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INFLUENCE OF STORAGE ON AROMA AND ACTIVITIES OF PHYTASE, PEROXIDASE, AMYLASE AND LIPASE IN BASMATI AND NON-BASMATI RICE GENOTYPES

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Introduction

Rice (*Oryza sativa L.*) occupies the enviable prime place among food crops. Increasing productivity and sustained production of rice are critical for food and nutritional security. Rice is consumed largely in the cooked form and is also nutritious and hypoallergenic which make rice products staple food ingredients. It is also used in many value added products due to some of its unique functional properties such as flavour carrying capacity and hypoallergenity. It is a common practice to age the freshly harvested rice at least for six months before consumption. Storage induced aging has both desirable and understable effects on the endproducts, depending upon storage conditions and rice variety[1]. Basmati rice is globally reputed aromatic group of rice and has been very popular and command premium price in domestic as well as in international market. In basmati rice, 2acetyl-1-pyrroline along with other aromatic compounds are responsible for scent. Inheritance of scent is regulated by different genetic interactions in aromatic rice in collaboration with environment[2-4]. Phytates and phytases have attracted interest in nutrition mostly owing to their relationship on mineral absorption[5,6]. The nutritional contribution of the plant foods could be significantly increased, if the phytates in these foods were reduced[7].

During storage, a number of enzyme linked biochemical changes occur such as changes in cooking properties, textual properties, pasting properties, nutrient composition, flavour, etc. Asatsuma *et al*[8] generated a series of transgenic rice plants with suppressed expression or over expression of α-amylase. Peroxidase activity in stored rice grains lost rapidly and is also related to storage temperature, duration and cultivar[9]. Lipases and lipoxygenase also contribute in ascertaining rice quality and result in considerable deterioration in taste and flavour in cooked and rancid odors in raw rice grains. This study reports changes in aroma and activities of phytase, peroxidase, amylase and lipase in eight basmati and four non basmati rice genotypes raised at regional rice research station, Kaul (Kaithal) of the university during 2003.

Materials and Methods

Paddy seeds of basmati rice genotypes namely Haryana Mehak, HKR-98-476, HBC-19, CSR-30, HKR-228, IET-18004, Super basmati, Pusa Sugandha and non-basmati namely Govind, IR-64, HKR-126, HKR-47 were procured from CCS Haryana Agricultural University, Regional Rice Station, Kaul (Kaithal) during December, 2003. The samples were dehusked and polished uniformly utilizing the local facilities available. The rice grains were cleaned for dust, stones, broken seeds and other foreign materials. One kg sample of each rice genotype was fumigated in air tight circular steel container of 20 kg capacity with one tablet of aluminium phosphide for 3 days as per the instructions mentioned in package of practices of Haryana Agricultural University, Hisar[10]. Rice grains (250g) of all the genotypes were stored in the dark for one year at room temperature. During one year of aging, rice samples were removed after four, eight and 12 months. Fresh and aged samples were ground to pass 80 mesh sieve having 0.2 mm particle size using Udytec cyclone sample mill. Rice aroma was determined as per the method described by Mahindru[11] and results are expressed as volatile reducing substances (VRS) in terms of mg $KMnO_4$/100g dry weight. Activity of amylase was monitored by the method of Bernfield[12] and of phytase by the method of Heinonen and Lahti[13]. Peroxidase and lipase were assayed by using the methods of Summer and Glessing[14] and Sadasivam and Manickam[15] respectively.

Results and Discussion

Volatile reducing substances

Table I summarises the aroma of eight basmati and four non-basmati rice at fresh and 4, 8 and 12 months of storage. The aroma has been expressed as VRS which is defined as mg $KMno_4$ required to oxidize aroma released by 100g rice. During storage, the maximum reduction in VRS values was recorded after 4 months of storage and with progress of aging, the reduction in aroma was comparatively less. IET-18004 retained higher VRS values throughout storage period than the remaining genotypes. Among non-basmati types, Govind exhibited higher VRS values throughout the aging period. After eight months of aging VRS values differed significantly from each other in both genotypes. Haryana MeHak, IET-18004 and Pusa Sugandha retained higher VRS content as compared to remaining genotypes. Mahindru[11] reported different VRS concentration varying from 73.9 to 95.2 for basmati, 64 . 2 for Dubra, 71.6 for Chinar and 35.4 for non scented. The present results are more or less in conformity to above values. The disagreement of results in rest of genotypes may be attributed due to differences in genetic make up of research material and various agroclimatic conditions provided to cultivate the crop. The present trend of decline in aroma during storage of basmati (Table I) is also being supported and authenticated[16,17]. During storage, the desirable volatile substances break down and diffuse out of rice grains to the extent of 40- 50 per cent into the environment that also lends support to the present results[18]. The

TABLE I

Effect of Aging on Volatile Reducing Substances (mg kmno$_4$/100g) In Basmati and Non-Basmati Rice Genotypes

Genotype	Fresh	4 months stored	8 months stored	12 months stored
Basmati				
Haryana Menak	72.73 ± 2.04	72.73 ± 2.04	69.53 ± 2.04	66.97 ± 1.59
HKR-98-476	61.53 ± 1.26	56.09 ± 1. 59	52.57 ± 1.42	50.33 ± 2.04
HBC-19	65.37 ± 1.42	59.93 ± 2.04	54.81 ± 2.04	51.93 ± 1.26
HKR-228	63.13 ± 2.04	56.73 ± 2.04	55.77 ± 1.42	50.33 ± 2.04
IET-18004	74.33 ± 3.44	76.03 ± 1.96	71.45 ± 1.30	66.33 ± 2.04
Super basmati	66.97 ± 1.59	62.73 ± 2.37	61.53 ± 1.42	58.95 ± 1.41
Pusa sugandha	72.73 ± 2.04	71.13 ± 1.26	67.93 ± 3.44	58.65 ± 1.30
CSR-30	67.93 ± 3.44	66.93 ± 1.61	60.57 ± 1.59	56.41 ± 1.80
Mean	68.09	65.28	61.77	57.48
Non-basmati				
Govind	32.00 ± 0.00	24.00 ± 1.60	12.40 ± 0.40	12.65 ± 0.15
IR-64	27.20 ± 1.60	16.00 ± 0.00	12.65 ± 0.15	11.35 ± 0.15
HKR-126	27.20 ± 0.32	15.90 ± 1.10	14.00 ± 0.00	11.51 ± 0.01
HKR-47	24.00 ± 1.60	14.72 ± 0.00	12.60 ± 0.20	10.75 ± 0.05
Mean	27.60	17.65	12.91	11.57
Mean (Basmati and non-basmati)	54.59	49.41	45.48	42.18
SE (m)	20.37	24.32	24.73	23.22
CD at 5%	3.37	2.73	2.71	2.38

* Each value is an average of two estimations

aroma is governed by number of genes[3] which is different in individual genotypes and also its expression is the outcome of genotype environment interaction[2]. Yoshihashi *et al.*[19] revealed that starch bound form and free forms of 2 acetyl- pyrroline may occur in aromatic rice and its biosynthesis before starch formation in rice kernel could play a key role in the aroma quality of aromatic rice. After 12 months of storage Haryana Mahak and Govind retained higher VRS values in basmati and non-basmati genotypes, respectively. The differential release and loss of aroma in stored rice may indicate its complexing with starch and/or it is being stabilized by some other cellular metabolites that remains yet to be investigated.

Phytase activity

Changes in phytase activity following rice aging are presented in Table II. Among basmati genotypes, the maximum phytase activity was noticed in HBC-19 (12.57 µ moles Pi released /g rice grain) and minimum in Haryana Mehak (10.92 µmoles Pi released/ g rice grain). HKR-98-426, HKR-228 and super basmati attained almost equal phytase activity. Similarly HKR-47 and Govind showed

TABLE II
Influence of Storage Period on Phytase Activity (µmoles Pi released/g grain) In Basmati and Non-Basmati Rice Genotypes

Genotype	Fresh	4 months stored	8 months stored	12 months stored
Basmati				
Haryana Menak	10.92 ± 0.07	12.82 ± 0.04	12.06 ± 0.03	12.14 ± 0.08
HKR-98-476	11.40 ± 0.21	12.17 ± 0.16	12.31 ± 0.08	12.43 ± 0.08
HBC-19	12.57 ± 0.07	I 2.96 ± 0.07	12.28 ± 0.07	12.34 ± 0.13
HKR-228	11.63 ± 0.14	13.67 ± 0.04	12.43 ± 0.05	12.31 ± 0.04
IET-18004	10.98 ± 0.11	14.24 ± 0.21	14.84 ± 0.06	12.54 ± 0.06
Super basmati	11.94 ± 0.07	12.40 ± 0.18	12.63 ± 0.12	12.37 ± 0.04
Pusa sugandha	12.16 ± 0.18	12.23 ± 0.22	12.26 ± 0.09	12.48 ± 0.10
CSR-30	12.09 ± 0.09	14.26 ± 0.02	14.32 ± 0.03	14.53 ± 0.02
Mean	11.71	13.09	12.89	12.64
Non-basmati				
Govind	12.25 ± 0.15	15.90 ± 0.08	14.84 ± 0.12	14.97 ± 0.12
IR-64	11.40 ± 0.10	14.77 ± 0.19	15.07 ± 0.03	14.60 ± 0.08
HKR-126	11.91 ± 0.10	15.32 ± 0.09	14.84 ± 0.06	15.08 ± 0.15
HKR-47	12.49 ± 0.15	14.60 ± 0.06	15.11 ± 0.06	15.38 ± 0.30
Mean	12.01	15.15	14.97	15.01
Mean (Basmati and non-basmati)	11.81	13.78	13.58	13.43
SE (m)	0.55	1.26	1.33	1.33
CD at 5%	0.21	0.25	0.12	0.22

* Each value is an average of two estimations

identical values but higher than IR-64 and HKR-126. Results also evinced that phytase activity increased maximum upto four months of storage and after eight months of storage, the enzyme activity remained almost static in all the genotypes. In fresh and after 4, 8 and 12 months the mean values for basmati rice were 11.71, 13.09, 12.89 and 12.64 respectively in comparison to 12.01, 15.15, 14.87 and 15.01 in non-basmati rice. The decline in phytic acid level during storage of rice earlier mentioned indirectly supports the present results that rice grain exhibit phytase action[17,20-23].

Perodixase activity

The peroxidase activity showed regular gradual decrease in 4, 8 and 12 months stored rice, however reduction in activity was much more among non-basmati rice as compared to basmati rice (Table III). The overall mean values in fresh 4, 8 and 12 months stored rice were 1.22, 0.93, 0.52 and 0.36 units mg^{-1} protein. IET-18004 and super basmati among basmati and Govind and HKR-47 among non-basmati exhibited identical values for this enzyme. Peroxidase is a key enzyme

TABLE III

Influence of Storage Period on Specific Activity of Peroxidase (units mg^{-1} protein)* In Basmati and Non-Basmati Rice Genotypes

Genotype	Fresh	4 months stored	8 months stored	12 months stored
Basmati				
Haryana Menak	1.23 ± 0.08	0.96 ± 0.06	0.50 ± 0.03	0.37 ± 0.01
HKR-98-476	1.22 ± 0.07	1.09 ± 0.06	0.58 ± 0.03	0.52 ± 0.01
HBC-19	1.23 ± 0.08	1.03 ± 0.06	0.45 ± 0.03	0.29 ± 0.02
HKR-228	1.10 ± 0.08	0.81 ± 0.06	0.42 ± 0.03	0.33 ± 0.02
IET-18004	1.25 ± 0.07	0.82 ± 0.06	0.64 ± 0.03	0.49 ± 0.01
Super Basmati	1.25 ± 0.07	1.17 ± 0.06	0.56 ± 0.03	0.42 ± 0.02
Pusa Sugandha	1.16 ± 0.08	0.96 ± 0.06	0.71 ± 0.03	0.28 ± 0.01
CSR-30	1.11 ± 0.07	0.74 ± 0.06	0.38 ± 0.03	0.27 ± 0.01
Mean	1.19	0.95	0.53	0.37
Non-basmati				
Govind	1.37 ± 0.08	0.98 ± 0.06	0.61 ± 0.03	0.30 ± 0.01
IR-64	1.19 ± 0.07	0.90 ± 0.06	0.44 ± 0.03	0.41 ± 0.01
HKR-126	1.13 ± 0.07	0.74 ± 0.06	0.51 ± 0.03	0.29 ± 0.01
HKR-47	1.36 ± 0.08	0.92 ± 0.06	0.42 ± 0.03	0.36 ± 0.02
Mean	1.26	0.89	0.50	0.17
Mean (Basmati and non-basmati)	1.22	0.93	0.52	0.36
SE (m)	0.09	0.13	0.10	0.09
CD at 5%	0.13	0.11	0.05	0.03

* Each value is an average of two estimations

involved in ROS (Reactive Oxygen Species) scavenging and involved in protection of rice grain against ROS attack during storage[3]. The aging induced decline in peroxide activity is linked to decreased freshness of rice[9, 24].

Amylase activity

Specific activity of β-amylase varied from 1.90 (HBC-19) to 2.72 (Pusa sugandha) and averaged to 2.24 in fresh basmati and in non-basmati from 1.31 (HKR-126) to 2.31 (Govind) and averaged to 1.73 (Table IV). In general β-amylase activity was higher in basmati rice than non-basmati during aging and it declined with advancement of storage period. The maximum decline observed was upto 4 months and also between 8 and 12 months of storage period. Appreciable decrease in amylase activity during storage in basmati and some fine varieties was also mentioned earlier[25].

Lipase activity

The results mentioned in Table V revealed very slight and feeble lipase activity in fresh and stored rice. Some genotypes both in basmati and non-basmati did not reveal any lipase activity in fresh as well as in four months aged rice. Chemical characteristics of lipids and associated enzyme activities

TABLE IV
Influence of Storage Period on Specific Activity of β-Amylase (mg maltose realsed mg⁻¹ Protein)* In Basmati and Non-Basmati Rice Genotypes

Genotype	Fresh	4 months stored	8 months stored	12 months stored
Basmati				
Haryana Menak	2.32 ± 0.06	2.08 ± 0.05	1.81 ± 0.05	1.07 ± 0.02
HKR-98-476	1.81 ± 0.06	1.81 ± 0.04	1.78 ± 0.07	1.64 ± 0.02
HBC-19	1.90 ± 0.06	1.57 ± 0.04	1.44 ± 0.05	0.86 ± 0.02
HKR-228	2.09 ± 0.06	1.67 ± 0.08	1.85 ± 0.05	0.97 ± 0.02
IET-18004	2.28 ± 0.06	1.83 ± 0.04	1.74 ± 0.05	1.34 ± 0.02
Super basmati	2.45 ± 0.06	2.11 ± 0.04	1.37 ± 0.05	0.93 ± 0.02
Pusa sugandha	2.72 ± 0.07	1.99 ± 0.04	1.52 ± 0.05	1.15 ± 0.08
CSR-30	2.31 ± 0.15	1.79 ± 0.04	1.93 ± 0.05	1.37 ± 0.03
Mean	2.24	1.86	1.69	1.16
Non-basmati				
Govind	2.31 ± 0.09	2.12 ± 0.04	1.71 ± 0.05	1.21 ± 0.04
IR-64	1.72 ± 0.06	1.65 ± 0.04	1.46 ± 0.05	0.74 ± 0.02
HKR-126	1.31 ± 0.06	1.38 ± 0.04	0.83 ± 0.05	0.99 ± 0.02
HKR-47	1.56 ± 0.06	1.04 ± 0.04	1.07 ± 0.05	1.24 ± 0.02
Mean	1.73	1.55	1.27	1.05
Mean (Basmati and non-basmati)	2.07	1.76	1.54	1.13
SE (m)	2.31	2.13	1.71	1.21
CD at 5%	0.12	0.08	0.09	0.06

* Each value is an average of two estimations

changes during storage result in affecting the market value of rice grains. Sources of lipases could be endogenous, however micro organisms populating in stored rice also elaborate lipases leading to hydrolysis of lipids and enzymatic oxidation of fatty acid by lipo oxygenase. Lipase activity was earlier visualized in oat but enzyme did not indicate substrate specificity[26].

Summary and Conclusion

Eight basmati and four non-basmati rice genotypes were evaluated for aroma and endogenous activities of phytase, peroxidase, amylase and lipase during one year of storage. Aroma in rice decreased gradually with progress of ageing. After all the storage periods, maximum VRS values were observed in Haryana Menak and minimum in HKR-98-476. Basmati rice genotypes in general possessed higher amylase activity than non-basmati types and activity declined with progress of ageing. Peroxidase activity showed regular declining trend and reduction in specific activity was much greater in non-basmati than basmati types. Phytase activity

TABLE V

Influence of Storage Period on Lipase Activity (meq/min/g grain)* In Basmati and Non-Basmati Rice Genotypes

Genotype	Fresh	4 months stored	8 months stored	12 months stored
Basmati				
Haryana Menak	ND	0.001 ± 0.00	0.002 ± 0.00	0.002 ± 0.00
HKR-98-476	0.014 ± 0.001	0.002 ± 0.00	0.003 ± 0.00	0.002 ± 0.00
HBC-19	ND	0.001 ± 0.00	0,003 ± 0.00	0.003 ± 0.00
HKR-228	ND	ND	0.003 ± 0.00	0.003 ± 0.00
IET-18004	ND	ND	0.005 ± 0.00	ND
Super Basmati	0.006 ± 0.000	0.002 ± 0.00	0.004 ± 0.00	0.003 ± 0.00
Pusa Sugandha	ND	0.001 ± 0.00	0.003 ± 0.00	0.002 ± 0.00
CSR-30	0.003 ± 0.001	0.002 ± 0.00	0.004 ± 0.00	ND
Non-basmati				
Govind	ND	ND	0.002 ± 0.00	0.002 ± 0.00
IR-64	ND	ND	0.002 ± 0.00	0.004 ± 0.00
HKR-126	ND	0.001 ± 0.00	0.003 ± 0.00	0.003 ± 0.00
HKR-47	ND	0.004 ± 0.00	0.003 ± 0.00	0.005 ± 0.00
Mean	0.002	0.001	0.003	0.002
SE (m)	0.004	0.001	0.001	0.001
CD at 5%	0.001	0.001	0.001	0.001

ND : Not detected

* Each value is an average of two estimations

peaked upto four months and thereafter it remained more or less static. The non-basmati rice exhibited comparatively higher phytase activity than their counterparts. Both in fresh and stored rice, very feeble lipase activity was recorded.

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