Experimental

Extraction of the Oil Under Nitrogen. Grinding of the seed was carried out in a one-gallon capacity, steel ball mill containing 50% of capacity of $\frac{1}{2}$ " stainless steel balls. A minimum of 12 hours was allowed for reducing the seed to a pulverized state. The ground seed was separated from the balls under nitrogen. This was done in a cardboard container covered with a plexi-glass top and fitted with one opening for the introduction of nitrogen and two openings, cut in the sides, for hands. The ground sample was stored under nitrogen prior to extraction in a 500-ml. capacity Soxhlet extractor with petroleum ether. Care was exercised to prevent exposure to air during the extraction process. The solvent was removed under vacuum at 15°C. and the crude oil was placed under nitrogen and stored in the dark. Oxygen was removed from the nitrogen used throughout the experiment by washing with Fieser's solution.

The freshly extracted Korean lespedeza seed oil was a clear, yellow-green oil. A yield of 7.5% of oil was obtained. This oil had a saponification number of 189.3, an acid number of 1.2, and a refractive index of $N_{20}^{D} = 1.4770$.

Determination of the Change of Saponification Number With Exposure to Air. Samples of approximately one g. of lespedeza seed oil obtained as above were weighed into 125-ml. Erlenmeyer flasks and exposed to sunlight and air. The saponification number of the oil was determined by A.O.C.S. Official Method Cd3-25 on duplicate samples at the following hourly intervals: 0.167, 1, 4, 10, 16, 24, 40, 64, 88, 110, 134, and 158. The data obtained are shown in Figure 1.

Rate of Formation of Cloudiness Under Nitrogen and in Presence of Hydroquinone. To each of three 3-in. test tubes was added a one-g, sample of clear Korean lespedeza seed oil. The first tube was stoppered after adding 0.1 g. hydroquinane. The second tube was flushed with oxygen-free nitrogen and stoppered, and the third tube was stoppered under air as a control. The tubes were observed at intervals for formation of cloudiness or a precipitate. After three hours the control had turned cloudy. The sample under nitrogen was clear for six days and cloudy on the seventh day. The sample stabilized with hydroquinone was clear for 13 days and on the 14th became cloudy. All deposited a precipitate a few days after cloudiness was first noted.

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The Buffalo Gourd, a Potential Oilseed Crop of the Southwestern Drylands¹

H. S. SHAHANI,² F. G. DOLLEAR, and K. S. MARKLEY, Southern Regional Research Laboratory,³ New Orleans, Louisiana, and

J. R. QUINBY, Texas Agricultural Experiment Station,⁴ Chillicothe, Texas

THE Buffalo gourd, *Cucurbita foetidissima* **H.B.K.,** is a wild gourd that is native to the Great Plains, the Southwestern United States, and Northam the Southwestern United States, and Northern Mexico. It was first described from growing specimens by Edward James in 1820, who stated that it was found in "arid and sandy wastes, along the base of the Rocky Mountains from the confluence of the Arkansa, the Boiling Spring Fork, to the sources of the Red River" (7). It is now known to be growing Wild in South Dakota, Nebraska, Kansas, Missouri, Oklahoma, Texas, New Mexico, Colorado, Utah, Arizona, Nevada, and California; and in Mexico as far south as Guanajuato (8).

This gourd is a long-lived perennial with dull green or gray stems and leaves. The stems and leaves are harsh to the touch, and the crushed foliage emits a fetid odor. After midsummer this odor disappears, but at the time of wheat harvest or cultivation of summer growing crops the odor is very pronounced when the plants are cut or bruised. The trailing vines grow to be 8 to 10 feet long, and a single plant in a good location can occupy 300 square feet of land area. Twenty or more vines usually emerge from a single root. The roots grow to great size, and roots six feet

long and 12 inches in diameter are not unusual. The plants persist for many years. The leaves are heartshaped and as large as a man's hand and are numerous enough to cover the ground completely. The sulfur-yellow flowers are numerous and conspicuous.

The gourds are usually about the size of a tennis ball, and two or three green fruits will weigh a pound. There is an abscission layer, at which the fruits separate from the vines at maturity. Pioneer women used the fruits as darning balls. When green, the fruit is hard and bitter to the taste and the seeds are attached to a mat of tough, coarse fibers. In the spring when the fruits are dry, they crush easily and the seeds separate readily from the fibrous mass. The flat, ovalshaped seeds, which resemble melon seeds, are about $\frac{1}{2}$ -inch long and about $\frac{5}{16}$ -inch wide. The fruits are eaten by cattle and horses, and the seeds are eaten by rodents. The literature contains many references relative to the use of the seeds for food by the American Indian. Curtis (9a) and Ault *et al.* (1a) have recently pointed out the potential value of the seeds of perennial cucurbits as a source of vegetable fat and protein.

Although this gourd has a wide natural geographic distribution, the plants are usually sparsely distributed on uncultivated land. The plants were very conspicuous in the short-grass territory, and before the South Plains area of Texas was put into cultivation 40 years ago, single isolated plants could be seen at the rate of two or three to each section: (square mile)

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³ One of the laboratories of the Bureau of Agricultural and Industrial

³ One of the laboratories of the

Association.

of land. Occasionally there are colonies where several hundred plants per acre occur over an area of several acres.

There is some evidence that plants of this species have become more numerous since the land has been cultivated. In the sandy soils of the Rolling Plains area of Texas many fence rows are seen which contain solid stands of this gourd, and the plants are known to persist in this area on heavy wheat land that has been in cultivation for 50 years. Even when the above-ground parts of the plants on wheat land are destroyed by cultivation after the wheat is harvested in June, they come up and grow until the land is plowed again. Seed is usually not produced under these circumstances, but the plants persist and grow so vigorously in the spring that the vines grow' into the wheat and the combine sickles must be lifted above the wheat heads to prevent the vines from "choking" the combines.

Plants growing in cultivated fields of cotton and sorghum will produce fruit with viable seeds after the crops have been "laid by." The large storage root accounts for the persistence of this gourd in cultivated land, and it appears that the number of plants have a tendency to increase in cultivated areas unless measures are taken to eradicate them. In the past this destruction has usually been done by digging to the large root with a post-hole digger and killing it with salt or kerosene oil.

If this gourd were brought under cultivation it would be a cheap crop to grow. At the Texas Agricultural Experiment Substation No. 12, Chillicothe, Texas, in the Rolling Plains area just south of the Red River and just east of the 100th meridian which is the eastern boundary of the Panhandle of Texas, the species starts spring growth early in April. Spring growth after the first year is very rapid. It appears that all the work necessary to produce a crop would be to harvest the gourds some time after a freeze in the fall and before growth starts in the spring and then give the land one cultivation with a one-way plow or implement that will do similar work. Nothing further would be necessary until the crop was to be harvested again.

The plant is resistant to the diseases found in the area of its adaptation and is immune to the cotton root rot fungus, *Pymatotrichum omnivorum (Shear)* Duggar (18). It is visited by insects of many kinds, but aphids do not persist on it and, although the Striped, *Diabrotica vittata,* Fabricius and the Spotted, *Diabrotica duodecimpunctata,* Fabricius cucumber beetles infest *C. foetidissima* to some extent, damage is relatively slight even to the flowers. It thrives when the other wild cucurbits, *C. digitata* and *C. palmata*, and the common cultivated annual squashes and pumpkins are killed completely by cucumber beetles.

Experimental Plantings

The Buffalo gourd is growing under cultivation at Texas Substation No. 12 at Chillicothe. It is easy to obtain a stand of plants from seed, but growth during the first summer is slow. As the season advances, growth is more rapid, but at. the end of one growing season the plants will usually consist of 1 to 4 vines less than 5 feet long and the roots will be two feet or more long and 2 or 3 inches in diameter. The top of the fleshy root is usually 4 or 5 inches below the surface of the ground. Stands can also be established

by setting out small roots that develop at the nodes of trailing vines when growing conditions are favorable. The growth from such roots is much more rapid than from seeds. If plants in their second year are spaced six feet apart in both directions, the entire surface of the ground will be covered with foliage in a month after growth starts in the spring. The proper spacing of plants commensurate with the largest yield of seed has not been determined. Probably the spacing can be varied considerably with only negligible influence on the yield of seed.

Plantings have been observed for three years, and only an occasional fruit is produced the first year from seed. In the second year of growth the plants appear as vigorous as much older plants. A small planting made in the spring of 1947 produced 37,400 gourds per acre in 1948. The yield of seed was 832 pounds per acre. In 1949 this same area produced 706 pounds of seed per acre. This difference in production is undoubtedly a normal seasonal fluctuation. Another planting made in 1948 produced a crop of seed in 1949. The average seed yield from six 1/500 acre plats was 1,287 pounds per acre. The lowest yield of the six was $1,000$ pounds and the highest, 1,977 pounds. Ten fruits from one plat yielded 55 grams of seed whereas the same number from another plat yielded 132 grams. With such a wide variation in the number of seeds per fruit and in the number of fruits per plant, there is considerable opportunity to improve yield through selection of high-yielding individuals. Since this gourd can be propagated asexually, first generation hybrids could be used to increase yields of seed.

Experimental

Material. The seed used in this investigation was harvested from wild plants growing in the vicinity of Chillicothe, Texas. The fruits, which were produced in the spring of 1948, were picked in the spring of 1949 along roadsides, on fence rows, and along the right-of-way of the Fort Worth and Denver City Railway. The dry fruits were passed through a grain thresher, and the seed was reeleaned with standard seed-cleaning equipment.

The' seed (145 lb.) as received at the laboratory contained 12.7% of foreign matter consisting of hull fragments, stones, sticks, and other foreign matter. The seed coats were yellowish-gray and the endosperms green in color. The composition of handcleaned seed is given in Table I.

Extraction of the Oil. Prior to extraction of the oil, the seed was cleaned by blowing with air and by separation on a fine shaking-screen to remove the bulk of the light material and dirt. The seed was cleaned further by screening through $\frac{1}{4}$ -in. screen to remove sticks and stones. The cleaned seed was

cracked between corrugated rolls set 0.02 in. apart and then flaked between smooth rolls set 0.002 in. apart. The flaked seed had an average thickness of 0.01 in. The flakes (121 lb.) containing 24.3% lipids and 9.6% moisture were charged into the batch extractor (16) and covered with commercial hexane (b.p. 63- 65°C.). After standing over-night the flakes were extracted for 12.5 hours at a temperature of 100°F. The miscella was concentrated to an oil content of 94.5% by evaporating the solvent under reduced pressure at 180° F. (82.2°C.). The remaining solvent was removed by heating the oil under reduced pressure and passing carbon dioxide through it. The yields of oil and meal were 27.5 lb. and 88 lb., or approximately 23% and 73%, respectively. The recovered oil was dark reddish brown in color with green tinge. The composition of the extracted meal is given in Table I.

Although the protein content of the undecorticated seed and extracted meal is relatively high, 31.7% and 42.1%, respectively, they contain a very high content of crude fiber, 26.5% and 35.2% , respectively, and a high ash content, 4.84% and $7.98\%,$ respectively. In order to be valuable as a stock feed the seed would have to be decorticated prior to extraction or the extracted meal processed further to reduce the crude fiber content.

Characteristics of the Crude Oil. The characteristics of the crude oil were determined by the methods prescribed by the American Oil Chemists' Society (1) except for the unsaponifiable matter (9), thiocyanogen value (13), and hydroxyl value (19), which were determined by the methods described in the references cited. The saturated acids were determined by the Pelikan and yon Mikusch (15) modification of the Bertram oxidation method except that sintered glass filter sticks were used for filtering the magnesium soaps. The characteristics of the crude oil are given in Table II.

When calculated from the iodine and thiocyanogen values (1), the composition of this oil was found to be linolein 66.7%, olein 23.9%, and saturated glycerides and unsaponifiable matter 9.4%.

The composition of the oil calculated from spectrophotometric absorption data after alkali isomerization (2) and by using the extinction coefficient for linoleie acid proposed by Swain *et al.* (17) was found to be as follows: linolein 65.3%, olein 24.1%, and saturated glycerides and unsaponifiables 9.6%. The spectrophotometric analysis of the unisomerized oil indicated the presence of 0.35%, 0.63%, and 0.016% of conjugated diene, triene, and tetraene constituents, respectively.

Saturated Acids. The crude oil was saponified with alcoholic potassium hydroxide. Without removing un-

1 : 1 **ratio of oil** in CCI4 in one-inch cell.

saponifiable matter, the soaps were converted into free fatty acids by warming with dilute sulfuric acid and the liberated fatty acids removed by extraction with ethyl ether and dried under reduced pressure on a steam bath. The mixed fatty acids (1,413 g.) were separated into solid and liquid acids by crystallizing from acetone at low temperatures, according to the procedure suggested by Earle and Milner (10) for the quantitative determination of the saturated fatty acids in soybean oil.

A 10% solution of the mixed fatty acids in acetone was cooled to -40° C. to separate the solid acids, which were then recrystallized twice from the same concentration of acetone solution at -40° C. The recovered solid acids (139.5 g.), which represented a yield of 9.9% of the total mixed fatty acids, had an iodine value of 10.8 and a neutralization equivalent of 283.0. On the assumption that the unsaturated acid was oleic the actual yield of saturated acids was 8.7%.

The solid acid fraction was converted into methyl esters and distilled at reduced pressure without attempting any fraetionation. The distillate was then fractionally distilled at 0.28 mm. pressure through a column 18 mm. I. D. and 56 cm. long, packed with $\frac{3}{16}$ -in., single-turn, glass helices. This column was surrounded with hot circulating Arochlor in a doublewalled Thiele-tube insulating jacket (14) to maintain the distillation as adiabatic as possible. Six fractions and a residue were collected. The iodine value and saponification equivalent of each fraction were determined and the composition of methyl esters calculated from these data with the results shown in Table III. From the data for the composition of the methyl esters the percentages of saturated glycerides in the oil were calculated to be as follows: C_{14} 0.17%, C_{16} 6.13%, C_{18} 2.22%, C_{20} 0.34%, and C_{22} 0.21%.

The composition of *C. foetidissima* oil, calculated from speetrophotometric absorption data for linolein and conjugated constituents and methyl ester distillation data for saturated glycerides, is given in Table IV. Olein has been calculated by difference.

^a Calculated from spectrophotometric absorption data.
^{b C}alculated from methyl ester distillation data.
[¢] Determined independently.

Refining. The crude solvent-extracted oil used in refining tests, contained 2.0% free fatty acids calculated as oleie. The color of the oil was too dark to be read on the Lovibond scale even in a one-inch cell. A 1:1 mixture of the crude oil and carbon tetrachloride had a Lovibond color of 35 yellow and 18.9 red measured in a one-inch cell. The oil was refined according to the procedure,of the American Oil Chemists' Society (1) except that in some cases only 200 grams were used together with variations in the time of stirring and the percentage of excess lye. The results of the refining tests are shown in Table V, from which it may be seen that neither the standard A.O.C.S. method or any modification thereof produced a refined oil of good

							Calculated composition					
Fraction No.	Weight. g.		Temperature in °C.		Saponifi- cation	Iodine value			Saturated			Unsatu- rated
		$_{\rm{Pot}}$	Column	Distillate	equiv.		C_{14} g.	$\mathrm{C_{16}}$ g.	C_{18} g.	C_{20} g.	C_{22} g.	C_{18} g.
Residue	18.12 22.42 17 8.31 20.42 7.67 4.20	194-196 196-201 201-206 206-211 211-230 230-240 	143-145 145 145 145-169 169-174 174-176 ********	119-122 122 122-124 124-140 140-143 143-154 	268.72 269.90 270.10 289.44 297.91 301.77 339.06	0.00 0.00 0.56 50.64 28.65 4.02 0.39	1.00 0.38 $_{0.28}$ AAAAAA44 	17.12 22.04 17.10 2.18 	 1.22 13.52 6.31 	 0.07 1.00 2.20	 1.98	 0.11 4.91 6.83 0.36 0.02
	98.63					********	1.66	58.44	21.05	3.27	1.98	12.23

TABLE III Fractionation Data for the Methyl Esters of Solid Acids of C. foetidissima Oil

color. Even on re-refining with the 40% lye as suggested by James (12), the color of the oil was too dark to be read in a 5.25-inch cell. Refined oil of lowest color was obtained with the slow break method using 0.65% excess of 16° Bé. lye. A batch of 12 lb. of oil was refined in a stainless steel laboratory refining kettle using 0.65% excess of 16° Bé. lye. The color of this refined oil was the same as obtained in the cup refining tests using the same lye.

Bleaching. All of the oils, regardless of method of refining, were much darker in color than oils generally used in edible products. The refined oils were bleached according to the procedure of the American Oil Chemists' Society (1) with the exception that the size of sample and the type of adsorbent used were varied in some cases. The American Oil Chemists' Society's official natural bleaching earth, official activated earth, a commercial activated earth (Activite⁵), and a combination of official activated earth and carbon (Nuchar C-115 N) were used. In one test, using the commercial activated earth, the oil was bleached at 82°C, which gave a product of slightly lighter color than that obtained at the usual bleaching temperature $(120^{\circ}C)$. The results of bleaching tests are recorded in Table VI. In addition to the bleaching tests given in Table VI a batch of 8 lb. of oil was bleached with 4% official activated earth. The color of the bleached oil, measured in a 5.25-inch cell, was 35 yellow and 3.2 red Lovibond units.

Deodorization. The refined and bleached oil was steam deodorized at 220°C. and 2 mm. pressure for one hour, in an all-glass laboratory deodorizer (6). The deodorized product had a bland flavor with a

 $\overline{\text{B}}$ in bleaching earth (Activite) and carbon (Nuchar O.115 N) are named as part of the specification of the exact experimental conditions, and do not imply that these products are particularly endorsed or recommend

TABLE VI Effect of Bleaching C. foetidissima Oil with Various Adsorbents

Oil.	Adsorbent	Color, Lovibond red units			
g.	Type	Per cent	Refined oila	Bleached oil ^b	
300	A.O.C.S. natural earth	5.25	4.1	5.5	
125	A.O.C.S. activated earth	4	4.1	4.2	
150	Activite	$\overline{\mathbf{4}}$	10.1	11,3	
150	A.O.C.S. activated earth	4	6.6	3.2	
150	Activite	4	6.6	10.1	
150	Activite ^c	$\overline{\bf{4}}$	6.6	9.1	
150	A.O.C.S. activated earth Carbon	4 0.2	29.0	3.2	
150	A.O.C.S. activated earth Carbon	4 0.2	3.2	2.9	
300	A.O.C.S. activated earth	$\overline{\bf 4}$	2.7	2.6	

^a Measured in one-inch cell with 70 yellow glass.

^b Measured in 5.25-inch cell with 35 yellow glass.

^c Temperature 82°C, instead of 120°C.

keeping time of six hours by the active oxygen method (3) at 97.7°C., using a peroxide value of 100 milliequivalents per kilogram of fat as the end point. Deodorization reduced the color of the oil from 35 yellow and 3.2 red to 35 yellow and 2.7 red Lovibond units. In some instances when the oil was deodorized at higher temperatures or for a longer time the color was either unaffected or darkened slightly.

The refined, bleached, and deodorized oil did not undergo flavor reversion when stored at room temperature (ca. 27° C.) in an open beaker for more than a month. The deodorized oil is a natural winter oil,

b Measured in 1-inch cell with 70 yellow glass.

"Refined oil was turbid.

"Lye separated from oil, loss could not be determined.

s Color of refined oil used, 70 yellow and 11.5 red.

passing the 5-hour cold test, and does not deposit crystals on long standing in refrigerator at *ca.* 3°C.

Hydrogenation. Eight pounds of the alkali-refined and bleached oil was hydrogenated under conditions of moderate selectivity. The hydrogenation was carried out at 149°C.(300°F.) under 15 p.s.i, hydrogen pressure, with 0.1% dry-reduced, electrolytically precipitated nickel catalyst (4) in the apparatus described by Bailey *et al.* (5). Progress of the hydrogenation was followed by periodically removing samples for analysis. The reduction in iodine value proceeded smoothly, but in contrast to the hydrogenation of cottonseed oil the temperature of the reaction decreased during hydrogenation and heat had to be supplied during the process.

Data with reference to the characteristics and composition of the hydrogenated oils are given in Table VII. No change occurred in the color of the hydrogenated oil until the iodine value was reduced to 69.0 when it changed suddenly from 35 yellow and 2.9 red to 20 yellow and 2.5 red Lovibond units. A plot of the iodine value *vs.* refractive index gave a straight line.

The compositions of the hydrogenated oils shown in Table VII were calculated on the basis of mixed glycerides of the individual fatty acids from the spectrophotometric adsorption after alkali isomerization (2) and from iodine and thioeyanogen values (1). The saturated acids were determined by the Bertram oxidation method (15) and the modified Twitchell lead salt-alcohol method (1). The iso-oleic acid was also determined by the lead salt-alcohol method (1).

Examination of the data in Table VII indicates that the agreement between the two methods of determining the composition is not very satisfactory. The content of linoleie acid calculated from the spectrophotometric data is, in all cases, 2 to 3 units lower than that found by calculation from the iodine and thiocyanogen values. This discrepancy is probably attributable to the presence of an iso-linoleic acid, formed during the hydrogenation, which either does not yield a conjugated system of double bonds upon alkali 'isomerization or conjugates to a lesser extent than either the natural or debrominated linoleic acid.

That calculation of the composition from iodinethioeyanogen values yields consistent results with regard for the hydrogenated fats is evident from Figure 1 in which the composition of the fatty acid glycerides is plotted as a function of iodine value of the fat.

The consistencies of the oils, hydrogenated to various iodine values, were determined according to the micropenetration technique described by Feuge and Bailey (11). Micropenetrations as a function of

FIG. 1. Glyceride composition of hydrogenated *C. foetidissima* oil as a function of iodine value.

temperature are plotted in Figure 2 for the oil hydrogenated to three different iodine values. The oll hydrogenated to an iodine value of 71.6 had a shortening consistency.

Absorption Spectra of Processed Oils. Curves for the visible absorption spectra of the crude, refined, bleached, and deodorized oils are shown in Figure 3. The absorption maximum at $418 \text{ m}\mu$ for the crude and refined oils may be attributable to the presence of an oxy-flavone. The maxima at 527, 567, and 590 m_{μ} may be attributed to an unidentified plant pigment which is responsible for the dark color of the oil. Maxima at 623, 635, and 670 m μ are caused by the presence of the green pigment, pheophytin.

The presence of abnormal pigments in the crude and refined oils and the inability to reduce the color of the processed oils to acceptable values may be the result of alteration of the original pigments in the seed through weathering in the field. Resistance to bleaching and the grayish cast of the hydrogenated oil are not unlike the related phenomena encountered in oils from frost-damaged soybeans and field-weathered cottonseed. If indeed this proves to be the case, it will be necessary to harvest the gourds in fall and dry them out of the weather.

Summary

The seed of the Buffalo gourd, *Cucurbita foetidissima*, one of the wild gourds native to Southwestern

^a Calculated from iodine and thioeyanogen values.
^b Calculated from spectrophotometric analysis, density of the salt separation method.

FIG. 2. Micropenetration of hydrogenated *C. foetidissima* oil.

United States and Northern Mexico, has been examined with respect to its potential use as a dryland oilseed crop.

The undecorticated seed was found to contain 9.6% moisture, 31.7% protein, 26.5% crude fiber, 4.8% ash, and 24.3% extractable lipids (crude fat). After extraction with a hydrocarbon solvent the meal was found to contain 8.6% moisture, 42.0% protein, 35.2% crude fiber, 7.98% ash, and 0.36% residual lipids.

Although both the seed and extracted meal are high in protein, they are also high in ash and crude fiber and very low in total sugar consequently the seed would have to be decorticated prior to extraction or the meal processed further to make it suitable as a stock feed.

The characteristics and composition of the crude extracted oil were examined. The oil had an iodine value of 136.1, which places it in the semi-drying class along with, but slightly above soybean oil.

The oil was found to have the following fatty acid composition, calculated as glycerides: olein 23.0%, linolein 65.3%, diene glycerides 0.37%, triene glycerides 0.66%, tetraene glycerides 0.02% myristin 0.17%, palmitin 6.13%, stearin 2.22%, C_{20} and higher glycerides 0.55% , and unsaponifiable 1.53% .

The crude oil was very dark in color and exceedingly resistant to bleaching. Refining, bleaching, and deodorization gave a bland oil with good stability and no tendency to revert in flavor. However the color at each stage of processing was inferior to that of other common edible oils such as cottonseed, peanut, and soybean. Hydrogenation to shortening consistency gave a fat of excellent stability (250 hours by the active oxygen method) but was likewise inferior color to other common hydrogenated oils. The abnormal pigmentation of the crude and processed oils may be attributed to alteration of the original color bodies of the seed as a result of weathering of the fruit in the field.

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