BIOCHEMICAL AND NUTRITIONAL STUDIES ON EAST PAKISTAN RICE AND RICE PRODUCTS

Part III.—Differential Titratable Acidity and Auto-esterase Activity of Raw and Parboiled Rice and Effect on their Storage

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The effect of parboiling treatment on the titratable acidity and on the activity of esterase group enzymes was investigated on sixteen varieties of rice. The results show that in the resting stage the parboiled rice contains comparatively higher titratable acidity than raw rice but the incubation of these samples for 5 hours at 37°C. causes greater percent increase in the case of raw rice than in parboiled one. On storage the increase of acidity was less in parboiled samples than in the raw ones. Incubation of the stored samples caused similar higher percentage increase in the acidity of raw rice than of the parboiled ones. The significance of the results has been discussed in the light of the apparent relationship between the lower esterase activity of parboiled rice and paddy and their longer storage life.

Introduction

In the previous communication by Qudrat-i-Khuda, De and Debnath,[1] it was shown that during parboiling of paddy the amylase of the rice grains is inactivated to a great extent resulting in the production of less quantity of reducing sugar in the parboiled rice in the resting stage and under incubation with water, buffer etc. for a considerable period. This was considered as one of the essential factors contributing to longer storage life of the parboiled rice and paddy.

In consideration of the above finding it was hoped that other enzymes like esterases etc. which lead to the production of titratable acids and rancidity in the raw rice causing infestation by organism, mouldy taste and bad appearance as reported by Kik and Williams,[2] Hunter *et al.*[3] Houston *et al.*[4] and others, may be severely inactivated by parboiling treatment. With this possibility in view the present work has been undertaken and the results of titratable acidity values of rice as affected by parboiling are presented in this communication. The effect of storage on the above values of raw and parboiled rice are also presented so as to correlate the parboiling treatment with the storage life of rice.

Experimental

Sixteen varieties of paddy were collected in the month of February 1961, and unmilled raw and parboiled rice samples were prepared from these varieties according to the traditional technique detailed in the previous communication.[5]

The rice samples, ground to 100 mesh powder, were then used in all the determinations detailed in the following:

Estimation of Titratable Acidity of Raw and Parboiled Samples.—5 g. of the powder were thoroughly shaken with 100 ml. distilled water for 2 hours with a 5 ml. of 1:1 alcohol and acetone mixture, previously neutralised with phenolphthalein, so as to inactivate the esterases and other enzymes and thus to prevent formation of acids during the period of shaking. The extracts were then titrated against 0.01N-NaOH and the acidity expressed in terms of ml. of NaOH required.

The general method of determining the titratable acidity by extraction with boiling water, which easily destroys the enzymic activity, was avoided for the reason that by such hot extraction non-enzymatic hydrolysis could also occur which would yield results that might not correlate with the values obtained by incubation of same samples at 37°C. as employed for assessment of autoesterase activity detailed in the following:

Evaluation of Auto-esterase Activity.—The titratable acids as mentioned above represent the inorganic acids mainly phosphoric acid, organic acid produced by the breakdown of starch through Embden Meyerhof and Kreb's cycles, and the fatty acids (mostly the short chain water dispersable ones). The enzymes involved in the process mainly belong to esterase groups like phosphatase (acid), hexose phosphatases, phytase and lipase. In the present investigation the measurement of the above group of esterases has been effected by determination of the titratable acidity after incubation of the samples at 37°C. Since the activity of the enzymes was not determined by measurement of the water dispersable titratable acidity after incubation of the exogenous substrates but only by allowing the tissue enzymes to

act on their own substrates present in the cell structures— a technique which was previously adopted in the determination of auto dehydrogenase activity of fish tissues,[6]—the term autoesterase has, therefore, been applied to the group of enzymes as investigated here. This is a part and parcel of autolysis with involvement of esterase activity only.

By following the same technique, 5 g. of the same sample of rice powder was incubated with 100 ml. distilled water for 5 hours at 37°C. After the completion of the incubation 5 ml. of 1:1 ethanol: acetone mixture, previously neutralised, was added to inhibit the enzymic activity and the titratable acidity was then determined in the same way as before. The resutls are shown in Table 1 (a).

Evaluation of Storage Effect.—For study of the storage effect, batches of both raw and parboiled rice samples were stored in small hessian bags in the natural atmospheric condition and after one year of storage these were again analysed with respect to the normal titratable acidity values and the auto-esterase activity.

Results

Titratable Acidity in Fresh Raw and Parboiled Rice.—The results of titratable acidity as per Table 1 (a) expressed as equivalent to the quantity of 0.01 N-NaOH reequired per 100 g. rice was found to vary from 14.8 to 44.51 ml. with an average value of 29.6 ml. in case of raw rice; and from 29.6 to 74.1 ml. with an average value of 51.9 ml. in the case of parboiled rice. The parboiling treatment thus effected an increase of the acidity values by 75 percent over the raw rice value.

The incubation of the samples for 5 hours at 37°C., which is related to the auto-esterase activity, has effected a large increase of the acidity values; the higher increase of 557 percent over the unincubated samples was noted in raw rice whereas comparatively less increase of 235 percent was observed in the case of parboiled samples.

Acidity in Stored Samples.—The results presented in Table 1 (b) show that storage caused an increase of the titratable acidity of the resting samples to a certain extent and the average values for raw and parboiled samples were found to be 51.9 and 73.6 ml., respectively. When compared with their unstored values described in the previous paragraph these are higher by 75.3 percent in case of raw riceand 41.2 percent in case of parboiled rice as calculated and shown in Table 2. Table 1 (b) further shows that the incubation of the stored samples, in the previous manner, effected an increase by 204 percent in the case of raw rice and 117 percent in the case parboiled rice samples.

Discussion

The parboiled rice even before storage (Table 1 (a) manifests higher titratable acidity values under ordinary conditions butcomparatively lower value due to incubation for 5 hours at 37°C. These characteristics of the parboiled rice may be explained as due to the fact that during the initial stage of parboiling treatment when the temperature is gradually raised perhaps some acids are formed by hydrolysis due to activation of the esterases upto its optimum temperature level with the net result of accumulation of more acids in the tissue cells of parboiled samples. At the later stage of parboiling, when the temperature is further raised beyond the above optimum level, perhaps some fraction of the esterase-group and other enzymes get inactivated, for which the parboiled samples produce comparatively less acidity when incubated for 5 hours at 37°C.

Comparison of the values of columns 8 and 10 apparently indicates a great variation in the esterase activity of parboiled samples. But it should be kept in view that the acidity values as shown in column 8 were measured by incubation of the sample in powder form for which the substrate and the enzyme present therein were brought closer together due to rupture of the cell with manifestation of high enzymic activity. The results of column 10 indicate acidity produced due to partial activity of the enzymes of the intact grain where the enzyme and the substrate were not brought to much closer contact even when the optimum temperature for enzymic activity is raised.

The existence of partial enzymic activity in the parboiled sample as evident from the result of column 8 may be explained as due to inactivation of the enzymes located on the pericarp and germ where the temperature is sufficiently raised leaving the others in the interior endosperm almost unaffected where the heat did not sufficiently penetrate tc raise the temperature to that extent to inactivate the enzymes there. This phenomena is substantiated by the previous[5] and other observations that parboiling of paddy causes dextrinisation of starch granules of the outer layer keeping those of the interior region almost unaffected because of lower temperature of the interior portion.

TABLE 1 (a).— (BEFORE STORAGE) SHOWING THE TITRATABLE ACIDITY UNDER NORMAL CONDITIONS AND AFTER INCUBATION AT 37°C. REPRESENTING THE ESTERASE ACTIVITY OF RAW AND PARBOILED RICE. THE FIGURES REPRESENT THE RANGES OF THE VALUE WITH THEIR AVERAGES IN THE PARENTHESES FOR SIXTEEN LOCAL VARIETIES.

0.01 N-NaOH required in ml. per 100 g. raw rice				0.01 N-NaOH required in ml. per 100 g. parboiled rice				Increase of 0.01 N-NaOH required due to parboiling only	
		Increase due to incubation				Increase due to incubation			
Normal value	After incubation for 5 hrs.	Total	As percent of normal value	Normal value	After incubation for 5 hrs.	Total	As percent of normal value	Total	As percent of raw rice normal value
(1)	(2)	(3) = (2-1)	(4)	(5)	(6)	(7) = (6-5)	(8)	(9) = (5-1)	(10)
14.8-44.5	118.6-266.8	103.8-222.3	266-1100	29.6-74.1	88.9-266.8	44.4-177.9	100-500	14.8-44.7	33-200
(29.6)	(194.5)	(164.9)	(557)	(51.9)	(174.2)	(122.3)	(235)	(22.3)	(75)

TABLE 1 (b).—(AFTER 1-YEAR STORAGE).

24.4-85.5	73.3-207.6	48.8-146.5	100-366	36.6-122.1	85.5-231.9	48.8-134.3	50-137	12.2-48.8	25-133
(51.9)	(157.6)	(105.7)	(204)	(73.6)	(160.2)	(86.6)	(117)	(21.7)	(41)

Foot Note: The values shown under columns 3, 7 and 9 are not the actual differences of the ranges shown under columns 1,2, 5 and 6 but represent the ranges of differences of individual variety and the percent as shown under columns 4, 8 and 10 were calculated from these individual differences.

Table 2.—Showing the Effect of Storage on the Normal Titratable Acidity of the Raw and Parboiled Rice. The Figures are Computed from the Average Values of Table 1 (a) and 1 (b).

Average acidity of raw rice in ml. of 0.01 N-NaOH required per 100 g. rice				Average acidity of parboiled rice in ml. of 0.01 N-NaOH required per 100 g. rice			
Before storage	After storage	Total increase due to storage	Percent increase due to storage	Before storage	After storage	Total increase due to storage	Percent increase due to storage
29.6	51.9	22.3	+75.3	51.9	73.6	21.4	+41.2

Regarding the effect of storage, as shown in Table 1(b) it appears that this caused an increase of the acidity values in the raw samples to a greater extent than in the parboiled ones indicating less autoesterase activity in the parboiled samples even on storage. For the same reason the stored parboiled samples showed less titratable acidity due to incubation for 5 hours at 37°C.

The present results of comparatively lower activity of esterases and other enzymes as compared to those of the raw ones coupled with the observations of lower amylase activity and moisture absorption in parboiled rice, supplement to our knowledge gathered so far in offering explanation as to the higher storage life of parboiled rice and paddy which generally remain free from attack by insects and microorganism and do not produce fermented off odour and rancidity on storage.

It is an established fact that parboiled rice is rich in vitamin B_1. It will not be unreasonable to correlate this with the higher titratable acidity in the parboiled rice, in its resting stage, which however conserves this vitamin to a great extent as compared to that by the raw rice where the titartable acidity is low.

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