter referred to as Ultrarice, has been attempted by the Bon Dente Company of Lynden, WA. This report describes the experiments performed to evaluate the physical and chemical stability of vitamin A fortified Ultrarice.

Ultrarice I was the first product made by Bon Dente using vitamin A oil. Ultrarice II and III were made using vitamin A 250 SD as recommended by Iowa State University to improve stability and initial fortification level. The Ultrarice was evaluated based on the initial vitamin A concentration in the product when it arrived at ISU, its rinse resistance, its cooking stability and its performance in an accelerated storage stability study at two water activities (Aw) or effective relative humidites. All experiments were done under yellow fluorescent lights to reduce degradation and isomerization to a minimum.

2. DESCRIPTION OF EXPERIMENTAL PROCEDURES

a) Vitamin A content was measured in each Ultrarice formulation received as soon as possible. Vitamin A content was measured by 1) grinding a weighed quantity of Ultrarice to a fine powder in a coffee/spice mill (Moulinex-Regal). Unground Ultrarice yielded no extractable vitamin A. The powder was saponified with alcoholic KOH for 40 minutes and the vitamin A was extracted into hexane. The hexane-extracts were chromatographed on a normal phase HPLC column with duplicate injections of each saponified sample. Vitamin A concentrations were calculated as IU/g rice.

b) The rinse stability of Ultrarice was evaluated by conducting a rinsing experiment suggested by Fred Barrett, USDA/OICD. Uncooked Ultrarice (~ 225g) was added to 2 L of tap water at room temperature. The ultrarice was was swirled to suspend rice in water for 30 seconds and then allowed to settle for 30 seconds. The water was decanted and the Ultrarice was blotted on paper towels. The Ultrarice was allowed to dry overnight. The dried Ultrarice was ground to a powder and assayed for remaining vitamin A content as described above. Control samples of Ultrarice were run at the same time. Results were expressed as a % of remaining vitamin A content compared to control.

c) The cooking stability of Ultrarice was evaluated by conducting a cooking simulation as suggested by Fred Barrett, USDA/OICD. Ultrarice was cooked in tap water at 1 part rice to 3 parts water and 1 part rice to 4 parts water (to establish if vitamin A was lost into excess water). The Ultrarice was boiled in water on a hot plate for 5 minutes, covered and allowed to cool for five minutes. Any excess water was poured off. Only when 1 part rice to 4 parts water were cooked together was there any excess water. After a number of attempts, we established that the cooked Ultrarice had to be homogenized to be able to extract the remaining vitamin A for analysis. Homogenization was performed for about 30 sec with a Tekmar tissuemizer-homogenizer or a Polytron homogenizer with added ethanol as solvent. The homogenizers provide extremely high rates of sheer with their insertable probes. Homogenization was done in the cooking beaker. Homogenization was judged to be complete when no discernable rice grains were observed in the suspension. The resulting slurry was quantitatively transfered to saponification flask, ethanol and KOH were added to the correct proportions and vitamin A analysis was performed as described above. Uncooked control samples were ground dry and assayed for vitamin A content at the same time. The cooking test was repeated four times.

Results were expressed as % remaining vitamin A content compared to the control. The vitamin A content is expressed as IU vitamin A / g dry Ultrarice.

d) Accelerated storage stability studies were performed to predict the storage life of Ultrarice (Labuza, T.P. in Food Chemistry, ed. O.W. Fennema, Dekker Publishing, 1985). All storage studies were performed in the dark. Each time-temperature-Aw point was done in triplicate. Ultrarice I was stored at 55°C and at Aw of 0.11 and 0.75. Ultrarice II was stored at two temperatures (45 & 65°C) and at Aw of 0.11 and 0.75. Storage tests for Ultrarice II were limited because most of the material was used to establish the best cooking test conditions. Ultrarice III was stored at four temperatures (35, 45, 55 & 65°C) and at Aw of 0.11 and 0.75. The Aw represents the effective relative humidity of the air over the rice during storage. The Ultrarices were stored in the dark for up to twelve weeks. The Ultrarice samples at each time-temperature-Aw point were ground to a powder and assayed for vitamin A as described above in (a). The percent remaining vitamin A was plotted versus storage time.

To estimate the storage life of the Ultrarices, the natural logarithm (ln) of the percent remaining was plotted versus time. The negative of the slope of this line is the rate constant (k) for the degradation of vitamin A at the temperature-Aw combination. A second plot of the natural log of the rate constant versus 1/T (°K) at each Aw allows us to extrapolate to other temperatures. The rate constant, k, at 25°C was the usual extrapolated value generated. The storage life of the Ultrarices was calculated by estimating the half-life ($t_{1/2}$) at 25°C with the following equation: $t_{1/2} = 0.693/ k$. The $t_{1/2}$ represents the amount of time required for one-half of the vitamin A content to be lost under a given set of storage conditions (Aw, T). The units for $t_{1/2}$ are the reciprocal of the units from k. These $t_{1/2}$'s were calcualted for each Aw. Statistical analysis allowed us to evaluate the error in our estimates.

3. RESULTS

a). The results of initial attempts to fortifiy ultrarice with vitamin A are presented in table 1. Ultrarice I was fortified with retinyl palmitate oil. Since the oil contains only minimal quantities of BHA and BHT as antioxidants, the resulting fortified product yielded unacceptabily low levels of fortification. Ultrarice II and III were prepared using retinyl palmitate 250 SD, a stabilized acacia matrix bead. These two preparations yielded vitamin A contents in the target range and demonstrated that the fortification could be reproducible.

b). The rinsing test was performed with Ultrarice II and III. The results of the rinsing test are presented in Table 2. This test was performed because rice is traditionally rinsed and washed prior to cooking in some cultures. Because we were running short of Ultrarice II, we diluted it with regular rice, and then ran the rinse test. Ultrarice III was rinsed without dilution with regular rice. In both cases, the Ultrarices retained most of the vitamin A content. Since the vitamin A was incorporated inside the simulated-rice grain, we did not expect to loose much vitamin due to rinsing.

c). The cooking test was designed to simulate cooking of Ultrarice. In these tests, only Ultrarice containing vitamin A was used. The Ultrarice was not diluted with regular rice nor nonfortified Ultrarice. The results of the cooking tests are presented in Table 3. In the cooking of Ultrarice II, the cooking trial was done without excess water. Visually, ULtrarice II and III look very similar to cooked regular rice. The results show that for Ultrarice II, all vitamin A was retained after cooking. In the second Ultrarice product made with retinyl palmitate 250 SD, Ultrarice III, the results were not as good

in the cooking test. Only about 40% of the vitamin A was retained after cooking. These results were obtained using just enough water to cook the Ultrarice and using about 33% excess water. We do not know if Bon Dente changed any of the fortification procedures or contents of Ultrarice III compared to Ultrarice II, but this should be clarified. Since these finding were the results of quadruplicates, we believe they are valid.

d). Stability estimates for vitamin A were made for Ultrarice I (Table 4), Ultrarice II (Table 5) and Ultrarice III (Table 6). The rate of % loss of vitamin A versus time for Ultrarice II and III are plotted in Figures 1-6. Ultrarice I had relatively short half-lives. This was not unexpected because pure retinyl palmitate oil is very unstable and subject to rapid oxidation. The relatively low initial content of vitamin A in the starting Ultrarice I suggested that rapid loss could be expected. Because of the limited amount of Ultrarice I, we only measured the stability at one temperature. Thus, we can only compare $t_{1/2}$ at 55° between the three Ultrarices. It appears that Ultrarice I had about half the stability of Ultrarices II.

The stability estimates for Ultrarice II were obtained at only two temperatures because we used most of the Ultrarice II to find the most appropriate cooking and extraction procedures. Ultrarice II appeared to have better stabilty than Ultrarice I. Unfortunately, Ultrarice II was not as stable as earlier fortified wheat products. Ultrarice III had greater stability, although, half the vitamin A was lost after 7-9 weeks. This, of course, is much less stable than fortified wheat made for a different project. Bon Dente has been able to, progressively, make a more stable vitamin A fortified rice.

4. Discussion

Ultrarice II and III demonstrate that the product can be made in replicate and that a rinse-stable product can be produced. Further work must be done to improve the cooking and storage stability. Improving one will most likely improve the other. The results of stability study extimates should not be viewed all negatively. The results of an earlier rice fortification study are presented in Table 7. The $t_{1/2}$s were longer than those reported for Ultrarice II and III by about a factor of 2. This product, however, did not have satisfactory rinse resistance. Cooking test were not performed. Early attempts to fortify wheat were not successful at first either. Only after about a dozen attempts was a fortified wheat produced that yielded $t_{1/2}$s of about 50 weeks.

In producing the Ultrarices, anything that tends to disrupt the 250 SD beads and separate the vitamin A from its protective antioxidants and shell of acacia, would tend to decrease the stability of vitamin A. Anything that Bon Dente can do to increase the content of antioxidants, reduce heat and oxygen during production and reduce oxygen exposure of the Ultrarice should improve the product.

We do not know what ingredients go into the production of Ultrarice except in a general way. One observation we have made is that with time at 55°C and Aw 0.75, the Ultrarice turns brown. AT 55°C, the kernals turn a tannish color. No color change is observed at lower temperatures nor any color change at any temperature at Aw=0.11. There may be nonenzymatic browning going on. Whether this has any effect on vitamin A degradation, we do not know. There must be free reducing sugar present, though, for nonenzymatic browning to happen. The browning will not be a problem for Ultrarice in the field, though, because such abusive conditions will not occur.

5. Figure Description

Figure 1. Degradation of Ultrarice II at 45°C. 1 = 0.11 Aw, 7 = 0.75 Aw, obersevations hidden = replicate points ontop of each other.

Figure 2. Degradation of Ultrarice II at 65°C. See figure 1 for symbol description.

Figure 3. Degradation of Ultrarice III at 35°C. See figure 1 for symbol description.

Figure 4. Degradation of Ultrarice III at 45°C. See figure 1 for symbol description.

Figure 5. Degradation of Ultrarice III at 55°C. See figure 1 for symbol description.

Figure 6. Degradation of Ultrarice III at 65°C. See figure 1 for symbol description.

Table 1. Vitamin A Content of Ultrarice

Bag	Ultrarice Vitamin A Content (IU/g)		
	I	II	III
1	737±14	2307±60	2204±32
2	733±15	2466±56	2230±26
3	737± 3	2406±47	2210±85
4	742± 5	---	2196±55
5	---	---	2093±73
6	---	---	2136±61

n = 6 per bag

Table 2. Rinse Test for Ultrarice II and III

Rinsing	Vitamin A Content of Ultrarice	
	II *	III
Before	245	2204±32
After	240±60	2006±119
% Retention	100	91

* One part Ultrarice II was diluted with 9 parts real rice for rinse test since almost all of this batch had been used to standardize cooking test.
n = 6

Table 3. Cooking Test for Ultrarice II and III

Cooking	Vitamin A Content of Ultrarice			
	II	%	III	%
Before	2307±60		2204±32	
After w/o excess H_2O	2691±208	100	1013±90	46
After w/ excess H_2O	---	-	856±90	39

n = 4

Table 4. Stability of Ultrarice I

Aw	Temperature	Rate ($days^{-1}$)	$t_{1/2}$ (days)
0.11	55	0.0506	14±2
0.75	55	0.1339	5±1

Table 5. Stability of Ultrarice II

Aw	Temperature	Rate ($days^{-1}$)	$t_{1/2}$ (days)
0.11	45	0.0321	22±2
	65	0.0423	16±2
	* 25	0.0235	29
0.75	45	0.0337	21±1
	65	0.0674	10±1
	* 25	0.0154	45

* = extrapolated from higher temperatures

Table 6. Stability of Ultrarice III

Aw	Temperature	Rate ($days^{-1}$)	$t_{1/2}$ (days)
0.11	35	0.0141	49±3
	45	0.0260	27±2
	55	0.0250	28±2
	65	0.0264	26±3
	* 25	0.0150	46
0.75	35	0.0107	65±4
	45	0.0251	28±1
	55	0.0517	13±1
	65	0.0970	7±1
	* 25	0.0113	62

Table 7. Stability of Fortified Rice

Aw	Temperature	$t_{1/2}$ (days) w/ iron	$t_{1/2}$ (days) w/o iron
0.11	35	44	--
	45	22	62
	55	15	47
	* 25	56	92
0.75	35	51	--
	45	27	42
	55	9	13
	* 25	71	98

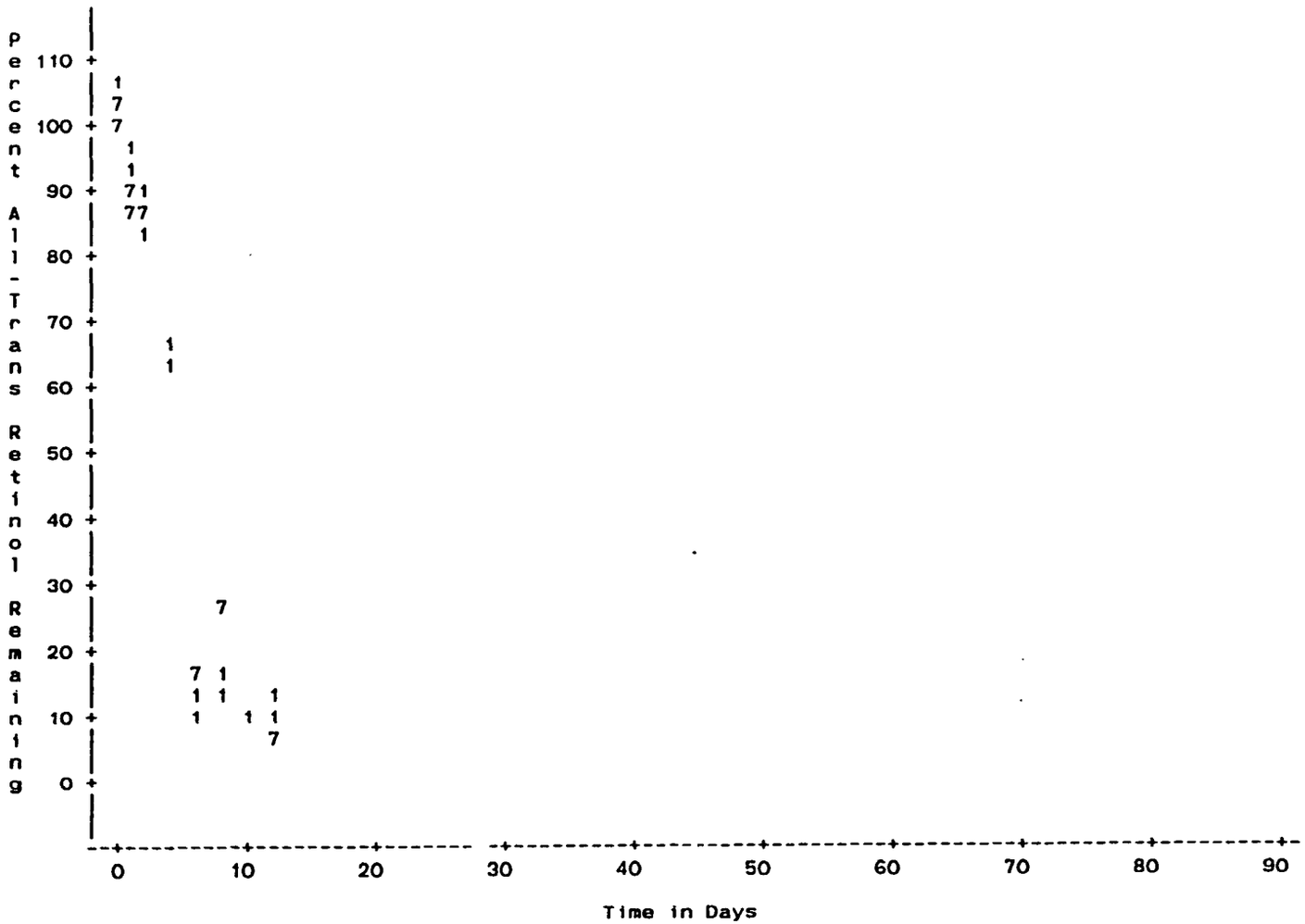
* extrapolated from higher temperatures

Fortified rice produced by Wright Enrichment, Inc.

ULTRA-RICE 2 DATA 14:32 SATURDAY, JANUARY 14, 1989 1

TEMP=45

PLOT OF PCTTRANS*TIME SYMBOL IS VALUE OF AW



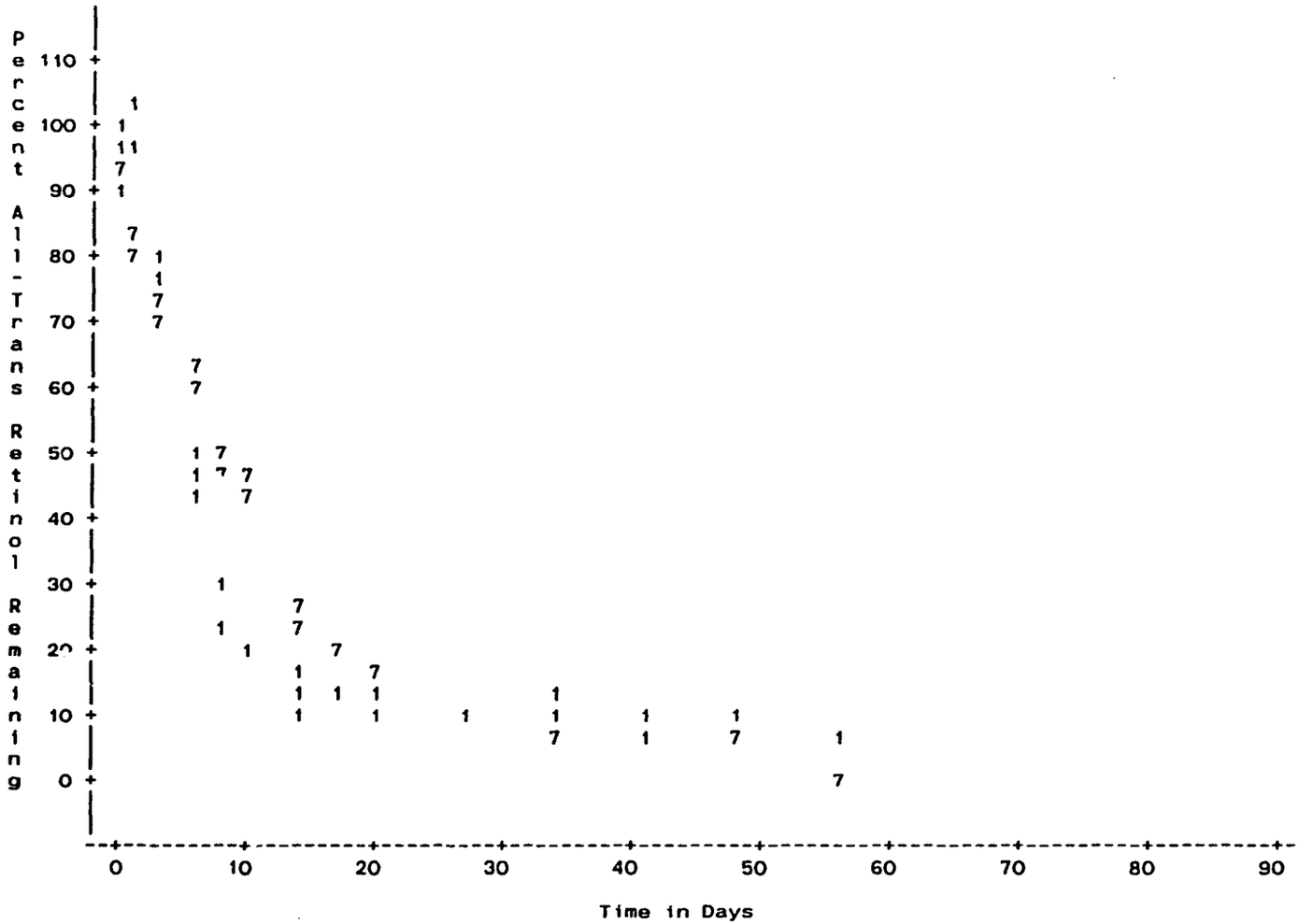
NOTE: 26 OBS HIDDEN

Figure 1

ULTRA-RICE 2 DATA 14:32 SATURDAY, JANUARY 14, 1989 2

TEMP=65

PLOT OF PCTTRANS*TIME SYMBOL IS VALUE OF AW



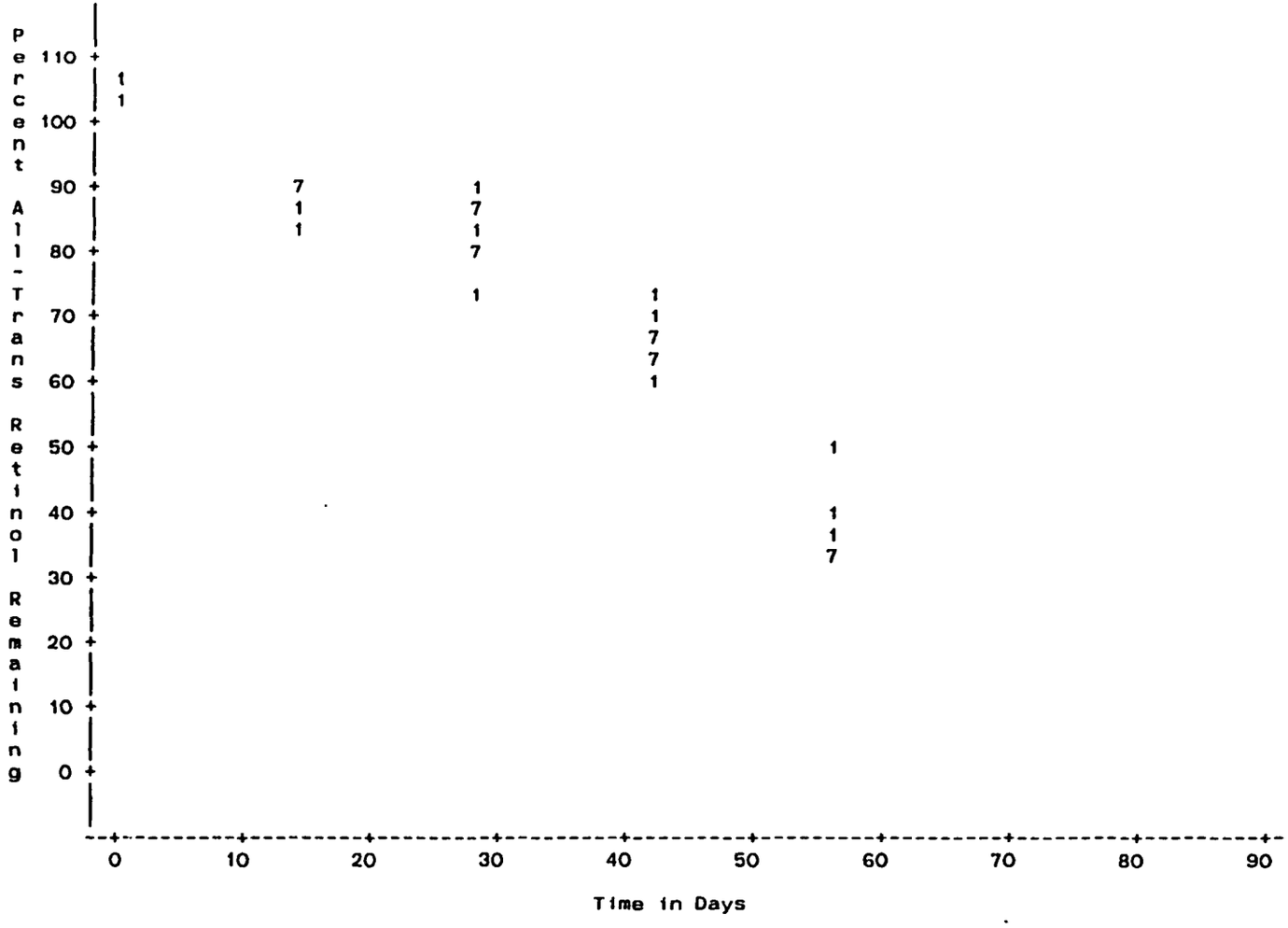
NOTE: 40 OBS HIDDEN

Figure 2

ULTRA-RICE 3 DATA 14:32 SATURDAY, JANUARY 14, 1989 3

TEMP=35

PLOT OF PCTTRANS*TIME SYMBOL IS VALUE OF AW



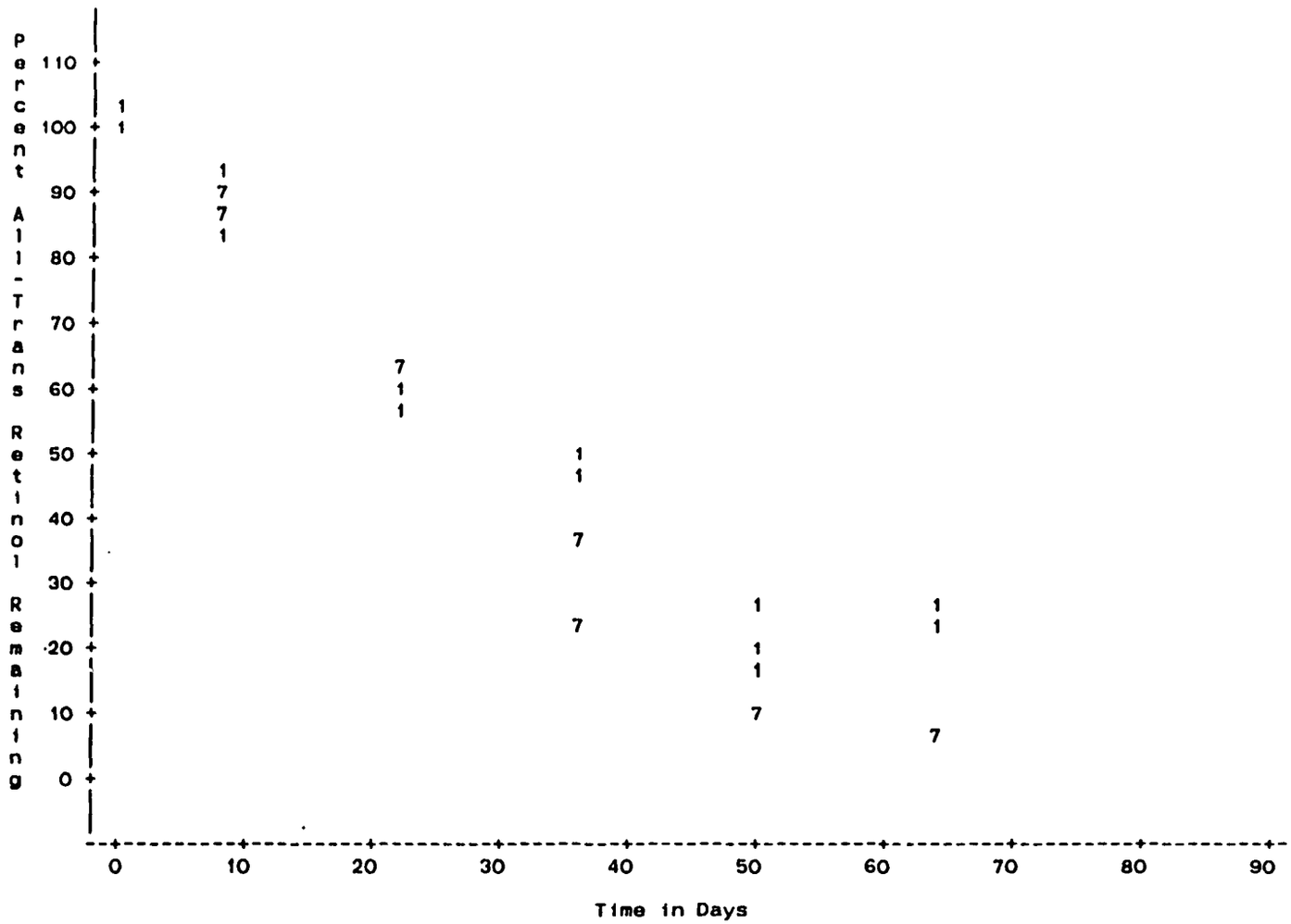
NOTE: 12 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 11 OBS HIDDEN

Figure 3

ULTRA-RICE 3 DATA 14:32 SATURDAY, JANUARY 14, 1989 4

TEMP=45

PLOT OF PCTTRANS*TIME SYMBOL IS VALUE OF AW

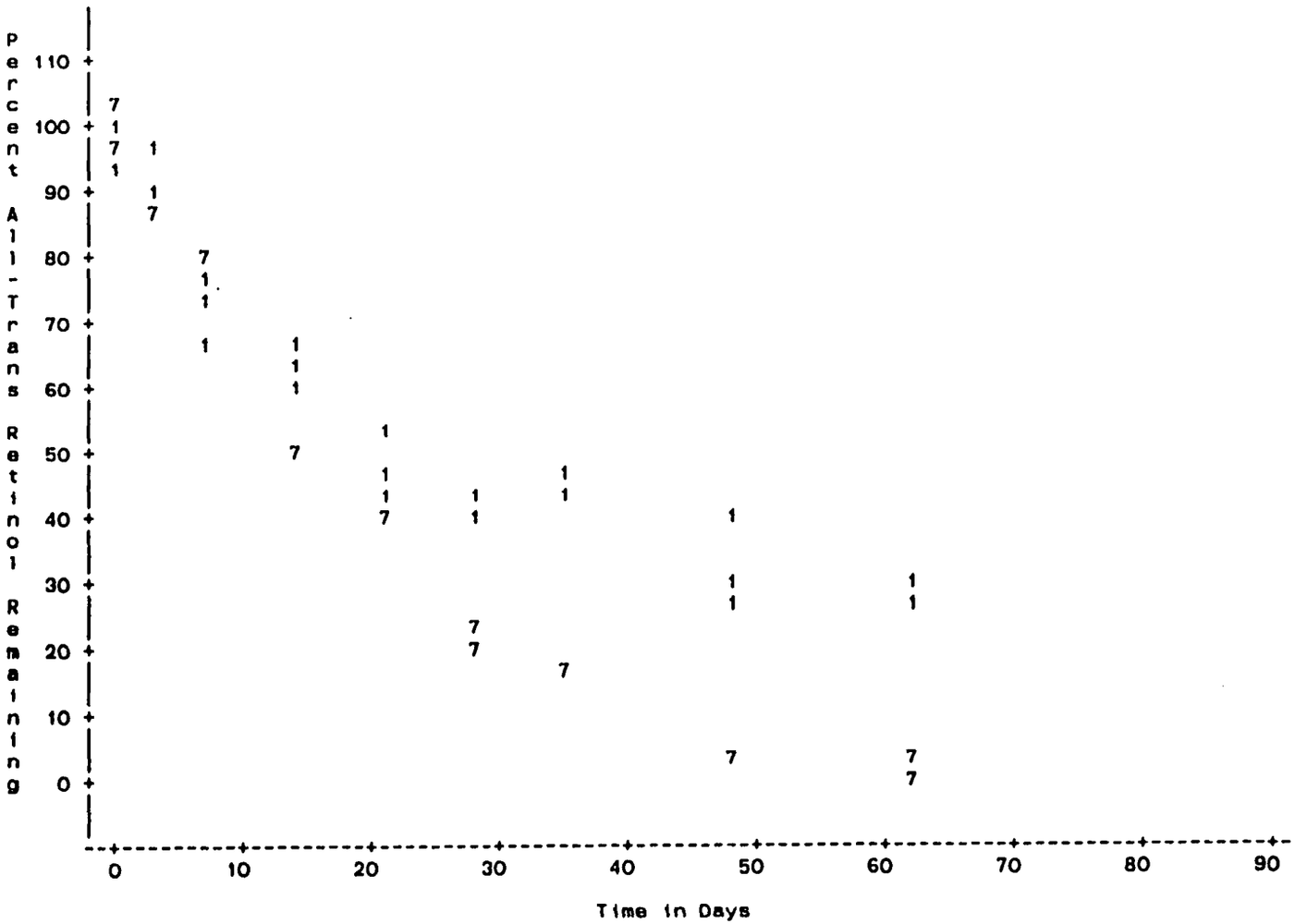


NOTE: 12 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 16 OBS HIDDEN

Figure 4

TEMP=55

PLOT OF PCTTRANS*TIME SYMBOL IS VALUE OF AW

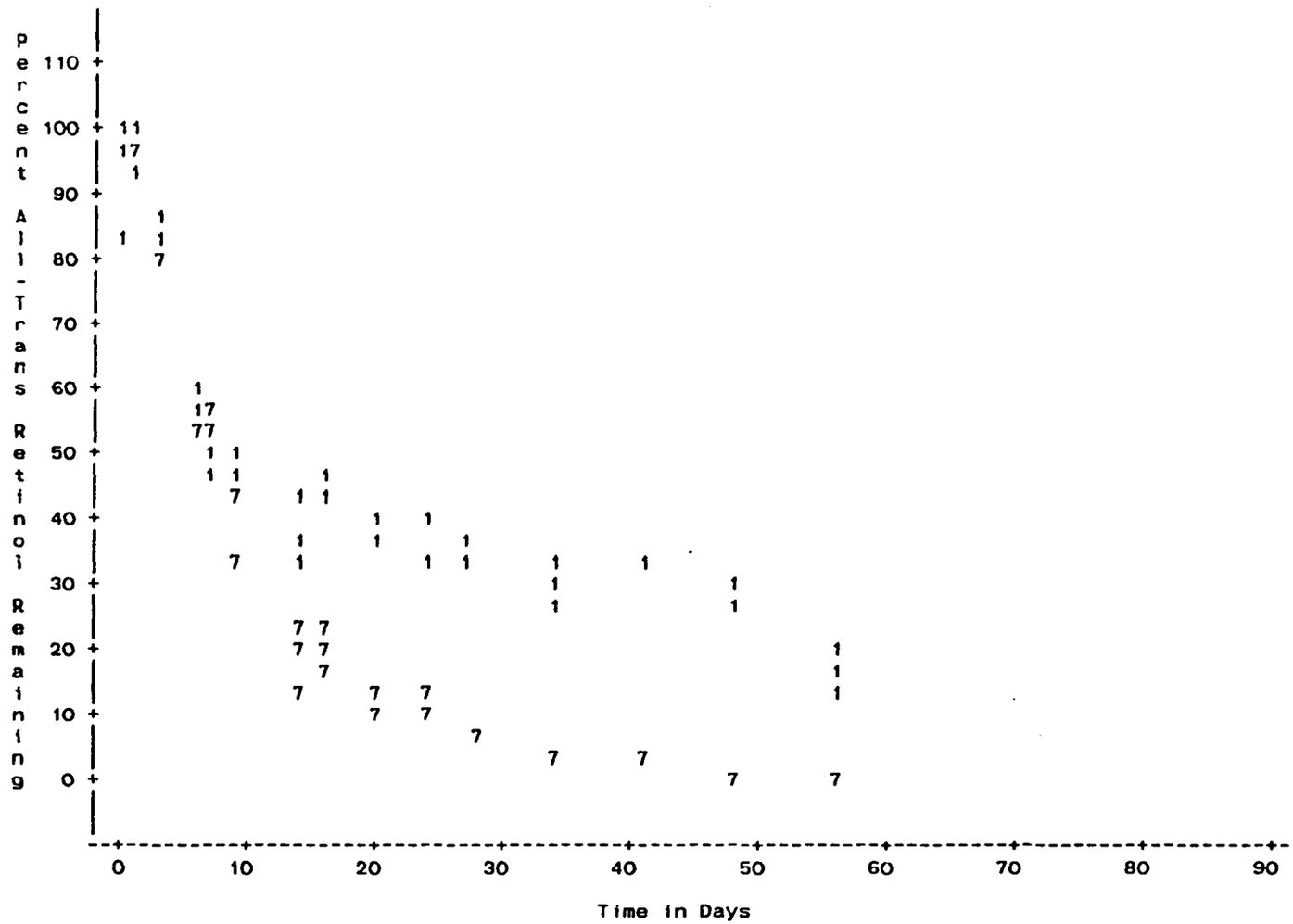


NOTE: 20 OBS HIDDEN

Figure 5

TEMP=65

PLOT OF PCTTRANS*TIME SYMBOL IS VALUE OF AW



NOTE: 35 OBS HIDDEN

Figure 6

Proposed Quality Assurance Guidelines for the "The Fortification of MSG with White Vitamin A in Indonesia" Project

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INTRODUCTION

The proposed Quality Assurance Guidelines for the "Fortification of Monosodium Glutamate with "White" Vitamin A in Indonesia" is broken down into 3 major sections.

A. Definition and Basis of Quality Assurance Programs

B. Steps to implement an MSG-White Vitamin A Quality Assurance Program* including:

1. Product Specification
2. Product Safety Analysis
3. Product Hazard/Quality Analysis
   a. critical tests
   b. physical check points
   c. environmental conditions
4. Physical Systems Hazard/Quality Control Program
   Flow Chart
   Summary of Critical Steps
5. Recall System
6. Audit System
7. Respond to complaints
8. Documentation

C. Appendixes containing general information on Hazard Analysis and Critical Control Point Procedures, QA/QC responsibilities and recall procedures.

*Specific suggestions are made where sufficient data exists. However, the fine tuning of the program must be made using the actual operating and storage conditions found in Indonesia.

Proposed Quality Assurance Guidelines for the
"Fortification of Monosodium Glutamate with "White" Vitamin A in Indonesia".

In order to insure that Vitamin A fortified MSG of the appropriate potency (Quality) reaches the Indonesian consumer on a regular basis a Quality Assurance/Control Program must be developed and implemented. This document proposes an appropriate procedure for the development of these guidelines.

A. Basis of Quality Assurance Programs.

The terms Quality Assurance and Quality Control are often used interchangeably to describe personnel or departments that audit the processing operation. However, Quality Assurance personnel develop programs to insure that the ingredients or foods manufactured by a company are safe, wholesome (not adulterated), properly labeled, and that they meet all company, city, state and government regulations, (quality, sensory, safety, grade, nutritive value etc.). The Quality Control function is involved with the application of the plan developed by the Quality Assurance.

The Quality Assurance system must include:

1) Ingredient Inspection and Control. Requires testing against written standards.

2) Manufacturing Control. This requires that hazards and quality factors be identified and critical control points (CCP) be established, monitored and any action taken duly recorded.

3) Distribution Control. This requires that the finished product be unadulterated, properly labeled and that the integrity of the finished product is protected by the environment.

The Quality Assurance and Control Guidelines recommended in the document are based upon the following ground rules:

1) Each company will produce safe products.

2) Accurate evidence (records) of the safety of all products will be available.

3) All products will be analyzed for potential hazards.

4) Product specifications will incorporate process safety requirements.

5) Specifications will govern plant processing operations.

6) Physical hazards will be identified and controlled.

7) Efficient Recall System will be established.

8) Customer complaints will be answered promptly and effectively.

9) Each company involved with this project will have Quality Assurance/Control Programs.

10) Quality Assurance will audit for compliance with the standards set for this project.

The success of this program is dependent upon a real commitment from all top executives and government agencies involved in this project.

In order to implement the Quality Assurance System mentioned on page 3 of this document a Hazard Analysis and Quality Analysis Critical Control Point System (HACCP and QACCP) will be developed by each ingredient supplier (supplier of MSG. Vitamin A and White Vitamin A) and the finished product processor (blender of MSG plus White Vitamin A) and distributor (same as the processor in this instance). This will include:

1) The identification of those control points in the processing operation which are important in the prevention of adulteration and maintenance of vitamin A potency, particle size and color

2) Identification of hazards/quality factors associated with each such point

3) Establish adequate controls at each such point

4) Establish adequate monitoring of the controls at each such point

5) Establish the means to document the adequacy of the controls

A Hazard and Quality Analysis procedure of this type is an evaluation of all ingredients, critical process controls, critical physical controls, human factors, manufacturing requirements, and distribution concerns involved in producing and distributing MSG/White Vitamin A (see Appendix A).

B. Steps to implement an MSG-White Vitamin A Quality Assurance Program.

Implementation of the Quality Assurance Hazard and Quality Analysis Critical Control Point procedure for MSG fortified with White Vitamin A.

1) Product Specifications. A formal document (in writing) defining white Vitamin A and White Vitamin A fortified MSG specifying particle size, color, potency and any other regulatory requirements should be developed.

Based upon the report by Murphy et al. (1987):

a) "White" Vitamin A is produced in the form of clusters or agglomerates of several coated particles held together by the coating binder. The acceptable size of the clusters should be defined in the QA document. For example, to insure proper size distribution of the White Vitamin A and minimize separation of the fortified MSG during blending and handling 35.5% of the White Vitamin A should pass a 18 mesh sieve but be held by a 30/35 mesh sieve, while 57.5% should pass a 30/35 mesh sieve but be held by a 45/60 mesh sieve.

b) White Vitamin A contains 175,000 International Units (IU) of Vitamin A per gram. An acceptable deviation from this value should be agreed upon and defined in the QA document (e.g. ±2% or a value appropriate based upon the test procedure used Part 3). White Vitamin A that falls outside of the acceptable range should be

rejected. Lower levels will eventually produce an underfortified product (nutritionally ineffective) and higher levels could produce a potentially toxic product for the consumer. In either case both extremes are an economical waste.

c) The White MSG should be snow-white in color. It should not have more than -2.84 ± 0.34 Hunter L units (darker) from unfortified MSG nor more than $+0.77 \pm 0.21$ Hunter b units (yellower) than unfortified MSG. A Munsell color chip or disc could be used in place of the Hunter Color Difference meter (we can provide assistance here if needed).

White Vitamin A that falls outside of these agreed upon specifications (a,b,c) due to yellowness (or atypical colors), particle size variations or too little or too much Vitamin A should be rejected.

d) The target fortification level of MSG for Indonesia is 3000 IU of vitamin A per gram of MSG [17.1 kg of White Vitamin A (175,000 IU per gram) blended with 982.9 kg of MSG] Murphy et al. (1987). Acceptable deviations from this target need to be agreed upon and defined in the QA document. Underfortification (nutritionally ineffective) and overfortification (potentially toxic to consumers) must be rejected.

Acceptable deviations ($\pm x\%$) from the target fortification levels will be dependent upon the accuracy/precision of the tests selected (Item 3) and random process variables (non-random process variables should be eliminated or defined and controlled) Item 4.

An acceptable target (upper and lower control limit) cannot be set smaller than the variation (% error) inherent in the testing procedure of choice and processing variation. (See Item 3 for further description of the Vitamin A analysis procedures.)

2) Product Safety Analaysis. This analysis includes an assessment of the microbiological, physical and chemical safety of the product.

The formulation, processing, distribution and recommended use levels have been evaluated in previous reports.

3) Product Hazard/Quality Analysis. This defines the following:

Sampling and testing of all ingredients and finished products must be carried out by Quality Control to insure that all ingredients and finished products are in fact meeting your specifications.

a) Critical tests that must be performed on ingredients and finished products

Vitamin A potency test

(1) High technology - the accurate/precise High Performance Liquid Chromotography (HPLC) method developed and used by Murphy and coworkers (Iowa State University) 1987. This technique can measure Vitamin A with less than 1% error. However, the equipment cost is high. Murphy et al. (1987) also found that

the blending/sampling procedure introduced errors up to 15%

(2) The quantitative spectrophotometric method of Dr. Muhilal (1986). This quantitative analysis of vitamin A in MSG was performed in the laboratory using a spectrophotometric method. A 2 gram sample of MSG-A is dissolved in 100 ml of water and isopropanol at the ratio 1:1. Five mls of this solution are further diluted with 50 ml of water and isopropanol at the ratio 1:3. The vitamin A level in the sample is measured using the following formula:

Vitamin A (IU) = A x 19 x dilution factor

Note: A = absorption or optic density
19 = derived from:

$$\frac{1{,}000{,}000}{1750} \times \frac{10}{3} \times \frac{1}{100}$$

(the ekstinksi coefficient value = 1750)

This technique has the advantage that it is currently being used in Indonesia and it is appropriate for a laboratory test procedure. However, we need to know the % error associated with this method in order to use it to set product specifications.

(3) The semi-quantitative method (modified Carr-Price reaction) developed by Dr. Muhilal (1986). A sample of about one gram of MSG-A was mixed with about 10 drops of tricholoroacetate (TCA) at 60% solution in chloroform. The blue color which resulted was immediately compared with the standard of the semi-quantitative color. The darker the resultant blue color the higher the vitamin A level in the MSG-A. The standard of this color is equivalent to successive Vitamin A levels of 500 IU, 1000 IU, 1500 IU, 2000 IU, and 3000 IU.

This test also has the advantage of currently being used in Indonesia. It is an appropriate field test (semi-quantitative), however we still need to know how accurate and precise the method is (% error). Appropriate color standards can then be used or developed (for example appropriate color chips could be used for the subjective color comparison eliminating the need to weigh out reagents, water and carry liquid color standards). We have the capability to help with this part of the project if needed.

(4) A qualitative UV-light (go/no go) test to look for the presence of White Vitamin A. This test could become semi-quantitative if appropriate studies were made relating (1) the number of beads (White Vitamin A) per specific sample size or (2) the amount of fluorescence per sample size. Further work with this procedure can be done if needed.

Particle Size Test

To insure proper size distribution of the White Vitamin A and minimize separation of the fortified MSG during blending and handling 35.3% the White Vitamin A should pass a 18 mesh sieve but

be held by a 30/35 mesh sieve, while 57.5% should pass a 30/35 mesh sieve but be held by a 45/60 mesh sieve.

Color Test

The White MSG should be snow-white in color. It should not have more than -2.84 ± 0.34 Hunter L units (darker) from unfortified MSG nor more than $+0.77 \pm 0.21$ Hunter b units (yellower) than unfortified MSG. A Munsell color ship or disc could be used in place of the Hunter Color Difference meter (we can provide assistance here if needed).

White Vitamin A that falls outside of these specifications due to yellowness (or atypical colors) or particle size variations should be rejected.

Other Tests

Concerning packaging integrity, net weight, freedom from adulteration, etc. should already be in place with the manufacturers in Indonesia and will not be further elaborated in this document. [Appendix B]

Standard Statistical Quality Control techniques should be used to obtain samples for determining the potency of the White Vitamin A and blended MSG/White Vitamin A.

b) Physical check points to prevent assembly/manufacturing hazards (see part 4)
c) Environmental conditions critical for the product. Potential for the product to be abused during storage and distribution to the customer

Based upon the report "Fortification of monosodium glutamate with "White" Vitamin A in Indonesia" by Patricia Murphy and coworkers (1987) environmental conditions that reduce the potency of the blend are high temperature, light, moisture and oxygen. Proper packaging (high barrier plastic) can reduce vitamin loss due to environmental oxygen and moisture. Since the consumers want to see the MSG (clear plastic sachets currently being used in Indonesia) the influence of light induced Vitamin A loss must be minimized as much as possible during storage, shipping and blending of the White Vitamin A (and the blended MSG/White Vitamin A). The temperature of storage of ingredients and blended finished product should also be controlled to minimize Vitamin A losses throughout the operation.

Storage Based upon Murphy et al. 1987 results

a) Packaged white vitamin A should be stored dry in the dark around 25°C to minimize Vitamin A loss. For example, assuming that no more than a 20% vitamin loss is acceptable, the White Vitamin A should be used within 29 weeks. [QA/QC must set the standards for acceptable loss.]
b) The packaged White vitamin A fortified MSG should also be stored dry in the dark around 25°C. Again assuming a maximum allowed 20% Vitamin A loss, the product should be distributed to retailers within 68 weeks. Clearly a shorter storage time

wi[illegible]ner potency product to reach the consumer. We encourage the companies to optimize their processing and marketing time tables to get the fortified MSG to the consumers with minimum storage. A good inventory control system is required.

c) Fortified MSG at the consumer level. Once the White Vitamin A fortified MSG reaches the retailers and consumers the potency of the fortified MSG can be drastically reduced by storage or display of the clear plastic packets in the sunlight or bright conditions (this could limit the useable shelf-life of the product to 2 weeks or less).
We recommend that retailers be encouraged to store the fortified MSG in the dark (e.g. opaque boxes) until needed for display/sales. Low light/shade conditions are also recommended in the fortified MSG display area. (Samples should be pulled under actual use conditions to better define the actual shelf-life of the fortified MSG.)

4) Physical Systems Hazard/Quality Control Program. In order to identify all of the critical control points that might adversely affect the product, a flow diagram of the total process (plant facility, equipment and environment) should be made. This should represent what actually happens in practice, not what should happen.

Since we cannot at this time make an accurate flow diagram for the companies involved in the project without actually being there, a simple flow diagram follows to demonstrate some of the control points that we feel are important.

Supplier of Agglomerated White Vitamin A → Quality Assurance Program with HCCP and QCCP

↓ → Validation inspection (potency of Vitamin A)

Shipping to Industry → Inspect Shipping conditions (physical and environmental)

↓

Industry → Quality Assurance Program HCCP and QCCP

a) Validate Potency, color and particle size of White Vitamin A upon arrival, and potency after storage
b) Monitor potency, color and particle size of blended White Vitamin A with MSG after blending and storage
c) Inventory Vitamin A use

↓

Storage → Verify Potency
Rotation of stock (Inventory control)

Retailer → Periodic sampling to verify potency
Rotation of Stock

Consumer → Periodic sampling to verify potency

Summary of Critical Steps

Summary of critical steps outlined in the MSG/White Vitamin A flow chart that Quality Control must verify:

a) Agglomorated White Vitamin A is being produced at the appropriate potency, color, particle size etc. (Product Ingredient Specifications).

b) Upon arrival the White Vitamin A has not been damaged, lost potency etc. during shipment. Color, particle size and potency should be evaluated at this point.

c) The packaging and environmental storage conditions should minimize loss of vitamin A potency both before and after blending with MSG. (How fast does the product turnover? Optimize production schedule to meet needs without potency loss.)

d) Proper blending of the MSG and White Vitamin A. The variation in Vitamin A potency in the MSG-White Vitamin A mix should be determined with the actual binder (Patterson-Kelly Cross Flow Tumble Blender) to be used in this process. The values obtained under this mini-study will determine the percent variation in Vitamin A levels to be allowed in your finished product specification. Once levels have been set use control charts to monitor the process. Post these so that the workers can view it. Clearly define the actions to be taken if the product fails the upper or lower control limits etc. (We can supply more information about the setting up and use of control charts if needed.)

e) The blended MSG/White Vitamin A potency meets specification. Due to the potential hazard of over fortification, a continuous inspection program (similar to USDA, Food Safety and Inspection Service Procedures in the U.S.A. would be desirable).

f) An inventory control program should be instituted (if it is not already in existence). To monitor Vitamin A usage in blending and storage operations.

Once the Hazard and Quality Critical Control points are established, document the system of controls and actions to be taken at each control point. Any hazard or factor that could lower the quality (e.g. potency of the White Vitamin A) should be eliminated if possible.

5) Recall System. In the event that a product must be recalled (recovered due to a safety hazard, product quality defect, labeling problem, etc.) you should be able to trace or identify the product by its code (i.e. day of production, shift, batch, lot, ingredient). (See the attached document on Recall Policy for more detailed information). - Appendix C.

6) Periodically audit your complete system for product compliance. Are steps 1-5 of this system in compliance. Is the product in the marketing chain still meeting your product specifications?

7) Respond to consumer and customer complaints as well as correct any safety or regulatory deficiencies discovered as a result of such complaints.

8) All aspects of this system should be documented and available to the appropriate individuals responsible for this product.

C. Summary

The development, refinement (using actual plant data from Indonesia) and implementation of the proposed Quality Assurance/Control System using Hazard and Quality Control Points will allow you to have an effective, practical and economical system to insure that the MSG is properly fortified and that it is delivered to consumers at the desired Vitamin A potency, and quality level (color, particle size etc.).

Appendix A

Hazard Analysis and Critical Control Point Identification
(HACCP Procedures)

Introduction:

The HACCP system is widely employed in food processing quality assurance programs for the control of food safety. It consists of hazard analysis and the identification of critical control points on the processing lines. Hazard analysis describes those elements of process equipment or environment which if not maintained and monitored can result in the introduction of hazardous materials into a food product. Critical control points are those elements which if not maintained within certain parameters can result in the production of food which contains pathogenic microorganisms or toxins. In contrast to traditional corrective quality control, HACCP is a preventive system which establishes and defines quality check points as well as accountability for each check point.

I. Hazard Assessment

The establishment and maintenance of a preventive system such as HACCP begins with thorough assessment of hazards. Hazards to the safety of food comprise three classes: a) chemical, b) microbiological and c) physical.

A. Chemical hazards
   1. pesticides
   2. toxic metals
   3. toxic chemicals found in environment
   4. cleaning and sanitizing agents

B. Microbiological hazards
   1. pathogenic organisms - disease causing
   2. spoilage organisms
   3. indicator organisms (indicating improper sanitation)

C. Physical hazards
   1. contributed by raw materials
   2. contributed by process environment
   3. contributed by personnel
   4. contributed by animals and insects

II. Hazard Control

A. Control of raw material hazards
   1. reliable suppliers of quality raw materials and adequate product specifications
   2. product inspection, sampling and testing programs
   3. in-process controls such as magnetic detectors, screens, filters, color detectors, etc.

B. Control of processing environment hazards
   1. building and grounds
   2. machinery and equipment
   3. processing area environment (handling in-process product; proper

storage and segregation of ingredients, product rework, and waste material; proper methods of cleaning and sanitizing; etc.)

C. Control of hazards from personnel
   1. proper grooming and attire (hair nets, uniforms, etc.)
   2. personal hygiene (washing, injuries, gloves, etc.)
   3. production line practices (hand tools and equipment, lunch, smoking, etc.)
   4. training programs

D. Control of hazards from animals and insects
   1. proper pest control management
   2. physical controls (good housekeeping, traps, etc.)
   3. chemical controls (pesticides)

III. HACCP Program - Process Line Development

The number of potential hazards in a food processing situation is very high. To monitor and control all hazards effectively can be expensive. In a HACCP system it is necessary to define the hazards at every step in the processing plant or line, then establish accountability for monitoring or controlling each one. Which hazards and critical points can be effectively monitored and controlled will then be determined based on product specifications, company philosophy, etc.

Developing a HACCP program is a five step procedure:

A. Develop a flow diagram for each process

B. Identify hazards and/or critical processing points and mark on the flow chart.

C. Specify effective control procedures for each hazard or critical point.

D. Prepare a HACCP analysis chart listing:
   1. all control points
   2. type of control implemented
   3. frequency of control
   4. who is accountable for the control

E. Document hazard control performance by developing a records system.

IV. HACCP Program - Implementation

Even though quality assurance designs and periodically audits the HACCP system, and quality control has the day to day responsibility for implementation of the system, it is evident from the vast array of potential hazards that product quality must be the responsibility of all those involved in processing. The HACCP system enables the distribution of quality accountability. A number of people throughout the production process, both supervisors and key employees, are responsible for monitoring or performing the HACCP controls in their specific area. HACCP control points become part of employee job responsibility. This should serve to create a greater awareness of the need for quality and aid in the control of hazards.

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Table 1—QUALITY CONTROL'S EIGHT MAJOR ELEMENTS

I. **Standards & Specifications** must be set for each ingredient and each finished product. Standards and specifications usually include
- A Physical Attributes
  - 1 Freezing & Boiling Points
  - 2 Shear Strength
  - 3 Color
  - 4 Density
  - 5 Solubility
- B Chemical Characteristics
  - 1. Food Chemicals Codex Specifications
  - 2. pH
  - 3. Nutrient Content
  - 4 Heavy Metal Limits
- C Organoleptic Qualities
  - 1. Flavor
  - 2. Aroma
  - 3 Texture
  - 4 Mouth-Feel

II. **Manufacturing Instructions** must be prepared for each product These include instructions for:
- A Specific Processing Equipment
- B Process & Product Times & Temperatures
- C Packaging Materials
- D. Package Fill Weight, or Volume Limits
- E. Product Labels and Labeling

III. **Specifications for In-Process Product** at key manufacturing points must be be prepared.

IV. **Sample Collection & Testing** must be established to insure that all standards are met.

V. **Information Feedback** procedures must be established to see that sample test results actually control production. This is where Quality Control gets its name

VI. **Recordkeeping & Record Reporting** procedures must be established and followed.

VII. **Plant Facilities Must Be Inspected** on a regular basis for:
- A. Sanitation & Good Manufacturing Practices
- B. Compliance with Corporate Standards
- C Safety
- D Environmental Control
- E. Energy Conservation

VIII. **Consumer Complaint Review & Action** procedures must be established and followed

Table 2—QUALITY ASSURANCE'S NINE MOST SIGNIFICANT FUNCTIONS

I. **Review Quality Control sampling schedules and procedures.**

II. **Develop Effective Market Withdrawal & Product Recall Systems** for those rare exceptions where the goal of Zero Defects has not been met.

III. **Prevent Criminal Indictments** of individuals not personally responsible for serious transgressions.

IV. **Design & Implement a Quality Control Exception Report** for periodic Quality Assurance reports to management

V. **Undertake a Hazard Analysis & Critical Control Point (HAACP) evaluation** for each product line in each plant.

VI. **Devise Statistical Quality Control methods** to minimize Quality Control costs and enhance consistency of product quality.

VII. **Seek Out & Develop Improved Laboratory methods** and instruments for use by Quality Control.

VIII. **Assist Quality Control Laboratories** with particular problems.

IX. **Serve as Expert Witness** in product liability cases.

From: Lushbough, C. H. 1978. Practical applications of the QA concept in an operating environment. Food Technol. 32: 46–47.

Appendix C

Product Recall

(Policy, Procedures, Industry Responsibilities)

Introduction: The following is a review of a product recall program for food processors. It is not intended to be an all inclusive recall strategy but rather an educational tool to inform food plant personnel of recall procedures and company's responsibilities for recall preparation and implementation. It is designed to stress the importance of routine plant activities such as raw material inspection, coding, processing data documentation, reporting, record keeping, etc.

Even though management personnel are usually directly involved with planning and implementing recall strategies, a recall may be initiated do to the failure of processing procedures, equipment, and/or personnel. The manner in which a company handles recall procedures can mean the difference between successfully correcting the problem (leaving the company's reputation untarnished) and unsuccessfully solving the situation (losing consumer confidence and compounding regulatory problems).

I. Recall Policy

There are basically three types of recall policies.

A. Voluntary recalls are the most common method of effectively removing or correcting consumer products in violation of FDA and USDA regulations.

B. Recalls may be requested by the regulatory agencies due to the product being harmful, a risk to the public, or out of compliance with food regulations.

C. Product seizures occur when manufacturers refuse to recall a product or when the recall is ineffective and the violation continues.

II. Recall Classification

Recalls are classified according to the potential hazard they impose to the public.

A. Class I recall is the situation in which there is reasonable probability that the use of, or exposure to, a violative product will cause serious adverse health consequences or death.

B. Class II recall is the situation in which use of, or exposure to, a violative product may cause temporary or medically reversible adverse health consequences or where the probability of serious adverse health consequences is remote.

C. Class III recall is the situation in which use of, or exposure to, a violative product is not likely to cause adverse health consequences.

III. Hazard Evaluation

An important step in a recall procedure is to evaluate the existing hazard to determine if a recall is warranted. The company or regulatory agency

should answer the following:

A. Has disease or injury already occurred?

B. Could exposure to humans be a health hazard?

C. Assess hazard according to population of greatest risk (children, hospitals, retirement comm., etc.)

D. Degrees of seriousness if exposed?

E. Likelihood of occurrence (exposure)?

F. Consequences of hazard of hazard (immediate or long term)?

G. Classify I, II, III, recall

IV. Recall Strategy

All companies should have a detailed plan to follow should the hazard evaluation warrant a recall. Some critical elements to consider in planning recall strategies are:

A. The depth of recall (warehouse, wholesale, retail, consumer levels).

B. Whether or not to issue a public warning, to what segment of the public the warning would be directed, and through what media (general: newspapers, TV, radio or through special groups: doctors, etc.).

C. Methods of recall effectiveness checks to determine how many have received the recall notice.

Effectiveness check level A = 100% notified
level B = between 10 and 100% notified
level C = 10% notified
level D = 2% notified

V. Companies Responsibilities During Product Recall

During recall procedures the company is expected to furnish upon request information as to the following:

A. Identify the product*
B. Reason for removal - date of defect discovery
C. Evaluation of risk due to defect
D. Total product quantity involved*
E. Information on product distribution*
F. Copy of company's recall strategy
G. Copy of company's recall communications
H. Name of a company employee to contact in reference to recall procedure.

*Information based on coding, recording, processing data, etc.

VI. Recall Communications

During a recall all communications to consumers, retailers, distributors, etc. should be handled properly.

tain:

1. name of product recalled
2. a statement that distribution and sales of product should cease
3. notification to customers (when appropriate)
4. explicit instructions on what to do with the product

B. Communications should be implemented by telegrams, telephone, mailgrams, or letters and documented.

C. Identification with product codes is important.

D. Explain the reason for recall; explain the hazard involved (if any).

VII. Other Recall Guidelines

A. Recall status reports keep everyone informed as to the recall progress. These reports would describe such things as numbers contacted, number responding, quantities of product recovered, and estimated time of completion.

B. Recall termination is necessary to end a product recall. When the product is recovered or all reasonable efforts have been made to recover it, a communication should be drafted requesting termination. This may go to FDA or USDA with the last status report.

VIII. Recall Summary

When the system fails and product retrieval is necessary:

A. Confirm product defect
   1. potential health risk
   2. potential consumer dissatisfaction
   3. probable amount and distribution of product

B. Decide whether to retrieve product and what level

C. Locate product

D. Isolate or recover product

E. Dispose of product

F. Maintain appropriate communications
   1. internal
   2. customer
   3. consumer/press
   4. regulatory agency

IX. Why Prepare for Recall?

A. Regulatory agencies can issue public warnings

B. Recalls and seizures are not uncommon. They occur at a rate of about 400/per year.

C. It is one of the major responsibilities of all food processors to produce safe and wholesome food for the consuming public.

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