Microwave Heating to Prevent Deterioration of Cottonseed During Storage

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Fuzzy cottonseed samples of 14.5% moisture and <1.0% free fatty acids (FFA) contents heated in a conventional, home-style microwave oven at 700W and 2450 MHz for intervals up to 2.0 min. The 2.0-min treatment reduced the moisture content to 13.1%. Examination of the seed immediately after microwave heating (MWH) indicated no differences in the proteins or in the quality or quantity of the cotton linters as compared with unheated seed. Neither the oil content of the seed nor the quality of the oil were affected by the microwave treatment. After nine weeks of storage at 50°C, the unheated seed had a FFA content of >3.0% while the FFA content of the 2.0-min microwave-heated seed remained <1.0%. During this storage period there was significant deterioration of the protein quality of the unheated seed. The 2.0-min MWH treatment, however, maintained the integrity of the protein during storage.

KEY WORDS: Cottonseed, free fatty acids, inhibition of deterioration, microwave heat, oil, protein.

Cottonseed is a source of high-quality edible oil; a residual meal that provides protein for animal and/or human consumption; linters, which are cellulosic materials used in the manufacture of many products, including cotton batting and bond paper; and hulls, which are used as roughage in animal feeds and as soil conditioners. Seed quality and economic value are based on the amount of foreign matter in the sample, contents of nitrogen (protein), moisture, oil, and the free fatty acid level in the oil. The oil, representing about 17% of the fuzzy seed, is the most important product.

Deterioration of cottonseed during storage prior to crushing for oil has been a problem for the cottonseed processing industry for more than a century. As deterioration occurs, the free fatty acid level of the oil contained in the seed increases along with a concomitant decrease in quality and economic value. Refrigeration of storage houses to keep seed temperatures below 10°C could prevent deterioration, but would not be cost effective, considering the volume of seed and the cost of energy. Altschul (1), in the 1940s and, recently, Lusas (2) demonstrated the applicability of chemical treatment prior to storage to prevent formation of free fatty acids in cottonseed during storage. These methods, however, have limited use because of safety hazards or adverse effects on seed components other than the oil. Today, oilseed processors draw air through the seed piles to reduce moisture content and temperature, and thus prevent free fatty acid formation. This is an inefficient and energy-intensive operation, particularly if the seed has a high moisture content when received at the oilseed mill.

In 1948, Lyman *et al.* (3) exposed moist cottonseed to a radio frequency electric field at 1 KW and 14 MHz for various intervals up to 5 min. Treatment times of more than 1 min reduced the formation of free fatty acids during subsequent storage of the seed. No further work was done along these lines until the 1970s when the use of microwave heating to dry seed cotton, *i.e.*, cottonseed with the lint still attached, was proposed. Wesley *et al.* (4) were concerned with the possibility of the penetrating microwave energy affecting cottonseed quality before efficient drying of the lint could be achieved. Therefore, they exposed cottonseed to 2–10 min of microwave heat at 200W, 400W or 600W at 2450MHz. No adverse effects were noted on the quality of the oil from these seeds. The germination potential of the seeds, however, was reduced to <10%. Anthony (5) dried seed cotton with microwave heat at 300W for 55 min or 600W for 27 min at 2450MHz without affecting oil quality of the seed. However, the germination potential was reduced to <1% and there was also an increase in the numbers of damaged seeds. Lyman *et al.* (3) did not evaluate the effect of the heat treatments on any other quality characteristics of the seed. Neither Wesley *et al.* (4) nor Anthony (5) evaluated the seed after storage.

Other investigators have studied the effects of microwave heat on various components of grains or seeds. Campaña *et al.* (6) used microwave heat to dry wheat without appreciable damage to the protein, provided the temperature of the grain did not exceed 65°C. Field corn can be dried with microwave heat provided the power input and/or the processing time are controlled to limit swelling of the kernels (7). Pour-el *et al.* (8) used microwave heat to produce soybean products of potentially high nutritional value. Microwave heating of some small-seeded legume species rendered these hard seeds permeable to water, thereby increasing germinability (9).

In the experiments reported here, cottonseed samples were exposed to microwave heat at 700W and 2450 MHz and then examined to evaluate the immediate effects on seed moisture, viability and microbial content, oil and protein content and quality, and linters content, appearance and quality. Selected parameters were evaluated after storage of the seed under conditions designed to accelerate seed deterioration. Results were compared with those from unheated cottonseed.

MATERIALS AND METHODS

Cottonseed from the 1988 and 1989 crop years in Mississippi were supplied by Yazoo Valley Oil Mill (Greenwood, MS). Moisture content of the seed as received at the laboratory was 8%.

The microwave oven was a Panasonic, Model NE-8070, 700W full power, 2450 MHz operating frequency, equipped with a turntable (Matsushita Electric Corp. of America, Seeaneas, NJ). The equilibration and storage unit was a wooden cabinet equipped with a heater and a

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forced-air blower thermostatically controlled to maintain a temperature of 50 ± 2°C.

Cottonseed was equilibrated to the desired higher moisture content by placing 500–1200 g of seed in a desiccator over a saturated solution of either $NH_4H_2PO_4$ or $CuSO_4.5H_2O$ for 72 hr in the cabinet at 50°C.

Microwave heat treatments. Microwave heating was conducted in either closed plastic bags or open glass containers. Heating times were 0.5, 1.0, 1.5 or 2.0 min. Immediately after removal from the microwave oven, the seeds were placed in a pile on thick paper towels. This separated the seeds from the moisture which collected in the bags or containers during the heating cycle and allowed the temperature of the samples to be measured by inserting a thermometer into the seed pile. The seeds were mixed thoroughly when each sample reached ambient temperature (22°C). A sample was collected to establish the moisture content and other seed characteristics at the start of the experiment. The remaining seeds were packed into sterilized Mason jars and stored in the cabinet at 50°C. One jar representative of each treatment was removed for analysis after three, six or nine weeks. Upon removal from storage, the samples were cooled to 22°C, then maintained at −18°C until analyzed.

Three successive experiments were conducted with fuzzy cottonseed of varying free fatty acid (FFA) and moisture contents.

Experiment 1. The seed/sample was 120 g; with 0.75% FFA; and 11.4% moisture. Microwave heat treatment consisted of closed plastic bags, for 1.0- and 2.0-min intervals. Storage was three and six weeks.

Experiment 2. The seed/sample was 200 g; with 0.24% FFA; and 13.8% moisture. The microwave heat treatment was closed plastic bags for 0.5-, 1.0- and 2.0-min. An open glass dish (190 × 100 mm) was used for 1.0- and 2.0-min. Storage was six and nine weeks.

Experiment 3. The seed/sample was 200 g; with 0.83% FFA; and 14.5% moisture. The microwave heat treatment consisted of open glass dish (190 × 100 mm), at 1.0-, and 1.5- and 2.0-min. Storage was six and nine weeks.

Analytical methods. Moisture contents were determined with a Sartorius Thermo Control Infrared Dryer (Sartorius Balances, Bohemia, NY). When using this unit, analysis of 5-6 g samples of cracked cottonseed provided data equivalent to that obtained by the official AOCS method (10). Free fatty acid (FFA) contents were determined by the official AOCS method (10), except that the oil was extracted with a Stomacher Lab-Blender 400 (Tekmar Co., Cincinnati, OH). Comparative oil contents of these cottonseed samples were determined by weighing the oil extracted for FFA determinations. Insufficient amounts of seed were available for replicate analyses of each sample from each treatment. However, five replicates of one cottonseed sample indicated the following standard deviations for FFA and oil contents, 1.20 ± 0.3% and 25.7 ± 1.7%, respectively. Triglyceride profiles were obtained by high-performance liquid chromatography (HPLC) according to Bland *et al.* (11). A Maxima 820 Chromatography Work Station, equipped with a Model 6000A solvent delivery system, a model U6K injector and a Model 410 Differential Refractometer, was used (Waters Assoc., Milford, MA). Two columns, 3.9 × 150 mm Nova-Pak C_{18}, in series, were maintained at 34°C. The Nova-Pak (Waters Assoc., Milford, MA) columns were preceded by a Micro-Guard ODS-10 guard column (Bio-Rad Labs, Richmond, CA). The eluent was acetone/acetonitrile (60:40, v/v) as an isocratic mixture at 1.5 mL/min and was degassed by sonication and cooled to 4°C. A 10-μL sample of a 10% solution of cottonseed oil in methylene chloride was injected.

Microbial content of the cottonseed was determined according to Klich *et al.* (12). Approximately 150 seeds were delinted with concentrated sulfuric acid, then rinsed three times with deionized water and air dried. The dry delinted seeds were surface-sterilized by stirring in an aqueous solution of sodium hypochlorite (2%, w/v) containing 0.001% (v/v) Triton X-100 for 2.0 min. The seeds were rinsed three times in sterile, deionized water. Using aseptic techniques, 100 seeds were placed on malt dextrose agar Petri plates (100 × 15 mm), five seeds per plate. After incubation at 25°C for seven days, the number of seeds visibly contaminated with mold or bacteria were counted. At the same time, the number of germinating seeds, *i.e.*, radicle clearly protruding from the seed coat, were counted. It had been established that this method of determining germination potential provided data equivalent to that of standard procedures.

Nitrogen content of the defatted meals was determined according to the official methods of the AOCS (10). Total protein content was calculated by multiplying the nitrogen content by 6.25. The meal was extracted with water, then with a 10% NaCl solution as described by Marshall and Conkerton (13). Nitrogen content of the salt-soluble fraction was determined by Kjeldahl analysis (10). Results were expressed as a percentage of total protein. The salt-soluble protein extracts were examined by HPLC (13).

Two additional samples of cottonseed were microwaved for 2 min in open containers. One sample, along with 200 g of unheated cottonseed, was forwarded to an independent, commercial testing laboratory for grading according to the procedures of the National Cottonseed Products Association (NCPA) (14), and for analysis of free gossypol content (10). The second sample, along with an equivalent amount of unheated cottonseed, was used for analysis of linters. Fiber cross sections were prepared from several seeds with a Hardy hand microtome (15). The fibers were carefully packed into the slot of the microtome, and the fiber bundle saturated with nitrocellulose lacquer. After the lacquer hardened, the bundle was trimmed flat and sections were cut by hand with a razor blade. Sections were mounted on glass slides in mineral oil for determination of the shape and size of cross sections by light microscopy. For measurement of external fiber structure, seeds were mounted on speciment stubs and coated by sputtering with gold-palladium (to prevent charging) prior to examination by scanning electron microscopy at several magnifications. The bulk of this sample of microwave-heated cottonseed and an equal amount of unheated cottonseed were forwarded to the testing laboratory for a determination of the amount of residual lint according to the official AOCS method (10).

Wherever possible, standard error of the mean for analytical determinations was determined. However, inherent variations from one seed lot to another, sample size restrictions in the microwave oven and/or sample size needed for analysis made statistical analysis of all data impractical.

RESULTS AND DISCUSSION

Demonstration of the potential of microwave heat. In Experiment 1, with cottonseed of 0.75% FFA and 11.4% moisture content, microwave heating in closed plastic bags for 1 min reduced the moisture content of the seed to 10.4%, while a 2.0-min heating period reduced the moisture content to 8.5%. After storage for three or six weeks in sealed glass jars, there was no change in the moisture contents of any samples. There was no apparent change in the FFA content of either the unheated or microwave-heated samples after three weeks of storage. After six weeks of storage, the FFA content of the unheated seed had increased to 2.30%, while that of the microwave-heated samples was 0.96%. Apparently, the three-week storage period at 50°C was not sufficient to allow deterioration of even the unheated seed. Therefore, to ascertain the sustained effectiveness of microwave heat treatments, additional experiments were designed to measure FFA formation after six and nine weeks of storage at 50°C.

Closed vs. *open containers for microwave heating of cottonseed.* In Experiment 2, seed of 0.24% FFA was adjusted to an initial moisture content of 13.8% and microwave treatments were varied. From the data in Table 1, for Experiment 2, it is apparent that moisture reduction induced by microwave heating was similar to that observed in the initial experiment and also that heating in open glass dishes paralleled the results obtained in the closed plastic bags. The data in Table 2 for these samples substantiate the FFA data obtained in Experiment 1. The additional three weeks of storage, *i.e.,* a total storage time of nine weeks, demonstrated the lasting effect of the microwave heating, particularly in the samples heated for 2.0 min, either in closed bags or open glass dishes. Since open microwave heating procedures would be more practical in oilseed processing mills, additional studies were conducted in the open glass dishes.

Effect of microwave heat on oil and other seed components. In Experiment 3, seed samples of 0.85% FFA and 14.5% moisture content received microwave heat treatments of 1.0-, 1.5- and 2.0-min in open glass dishes. In addition to determining the effects of microwave heating on the moisture and FFA contents, a more in-depth study on potential positive or negative effects of microwave heat on oil composition and other seed components was conducted.

From Experiment 3 (Table 1), it is apparent that reductions in moisture contents were similar to those noted earlier. The 20% reduction noted in the 2.0-min microwave-heated sample was slightly less than the 25% noted in the two earlier experiments, and the temperature reached in the seed pile was 78°C rather than the 85°C observed in the previous experiment. While there is no specific reason for this temperature difference, the lower temperature may account for the 5% differential in moisture loss.

Total oil content of the unheated cottonseed was 23%. Samples which were microwave-heated had oil contents ranging from 21–24%. Similar variations were noted in the oil content of stored samples. The variations appeared to be associated with experimental error rather than with treatment or length of storage.

Because of the limited amount of oil available in these experiments, it was not possible to determine any effect of microwave heat treatments on the refining characteristics of the oil. However, it was possible to evaluate the effect of microwave heat on the glyceride composition of the oil. Based on HPLC analysis, crude cottonseed oil contains six major triglycerides composed of mixtures of linoleic (L), oleic (O) and palmitic (P) fatty acids; several minor triglycerides containing mixtures of palmitic, oleic and stearic fatty acids; plus a fraction containing mono- and diglycerides and free fatty acids (11). In the oil from unheated seed and the 2.0-min microwave-heated seed, the free fatty acids, mono- and diglyceride fraction and the minor triglycerides represented 2.5% and 10.5% of the glyceride fraction, respectively. The relative composition of the major triglycerides was PLL, 28.3%; LLL, 17.4%; POL, 15.5%; PPL, 12.4%; OLL, 9.8% and OOL, 3.4%. During storage there were slight decreases in each of the major and minor triglycerides with a corresponding increase in the fraction containing free fatty acids and mono- and diglycerides. This fraction increased to 7.2% in oil from the unheated seed, but only to 5.3% in oil from the 2.0-min microwave-heated seed.

From Figure 1 it can be seen that there were significant differences in the development of FFA in the unheated seed *vs.* the microwave-heated seed. After nine weeks storage, there was a 2.29% increase (0.83% to 3.12%) in

TABLE 1

Temperatures and Moisture Contents of Cottonseed (200 g) Exposed to Microwave Heat (700W, 2450 MHz) in Closed Plastic Bags or Open Glass Containers

Treatment	Temperature (°C)	Moisture content[a] (%)
Experiment 2		
None	—	13.8
0.5 Min, closed	45	12.9
1.0 Min, closed	65	12.2
1.0 Min, open	55	11.8
2.0 Min, closed	95	10.6
2.0 Min, open	85	10.3
Experiment 3		
None	—	14.5
1.0 Min, open	60	13.1
1.5 Min, open	70	12.3
2.0 Min, open	78	11.7

[a]± 0.1%, Std. error of the mean.

TABLE 2

Free Fatty Acid Content[a] of Oil from Unheated and Microwave-Heated (700W, 2450 MHz) Cottonseed After Storage at 50°C

Heating conditions	Storage time 6 wk	9 wk
None	1.36	2.42
0.5 Min, closed	1.17	1.54
1.0 Min, closed	0.66	1.15
1.0 Min, open	0.74	1.37
2.0 Min, closed	0.54	0.72
2.0 Min, open	0.43	0.78

[a]% As oleic, initial FFA content of all samples = 0.24 ± 0.08%.

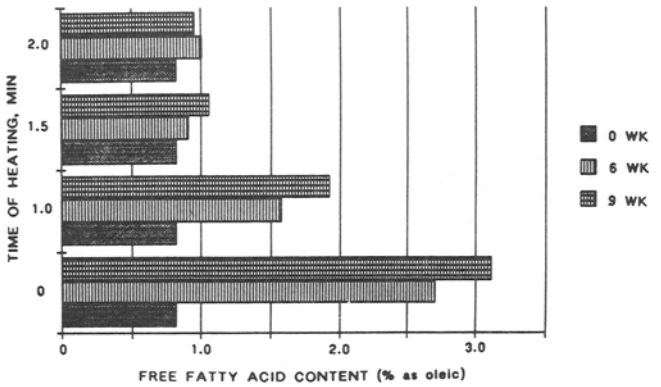

FIG. 1. Effect of microwave heat treatments (700W, 2450 MHz) on the development of free fatty acids in cottonseed during storage at 50°C.

the FFA content of the unheated seed. The FFA content of seed heated for 1.0 min increased 1.0%, while that of the 1.5- and 2.0-min microwave-heated samples increased 0.23% and 0.13%, respectively.

Lyman *et al.* (3) proposed that the prevention of FFA formation in cottonseed after microwave heating was the result of two mechanisms—rapid removal of moisture and destruction of the enzymes responsible for lipolysis. He based the latter conclusion on data from microwave-heated samples that had been rehumidified to 10, 16 or 20% moisture content after the microwave heat treatment. Lyman *et al.* (3) used 255-g fuzzy seed samples with an initial FFA content of 1.9%. After storage in sealed containers for 51 days at 38.5°C, the seed of 10, 16 and 20% moisture content, which had been microwave heated for 2 min but not rehumidified, had FFA contents of 3.5, 4.0 and 4.0%, respectively. Similar seed that had been rehumidified to 10, 16 and 20% moisture content after microwave heating had FFA contents of 3.7, 4.1 and 5.0%, respectively. There was, therefore, some FFA formation in all samples, possibly indicating an inactivation rather than a destruction of lipolytic enzyme activity. Lyman *et al.* (3) also noted that, in repeated experiments, treatments of 0.5 or 1.0 min increased the formation of FFA. They proposed that, under these conditions, the enzymes were not destroyed, but that their activity was actually stimulated. Data reported here does not support the proposal of enzymatic activation by short-term exposure to microwave heat. Treatment times of 0.5 and 1.0 min were less effective than longer exposure times, but, in all cases, microwave-heated samples developed less FFA during storage than unheated samples. This also suggests reduction or inactivation of lipolytic enzyme activity rather than destruction.

From the data in Table 3, it is apparent that microwave heat treatment for 1 min caused a significant reduction in the germinating capacity of the cottonseed. Longer microwave heat treatments completely inhibited the germinating capacity of the seeds. These data are consistent with the data reported earlier (4,5). Although there appeared to be a slight reduction in the microbial content of the seed as a result of microwave heat treatments, there was no consistency in the reduction. No attempt was made

TABLE 3

Effect of Microwave Heat Treatments (700W, 2450 MHz) on the Germination and Microbial Content of Cottonseed

Time of heating (min)	Germination[a] (%)	Microbial content[b] (%)
None	25	42
1.0	10	23
1.5	3	35
2.0	1	29

[a]± 6%, Std. error of the mean.

[b]± 11%, Std. error of the mean.

to differentiate between fungal and bacterial content; nor was there any attempt to determine the type of fungi present in the seed. After storage for six and nine weeks, germination of all samples was 0%. Microbial content, however, remained similar to the values reported on the unstored samples.

The increase in the number of damaged seeds noted by Anthony (5) in the microwave heat treatment of seed cotton could result in abnormalities in seed hulls or linters. Although the condition of the hulls is not important to their utilization, any changes in linters could cause a reduction in their use and an economic loss to the oil processor. Shape and size of cross sections of linters from unheated and microwave-heated cottonseed varied, but no real differences could be determined. The variations can be attributed to differences in maturity and to the presence of linters and lint fibers in these cross-sections. Scanning electron microscopy can reflect changes either in the external fiber structure or in the non-cellulose coating on the fiber surface. No differences were noted in the external fiber structure of linters from microwave heat-treated seed as compared with linters from unheated seed. In addition, there was no change in the nature of the non-cellulose coating on the fiber surface as a result of microwave heating. Determination of residual lint also indicated no effect on the amount of linters. Residual lint on the unheated seed and the microwave heat-treated seed was 10.50 and 10.70%, respectively.

If cottonseed meals are to be used in animal feeds, it is necessary to determine any effect of microwave heat on the protein content of the seed. Examination of the protein content of these cottonseed samples indicated no initial effect of microwave heat on either the total protein content or the amount of protein that was soluble in 10% NaCl (Table 4). The salt-soluble protein represents the globulin content of the cottonseed meals and has been associated with nutritive value of the meal as a feed for nonruminant animals (16). Although there was no change in the total protein content of the meals as a result of storage, there was a dramatic reduction in the salt-soluble protein content of the unheated seed during storage. This reduction in the salt-soluble protein also was apparent in seed that had been microwave-treated for 1.0 min. The 1.5-mn treatment appeared to maintain the integrity of the salt-soluble protein for six weeks, but only the 2.0-min microwave heat treatment was effective for the nine-week storage period.

These results were substantiated by the chromatographic examination of the soluble protein

TABLE 4

Effect of Microwave Heat Treatment (700W, 2450 MHz) of Cottonseed on the Protein Content of Their Defatted Meals

Time of heating (min)	Storage (wk) 0		6		9	
	A[a]	B[b]	A	B	A	B
None	53.9	51.9	51.3	10.9	53.6	3.5
1.0	53.4	52.0	52.6	32.6	52.8	5.6
1.5	51.3	55.4	52.6	51.6	53.4	27.8
2.0	51.9	51.5	52.1	48.1	53.1	45.4

[a]A = total protein; % N (by weight) × 6.25.

[b]B = salt-soluble protein: % total protein soluble in 10% NaCl.

TABLE 5

Comparison of Grade Certification of Unheated and Microwave-Heated Cottonseed[a,b]

	Unheated	2.0 Min MWH[c]
Foreign matter (%)	0.00	0.10
Moisture (%)	8.80	8.10
FFA in oil (% as oleic)	1.80	1.60
Oil (%)	17.80	18.10
Ammonia (%)	4.05	4.14
Grade	100.5	102

[a]Woodson-Tenent Laboratories (No Little Rock, AK).

[b]Except for "Grade", values are % by weight as is.

[c]MWH, microwave heat (700W, 2450 MHz).

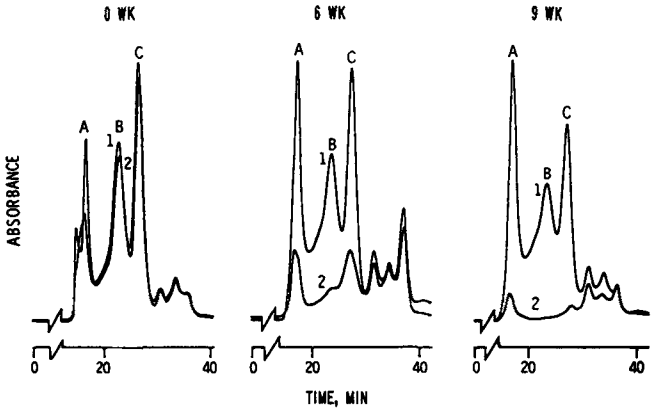

FIG. 2. High-performance liquid chromatographic separation of the salt-soluble proteins of defatted meals from unheated and microwave-heated (MWH) cottonseed before and after six and nine weeks storage at 50°C. 1, Tracing from 2 min MWH seed; 2, tracing from unheated seed. A, aggregate of high-molecular-weight proteins; B, 11 S protein component; and C, 7 S protein component.

fraction. Earlier work had shown that the globulin fraction of cottonseed protein consists of two main components, 7S and 11S (17). In Figure 2, the tracing at 0 weeks typifies the pattern associated with cottonseed proteins (18, 19) with peaks B and C corresponding to the 11S and 7S components. Peak A represents an aggregate of large-molecular-weight proteins. As storage time increased, peaks B and C disappeared in proteins isolated from the unheated seed. Although data for the 1.0- and 1.5-min microwave-heated samples are not shown, tracings for these samples also indicated decreasing amounts of the 11S and 7S components as storage time increased. The cluster of three peaks eluting between 30 and 40 min seems to vary; however, a determination of the ratio of the area under this cluster to total area showed values of 0.11, 0.16 and 0.13 for the 2-min microwave-heated samples at 0, 6 and 9 weeks of storage, respectively. The ratios for the unheated seed were 0.11, 0.47 and 0.67, respectively, suggesting a faster rate of destruction of the 11S and 7S components than of the cluster.

Standards have been established by the cottonseed industry for grading seed and for use as an indicator of the quality of the oil and protein that will be produced as the seed is crushed. Grading is done by certified commercial laboratories on representative samples of seed (minimum, 200 g per sample). Therefore, to corroborate the experimental results, samples of unheated and 2.0-min microwave-heated cottonseed were submitted to an independent laboratory for grading. From the data in Table 5 it is apparent that microwave heating had no effect on the grade of the seed. In addition, analyses indicated a similarity in the free gossypol content of the unheated and the microwave-treated seed (1.06% and 1.12%, respectively).

These data demonstrate the lack of any adverse effects of microwave heat treatments on cottonseed except for the reduction in germinability. Therefore, this type of treatment could be used for seed destined for processing into oil and meal, but not with seed intended for use as planting seed. This should not pose a problem, however, because planting seed is segregated before cottonseed is shipped from the ginner to the processor.

In the experiments reported here, the most dramatic effect of microwave heat was the rapid reduction in the moisture content of the seed—2.0 min exposure to 700W (3.5 W/g seed) at 2450 MHz reduced the moisture content of the seed 20%. These results also substantiate the work of Lyman *et al.* (3), *i.e.,* microwave heat treatments of cottonseed prior to storage retard the development of FFA in the seed during storage. However, the most provocative result of these studies is the added advantage of inhibiting deterioration of the salt-soluble proteins of the seed during storage by microwave heat.

Advances in microwave heating technology should make it possible to apply microwave heat to materials as they pass through a covered conveyor system (20). Since conveyor sysems are used in oilseed processing mills to transfer seed from trucks on arrival at the mill to storage houses or silos, the application of microwave heat seems to have potential. Because microwave heat does not have any adverse effects on seed or oil quality, the use of short-term exposure to microwave heat to reduce seed moisture and retard seed deterioration is technically feasible and should be examined for economic feasibility at oilseed processing mills.

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