EFFECT OF SOME VOLATILE CHEMICALS ON THE MICROBIAL SPOILAGE OF MOIST KAFIR CORN (Andropogon sorghum) UNDER AIRTIGHT STORAGE[1]

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ABSTRACT

Kafir corn at 20% moisture was fumigated with methyl bromide and ethylene dibromide and hermetically stored for 60 days at 25°–29°C. Molds and bacteria were destroyed. Changes in fat acidity, water-soluble acidity, reducing sugars, amino nitrogen, and weight/volume ratio were retarded. Similar results were obtained with chloropicrin and ethylene oxide. The possibility of cold sterilization of moist grain and storage under aseptic conditions in hermetic storage is discussed.

The critical role of moisture in the deterioration of stored grain has been widely recognized. Kolkwitz (9), Swanson (24), Milner and Geddes (17,18) and Semeniuk *et al.* (22) have established the interaction of time, temperature, and moisture on the degree of deterioration of grains. Microorganisms are considered to be mainly responsible for the deterioration of moist grain, owing to their lipolytic, amylolytic, and proteolytic activities (5,15).

Kafir corn *(Andropogon sorghum)* is generally stored in underground pits in India. Although insect infestation is prevented through depletion of oxygen and accumulation of carbon dioxide under airtight storage (4), biochemical and microbiological degradation of certain kinds proceeds unhindered in the stored moist grain (7). The degree of spoilage in kafir corn stored in underground pits is quite high.

Several fungicidal agents (14,16,28) have been used to inhibit microbial activity on moist grain. Unfortunately, chemicals (14,16,24) such as thiourea, mercuric chloride, ceresan, and arasan, hitherto found suitable for the storage of seed grains, cannot be employed on food grains for toxicological reasons.

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Previous investigations of Majumder *et al.* (11,12) on different volatile organic chemicals have shown the usefulness of methyl bromide and a 3:1 mixture of ethylbromide-ethylenedibromide (v/v) for an initial reduction in microbial population and beneficial effects on subsequent storage. Matz and Milner (13) reported that a 1:1 mixture of propylene oxide and carbon tetrachloride was effective in reducing the deleterious changes which occur in damp grain; but the results obtained with this mixture suggested that it could be used as a short-term measure until the grain could be dried to safe moisture level. Majumder *et al.* (10) have used methyl bromide fumigation as an adjunct to drying chips of moist tapioca (*Manihot utillissima*) to safe moisture levels; the microbial count was brought down from an initial 125 million to 1,500 per g. at a dosage of 5 lb. per 1,000 cu. ft. and exposure period of 48 hours. Under the same conditions in wheat (25% moisture) the population was brought down from 2.4 millions to 720 per g.

In the present investigation, attempts have been made to study the deteriorative changes in moist kafir corn *(Andropogon sorghum)* under airtight storage and the effects of some fumigants such as ethylene dibromide, methyl bromide, chloropicrin, and ethylene oxide in preventing these undesirable changes.

Materials and Methods

Freshly harvested kafir corn (variety Narkin) was dried to initial moisture of 11%. The samples were distributed in 700-g. lots in 1-liter wide-mouthed glass-stoppered bottles, and moisture contents were adjusted to 11, 15, 20, and 30% by adding the required amount of sterile water, mixing thoroughly, and shaking intermittently for 24 hours. The bottles were tightly stoppered (silicone grease used as lubricant), sealed with beeswax, and incubated at 30°C.

In a second experiment, ethylene oxide, ethylene dibromide, methyl bromide, and chloropicrin were used at dosages of 32, 64, and 96 mg. per liter on 10-kg. grain lots in properly clamped 16-liter desiccators. Fumigants ethylene oxide and methyl bromide were applied from a microburet at −10°C.; chloropicrin and ethylene dibromide were introduced into the desiccators at room temperature (26°C.). The moisture level of the grain was brought to 20% before the fumigants were introduced into the desiccators. The samples were analyzed for fat acidity, water-soluble acidity, reducing sugars, amino nitrogen, germination, microbial counts, fumigant residues, and organoleptic qualities at the end of storage period, 60 days.

The following methods were adopted for analysis of the samples: *Fat acidity* was determined according to the method of Zeleny and

Coleman (28). *Water-soluble acidity* was determined by the AOAC method (3). *Total reducing sugars* were estimated by the micro method of Somogyi (23). *Amino nitrogen* was determined by Samuel's method (21). *Weight/volume (w/v) ratio* was determined by measuring the displacement of water by the grain in a measuring cylinder.

Total microbial counts were determined on seeds ground aseptically in a laboratory grinder. The grinder was sterilized and the ground grains were sampled aseptically according to Teunisson (25). Counts were taken from colonies developed on potato-dextrose agar plates adjusted to pH 6.8 and incubated at 30°C. for 5 days. For anaerobic counts, serial dilution plates were incubated at 30°C. in desiccators containing alkaline pyrogallol. Methylene blue tests (20) indicated the absence of oxygen in the desiccators used for incubating plates for anaerobic counts.

Moisture was determined by the two-stage air-oven method (1) with ground samples at 130°C. for 1 hour. The results are reported on moisture-free basis.

Germination of kafir corn was ascertained by counting the number of germinated kernels on salt-free malt agar in Petri dishes after 5 days of incubation at 26°–29°C.

Fumigant residues were estimated for ethylene dibromide and methyl bromide according to the method of Young, Carter, and Soloway (27) after aeration of the samples in still air on shallow enameled trays at room temperature for 7 days. Residues of chloropicrin and ethylene oxide were not determined.

Organoleptic qualities of the samples were judged by a panel of judges on the aerated grains, flour and *roti* (conventional unleavened bread) by triangle difference test method (19). *Roti* was prepared by rolling 24 g. dough to a circle 4 in. in diameter and baking it on a hot plate until it attained a light brown color.

Results and Discussion

At the end of 30, 90, and 150 days, kafir corn samples were analyzed for fat acidity, water-soluble acidity, reducing sugar, amino nitrogen, viability, microbial count, and weight/volume ratio. The results are presented in Table I.

These data indicate rapid increase with time of fat acidity, water-soluble acidity, reducing sugar, amino nitrogen, and microbial count in grains stored at 15, 20, and 30% moisture. There is an appreciable increase in the fat acidity values between 15 and 20% moisture as compared to values between 20 and 30% moisture. Water-soluble acidity of the samples, on the other hand, shows a progressive increase

TABLE I

BIOCHEMICAL AND MICROBIAL CHANGES IN STORED KAFIR CORN

(Average values of five replicates)

STORAGE PERIOD AND MOISTURE PERCENTAGE		FAT ACIDITY [a]	WATER-SOLUBLE ACIDITY [b]	REDUCING SUGAR	AMINO NITROGEN	GERMINATION	MICROBIAL COUNT (per g. × 10^5)		WT./VOL. RATIO
							Aerobic	Anaerobic	
		mg/100g	*mg/100g*	*mg/100g*	*mg/100g*	%			
Control —	11[c]	31	63	160	10	96	1.1	0.6	0.81
30 days —	11	40	84	186	12	93	1.9	2.0	.79
	15	64	90	200	16	81	3.2	4.0	.77
	20	144	112	310	20	56	5.4	8.4	.75
	30	151	208	350	42	nil	9.4	10.0	.74
90 days —	11	54	124	189	12	90	2.4	3.7	.78
	15	82	129	235	21	30	6.5	9.4	.76
	20	169	148	385	26	nil	20.0	22.0	.74
	30	170	288	490	70	nil	22.0	23.0	.73
150 days —	11	59	90	250	14	89	4.0	6.4	.75
	15	94	115	270	25	6	12.0	44.0	.73
	20	180	130	405	50	nil	25.0	54.0	.72
	30	208	266	470	120	nil	18.0	74.0	0.69

[a] Milligrams of potassium hydroxide per 100 g.
[b] Milligrams of sodium hydroxide per 100 g.
[c] Analyses at the start of the experiment.

with time and proportional to the increase in moisture levels. Reducing sugars showed a progressive increase with time and moisture contents. At 20% moisture level and above, the germination percent was nil. With 15% moisture after 150 days of storage, germination percentage dropped as low as 6%. The total microbial count increased with the period of incubation. The anaerobic population was comparatively higher than the aerobic. There was considerable change in the density of the seeds at 30% moisture level on storage for 150 days. All the above data indicated that kafir corn stored hermetically at 11, 15, 20, and 30% moisture underwent biochemical and microbiological degradation. Lipolytic, amylolytic, and proteolytic activities were indicated by the changes in fat acidity, reducing sugars, and amino nitrogen respectively.

The data on the effects of fumigants — ethylene oxide, ethylenedibromide, methyl bromide, and chloropicrin used at dosages of 32, 64, and 96 mg. per liter on grain containing 20% moisture — are presented in Table II.

The results reveal that, in contrast to the changes in the control sample containing 20% moisture, the fumigants appreciably retarded adverse changes in treated moist grains. The criteria for the deteriorative changes in grains fumigated with methyl bromide, chloropicrin, and ethylene oxide maintained almost the same values as the control

TABLE II

EFFECT OF CERTAIN VOLATILE CHEMICALS ON THE DETERIORATIVE CHANGES IN KAFIR CORN

(Average values of five replicates)

Moisture, 20%; period of storage, 2 months; temperature, 25°–29°C.

CHEMICAL	FAT ACIDITY	WATER-SOLUBLE ACIDITY	REDUCING SUGARS	AMINO NITROGEN	MICROBIAL COUNT (per g. × 10^5)		FUMIGANT RESIDUES	ORGANOLEPTIC QUALITY [c]
					Aerobic	Anaerobic		
	mg/100g	*mg/100g*	*mg/100g*	*mg/100g*			*ppm Br*	
Control, 11%M [a,b]	28	63	165	10	1.2	0.5	..	G
Control, 20%M [a]	150	115	320	25	20	21	..	MS
Ethylene dibromide, mg/l.								
32	56	94	260	15	4.4	3.6	12	FO
64	38	77	180	13	0.6	0.2	22	SF
96	28	70	170	12	0.2	0.1	23	A
Methyl bromide, mg/l.								
32	41	76	210	14	1.0	0.3	15	SF
64	32	69	182	14	0.1	...	23	A
96	28	68	175	11	...	...	27	A
Chloropicrin, mg/l.								
32	42	80	214	15	1.1	...	..	SF
64	33	72	183	14	0.5	...	..	A
96	28	69	169	12	...	...	..	A
Ethylene oxide, mg/l.								
32	39	74	199	15	0.6	...	..	SO
64	36	66	182	12	...	...	..	A
96	30	64	171	11	...	...	..	A

[a] Analyses at the start of the experiment. (M, moisture.)
[b] Germination in control, 11% moisture, 96%; otherwise, none.
[c] Dough and unleavened bread. G, good; MS, musty; FO, fermented odor; SF, slightly fermented; A, acceptable; SO, slight odor.

sample at 11% moisture. Although ethylene dibromide retarded microbial activity, absolute sterilization of the grain could not be achieved even at a dosage of 96 mg. per liter under the experimental conditions. Surface mycelial growth was visible in top layers in the grain samples fumigated with ethylene dibromide within 30 days of incubation. Methyl bromide and chloropicrin at 96 mg. per liter and ethylene oxide at 64 mg. per liter brought down the microbial population to zero under sustained fumigation for the whole incubation period of 60 days. Owing to sterilization of the grain at these concentrations by the fumigants, biological decomposition was retarded. The organoleptic tests indicated the soundness of the hermetically stored moist grain fumigated with methyl bromide, chloropicrin, and ethylene oxide, whereas the control sample was unacceptable. Usually a characteristic taint develops in wheat flour fumigated with methyl bromide

(26), but no off-odor could be detected by the test panel on the kafir corn samples experimentally fumigated.

Grain may be stored satisfactorily in metal and concrete silos in temperate climates. In the tropics, however, wide fluctuations in diurnal temperature, high average temperature, translocation of moisture, and sweating accelerate the rate of microbiological and biochemical degradation. This investigation indicates the possibility of using volatile sterilizing agents for suppressing deteriorative changes and preventing external microbial contamination during storage.

Methyl bromide, ethylene oxide, and chloropicrin were the best fumigants for sterilizing the grain under hermetic storage. Further information on the residues and their effects on the various chemical constituents of the grain under such high dosages and exposure periods will be required. In the present study, the residues of methyl bromide were within the permissible limits as prescribed in Canada (6) and the United States (2) for cereals. Precise information is available on the toxicological aspects of the residues and reaction products of methyl bromide (26) and ethylene oxide (8), but chloropicrin requires critical studies on the residues and toxicological aspects for its use under these conditions.

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