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Full Length Research Paper

Effects of planting location and storage time on lipids and fatty acids contents of some Madagascan rice varieties

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Lipid and fatty acid composition of some Madagascan rice varieties were analysed by both Soxhlet method and Gas Chromatography based on variety, planting location and the storage time of rice grain. The results showed that there was a significant variety effect. This work also highlighted an agro-ecological site influence on the lipid content, the oleic and arachidic acids. Finally, fatty acid composition of Madagascan rice obeyed Keys's rule few time (14 days and 30 days) after harvest and after one year of storage for one variety out of three. Keys's rule was related to variety, to site and to storage time. Mono- and polyunsaturated fatty acids were significantly influenced by storage time.

Key words: Lipid, fatty acids, Madagascan rice, site, storage time.

INTRODUCTION

Madagascar is at 85 % an agricultural and rural land, the rice of which holds an important place among the Magadascan households. 73 % of households are rice cultivators. Since 1980, FO.FI.FA. has taken part to the rice ameliorating research in many fields. The present work is undertaken as part of evaluation for the application of ameliorated varieties to be selected in vulgarisation. For that study, three parameters are chosen: varieties, plant-ing location and storage time.

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Abbreviations : FO.FI.FA National center applied for rural development ; PCA Principal component analysis ;AHC ascendant hierarchic classification ;ToxV2=tv2 = Tox3217-69-3-1, Tox V5= tv5= Tox3233 -31-6 -2-1-1A,Tox V3= Tox3217-71-2-1=tv3 if from Marovoay and tv3u if from Toliara, ToxV4= Tox3219-51-1-3-1-2B= tv4, if from Marovoay and tv4u if from Toliara, P882=P882-2 -1-B-4-1-1, SPR= SPR7216-RST ; GC Gas chromatography; FC lipids contents, SFA saturated fatty acids, MFA monounsaturated fatty acids; PFA polyunsaturated fatty acids; M Marovoay; U Toliara; T Mahitsy.

Rice contains the least lipid content among cereals. However, lipid content is one factor that makes rice tasty or not (Kutsukake, 1986). Fatty rice is rather gelatinous when cooked in mush. After a long storage period, it might smell and taste rank.

Ramarathnam and Kulkarni (1983) found that volatile fatty acids (capric C10:0, lauric C12:0 acids) in freshly harvested Indian rice grain disappeared over storage time (four months) for most of the studied varieties.

Palmitic C16:0 and oleic C18:1 acids also decreased after four months of storage while arachidic acid C20:0 disappeared. Linoleic acid C18:2, however, increased.

The determination of lipids and fatty acids in rice grain became a useful means for breeders and consumers to guide them in the choice of the most convenient varieties for momentary consumption or for storage. Furthermore, it helps to settle the critical stage of taste alteration.

The purpose of this work was firstly to study the effects of planting location and storage time on lipids and fatty acids contents of Madagascan rice varieties. The varia-bility of fatty acids in Madagascan varieties of rice was compared in the present article with the results of Ramarathnam and Kulkarni (1983). Furthermore, the aim is to verify Keys's rule from the methyl esters of rice grain fatty acids.

Keys's rule is a criterion defined by 1/3, 1/3, 1/3 proportion of saturated, monounsaturated and poly-unsaturated fatty acids, which reflects good alimentary oil.

MATERIALS AND METHODS

Planting locations

We studied samples from three sites: Marovoay, Mahitsy and Toliara. The area of Marovoay under a tropical climate is a plain with ferruginous soil in the North-west of the island (Madagascar). The area of Toliara in the South- west part has a dry tropical climate and alluvial soil. Mahitsy culture site has also an alluvial soil inside the Betsimitatatra area in the neighbourhood of Antananarivo, with an altitude tropical climate.

Rice varieties

The material used in our experimentation included a total of 22 samples (milled rice) from FO.FI.FA according to the three sites as follows:

1). Marovoay planting location, 9 varieties: X372, NDR80, X398, X415, X360, IR38, ToxV3, ToxV4, ToxV5
2). Toliara planting location, 10 varieties: X21, 2798, 2787, P882-2-1-B-4-1-1, HB96, SPR7216-RST, ToxV3, ToxV4, ToxV2, IR50
3). Mahitsy planting location, 3 varieties: Soameva, MR10309-1-2-2, X1580

Storage time study

It concerned the varieties of Mahitsy. Storage time study was carried out 14 days (t_0), one month (t_1), three months (t_2) and one year (t_3) after harvest. Samples were kept in small plastic bags and stored at room temperature.

Chemical analysis

Oil extraction: The extraction was carried out in duplicate. Rice samples (5 g) were milled samples ground with a laboratory hammer mill UDY cyclone, at about 12% moisture (as determined by weighing after 6 h in a drying stove at 105°C) . Lipids were extracted with a soxhlet by using 150 ml of n-hexane d = 0.68, Eb: 70°C (Labosi, Oulchy-Le Château and France) according to the usual procedure (Wolff, 1968). Then the solvent was evaporated to dryness under reduced pressure and the residue (FC) dried in an oven at 105°C during one hour.

Lipids saponification

Prior to G.C. analysis, the lipids (50 mg) were first saponified with a 2N ethanolic solution of potassium (2 ml) (potassium hydroxide Merck, Darmstadt, Germany); absolute ethanol (Riedel-De-Haen AG Seelze-Hannover, Germany) at 80°C for 20 minutes. 1ml of distilled water, then 2 ml of hexane d = 0.68, Eb: 70°C (Labosi, Oulchy-Le Château, France) were respectively added to the reaction after cooling and the unsaponifiable part was removed three times with 2 ml hexane. 3 ml of 6N hydrochloric acid was added to the aqueous part. The fatty acids were extracted three times with 2 ml hexane. About 30 mg of anhydrous sodium sulphate was added into the solution. After filtration, the solvent was evaporated under reduced pressure.

Methyl esters preparation

The fatty acids were hydrolysed with a 2 ml of methanol hydrochloric acid 2N (hydrochloric acid d =1.19, Merck); methanol (99.9% Fisher Labosi, Zac clé de St Pierre, France) at 80°C for five minutes in an oven, then extracted with hexane.

The obtained methyl esters were tested by thin layer chromatography with hexane/ether (90/10) as eluant revealed by sprying with eosine solution in ethanol (0.5 %0) and observed under UV light after drying. Their spot was expected to migrate into a high position.

Gas chromatography analysis of the methyl esters

Each sample (30 mg in 0.5 ml of hexane) was analysed in triplicate on a Shimadzu GC-14A device equipped with a flame ionisation detector (230°C) and a glass injector (240°C). The separation was performed on a glass capillary column (30m x 0.53 mm) coated with carbowax 20 M (1 µm thickness) at 180°C. The flow rate of nitrogen as carrier gas was 0.3 bar.

The G.C. column allowed the following methyl esters analysis identified by comparison with standard compounds:
1). Saturated fatty acids: myristic C14:0, palmitic C16:0, arachidic C20:0 acids.
2). Monounsaturated fatty acids: oleic acid C18:1.
3). Polyunsaturated fatty acids: linoleic C18:2 and C18:3 linolenic acids.
They were quantified as relative peak area percentages.

Statistical analysis

Principal Component Analysis (PCA) was done for varietal study. The considered variables were FC, C14:0, C16:0, C18:0, C18:1, C18:2, C18:3 and C20:0. It was coupled with Ascendant Hierarchic classification (AHC) to classify the varieties (complete linkage, Euclidian distance).

Non parametric Kruskal -Wallis test or Mann-Whitney test was applied for all the analysis to study the difference between the two sites (Marovoay and Toliara) or among the storage periods (t_0, t_1, t_2 and t_3) with a 0.050 probability of error. The dependent variables were: FC, C14:0, C16:0, C18:1, C18:2, C18:3 and C20:0. To define the varieties complying with Keys's rule, we have chosen among the smallest values (<3.5) the standard deviation of he absolute values deviation around the ideal proportion 1/3, 1/3, 1/3 (Table 2).

RESULTS AND DISCUSSION

We gave the result for lipids contents, then for fatty acids contents, first according to varietal analysis, second to planting locations effects and finally to storage time.

The main fatty acids of the rice grain were identified as C14:0, C16:0, C18:0, C18:1, C18:2, C18:3 and C20:0.

About varieties : Lipids contents of Marovoay varieties got an average 1.08% (higher than Toliara lipids average which was 0.75%); Mahitsy varieties got an average 1.08%. The standard deviations were the minima for this last one (0.098) whereas the Marovoay and Toliara varie-

Table 1. Lipids contents of rice varieties.

Site	Marovoay	Toliara	Mahitsy
Number of samples	9	10	3
Average FC %	1.08	0.75	1.08
Standard deviation	0.261	0.266	0.098

ties showed almost the same value 0.261 (Table 1). It appeared that Marovoay varieties were fattier than that of Toliara.

Fatty acids composition was uniformly characterised with high contents of palmitic, oleic and linoleic acids. Saturated fatty acids varied from 20.54 to 36.03%, mono unsaturated ones from 29.57 to 58.17% and polyunsaturated ones from 14.96 to 48.31% (Table 2). Furthermore, high values of monounsaturated occurred where low values of polyunsaturated were observed.

Statistical analysis by principal component analysis according to varieties

The first two Principal Components explained 65.27% of the variability including 48% for F1 (1st Principal Component) and 17% for F2 (2nd Principal Component) according to Figure 1. F1 axis (48%) was essentially determined by the variables C18:1 (13.05%), C18:2 (17.5%), C18:3 (11.03%) and PFA (17.98%). PFA almost coincided with C18:2 and MFA with C18:1. F1 separated the varieties rich in PFA to those poor in PFA but rich in MFA. MFA and PFA were negatively correlated. F2 axis (17%) was essentially determined by the palmitic acid C16:0 (18.2%), the arachidic acid C20:0 (16.5%), and the SFA (19.3%). SFA coincided with C16:0 (r = 0.940). F2 separated the varieties rich in palmitic acid C16:0 to those poor one. There was also a negative correlation between SFA and C18:2 (r =-0.630). The coefficient of correlation was the highest for the oleic and linoleic acids (r = -0.904). Like Taiwanese rice studied by Taira (1986), for which the oleic and the linoleic acids were strongly correlated, the Madagascan rice (r = -0.904) was near the Indica-type (r = -0.942) and was not glutinous.

AHC coupled with PCA defined two groups of individuals:

1).The first group was formed by the Tox varieties, the Mahitsy varieties and IR50 from Toliara; they were rather rich in polyunsaturated fatty acids (C18:2 and C18:3), and poor in oleic C18:1 acid.

2).The second group was formed by SPR7216, HB96, 2787, 2798, X360, X415 X398, NDR80 and X372. They were poor in C18:2 and C18:3 and rich in C18:1. Whereas P882-2-1- B-4-1-1 and IR 38 far from the circle were rather rich in arachidic acid, X21 was rich in stearic acid C18:0.

Table 2. Variation of fatty acids contents according to varieties on the basis of Keys's rule.

Site	Variety	SFA (%)	MFA (%)	PFA (%)	Standard Deviation
M	IR38	34.24	47.14	18.62	7.721
M	NDR80	30.85	46.52	22.63	5.605
M	ToxV3	28.92	36.20	34.88	1.431
M	ToxV4	22.73	39.7	37.57	2.772
M	ToxV5	23.96	38.64	37.4	3.237
M	X360	29.54	49.53	20.93	6.358
M	X372	25.6	56.88	17.52	7.911
M	X398	36.03	46.2	17.77	6.783
M	X415	27.72	48.78	23.5	4.937
U	HB96	31.05	44.29	24.66	4.498
U	P882	29.1	39.2	31.7	2.138
U	SPR.7216	31.82	53.22	14.96	10.201
U	2787	28.58	45.21	26.21	3.631
U	2798	23.72	50.31	25.97	5.032
U	X21	20.54	58.17	21.29	7.183
U	ToxV3	23.67	33.83	42.5	5.153
U	ToxV4	22.12	29.57	48.31	5.710
U	ToxV2	24.56	37.33	38.11	2.559
U	IR50	25.14	38.02	36.84	2.434
T	MR10309	24.07	42.57	33.36	5.323
T	Soameva	25.09	37.24	37.67	2.386
T	X1580	24.35	42.44	33.21	5.153

Significant standard deviation<3.5

Fatty acid keys proportions

According to varieties study performed in Table 2, only the IR50, the ToxV2 and P882-2-1-B-4-1-1 varieties from Toliara, ToxV3,ToxV4, ToxV5 from Marovoay and Soameva from Mahitsy obeyed Keys's rule (1/31/3-1/3) of saturated, monounsaturated and polyunsaturated fatty acids, with values, nearing these optima (22.73 - 29.10/36.20 - 39.70 /31.7 - 38.11). The other varieties got a rather big difference in saturated, mono- and polyunsaturated proportions (20.5436.03/ 29.57-58.17/ 14.96-48.31).

Through the variety of rice, differences in lipids and fatty acids contents were observed; the Marovoay varieties were as high as the Mahitsy varieties, while the Toliara varieties were lower in lipids contents; its Tox varieties were richer in PFA. Most of the Tox varieties answered the Keys's rule. Only few varieties of the whole sample satisfied that ideal proportion and its occurrence depended on the planting locations. Soameva was among the interesting variety with good alimentary lipid content. The Tox varieties from Marovoay also got a nutritional interest to agree with Keys's rule. X360 and X415

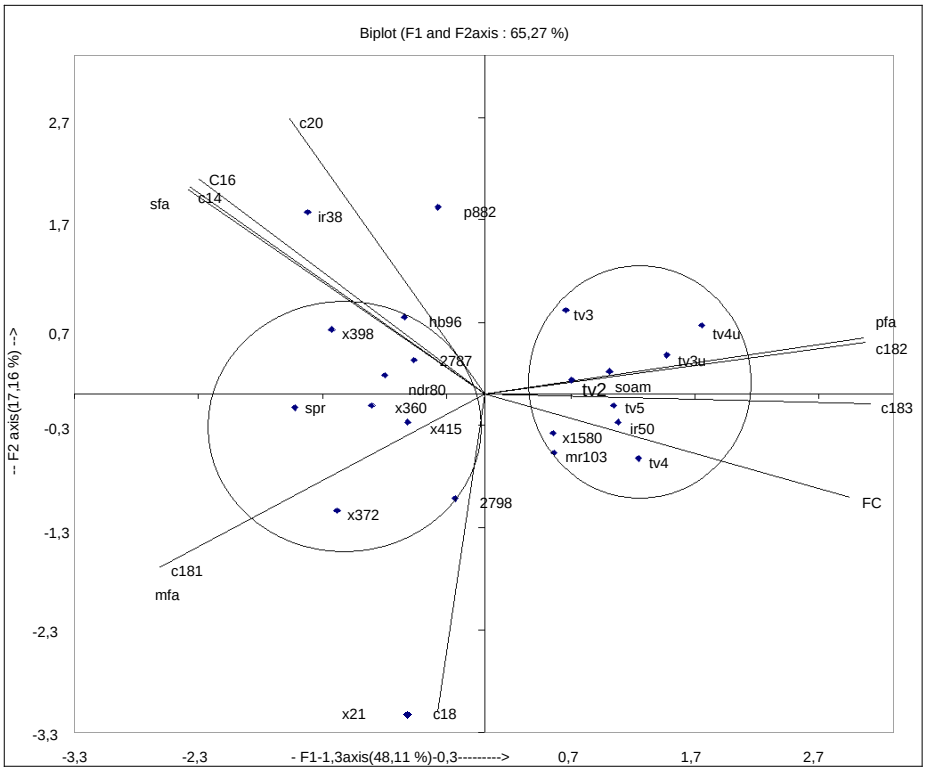

Figure 1. Statistical analysis by Principal Component Analysis according to varieties.

contained less fat but were rich in oleic acid C18:1 which is an important fatty acid, reducing the LDL (bad) cholesterol.

About planting location

Fat content: The two studied varieties ToxV3 and ToxV4 cultivated on two sites gave in Mann-Whitney non parametric test a slight difference between the two factors. (p 0.05): Table 3.

Fatty acids content: According to Table 2, the Tox varieties were different in their fatty acids composition between the two sites. The Marovoay Tox varieties showed fatty acids proportion nearing the Keys's rule proportion, whereas the Toliara Tox varieties were rather richer in polyunsaturated fatty acids.

The non parametric test of Mann-Whitney displayed a slight difference between the two factors for the oleic C18:1 and arachidic C20:0 acids of Tox V4 (respectively p = 0.050 and p= 0.043). There was no effect of the site referring to the other fatty acids (Table 3).

The lipid content was essentially discriminator on the site effect between the two varieties ToxV3 and ToxV4.

As far as site effects were concerned, lipid contents varied from an agro-ecological site to another (1.28 to 1.52% in Marovoay against 0.80% in Toliara). Between the two sites (Toliara and Marovoay), lipid enrichment may be a result of the weaker daily temperature amplitude, that prevailed in Marovoay. The formation of Keys's rule proportion under this climate was a varietal characteristic of the Tox Marovoay varieties.

About storage period

This study concerned the Mahitsy varieties.

Kruskal Wallis test on fat content (Table 3)

Concerning storage time, all the lipid contents showed little or no variation according to Kruskal Wallis test (p>0.050). The three varieties were characterized by insignificant difference in lipid contents (1.06 to 1.02% for MR10309, 1.14 to 0.92% for Soameva, 0.87 to 1.03% for X1580) but fatty acids varied (Table 4).

Variation of fatty acids content:

There was some difference across varieties over storage time. For the Soameva and X1580 varieties, saturated fatty acids (SFA) decreased until t_2 (one month). The saturated fatty acids were the weakest among the three

Table 3. Mann-Whitney test on site effect.

Varieties	Sites	Re	FC	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0
ToxV3	U	3	0.80	0.63	22.9	0.89	33.4	39.63	2.68	0.14
	M	3	1.28	1.59	24.7	1.61	36.2	32.8	1.94	0.37
	p-value		**0.050**	0.513	0.184	0.513	0.376	0.077	0.275	0.658
ToxV4	U	3	0.80	0.50	21.1	0.70	29.6	47.40	0.91	0.17
	M	3	1.52	0.42	21.8	0.69	39.7	35.67	1.91	0.22
	p-value		0.**050**	0.513	0.827	0.513	**0.050**	0.275	0.275	**0.043**

Re= Replication p 0.050: significant

Table 4. Kruskal Wallis test on storage time**.**

Variety	Time	Re	FC	C14 :0	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	SFA	MFA	PFA
MR10309	t_0	3	1.05 a	0.17 a	19.00 a	1.10 a	40.23 a	36.52 a	2.50 a	0.42 a	20.68 a	40.29 a	39.02 a
	t_1	3	1.06a	0.73 a	20.70 b	0.17 b	40.57 a	36.57 a	1.21 b	0.06 b	21.66 a	40.5 a	37.77 a
	t_2	3	1.02 a	0.51 a	18.33 a	0.27 b	45.03 b	33.11 b	2.12 c	0.62 c	19.74 a	45.0 b	35.23 a
	t_3	3	1.02 a	0.75 a	18.96 a	0.13 b	42.94 b	33.01 b	3.71 d	0.50 c	20.34 a	42.94 c	36.72 a
p-value			0.284	0.108	**0.028**	0.083	**0.024**	**0.039**	**0.016**	**0.041**	0.282	**0.024**	0.069
Soameva	t_0	3	1.14 a	0.15 a	25.94 a	1.64 a	35.17 a	35.77 a	2.39 a	0.13 a	26.68 a	35.17 a	38.15 a
	t_1	3	1.19 a	0.00 a	23.99 a	3.75 a	34.2 a	38.23 a	1.74 b	0.00 a	25.8 a	34.2 a	39.97 a
	t_2	3	1.34 a	0.02 a	14.42 b	0 a	61.3 a	22.1 a	1.13 b	0.37 a	15.47 b	61.3 a	23.23 a
	t_3	3	0.92 a	0.40 a	19.59 c	0.85 a	39.07 a	36.8 a	2.68 a	1.03 a	21.45 a	39.07 a	39.48 a
p-value			0.128	0.061	**0.024**	0.51	0.063	0.054	**0.034**	0.077	**0.041**	0.063	0.053
X1580	t_0	3	0.87 a	0.51 a	22.46 a	1.13 a	40.13 a	34.17 a	1.11 a	0.48 a	24.59 a	40.13 a	35.28 a
	t_1	3	1.20 a	0.56 a	21.33 a	0.00 a	31.13 b	44.57 b	2.40 a	0.00 a	21.89 a	31.13 b	46.97 b
	t_2	3	1.15 a	0.20 a	15.43 a	0.67 a	60.40 c	22.04 c	0.86 a	0.40 a	16.70 b	60.40 c	22.9 c
	t_3	3	1.03 a	0.11 a	18.63 a	0.60 a	35.01 d	41.42 d	4.03 a	0.00 a	19.35 b	35.20 d	45.45 b
p-value			0.099	0.052	0.055	0.211	**0.016**	**0.016**	0.053	0.065	**0.05**	**0.015**	**0.022**

Re: Replication P 0.05: significant.

types of fatty acids (average 21.20% upon the three varieties). The monounsaturated fatty acids were with the highest values (average 42.12%). The polyunsaturated fatty acids were almost slightly superior to 33.33 % (general average 36.68%).

Kruskal Wallis test (Table 4) showed the variance on the lipids or on the fatty acids contents. The letters following the values referred to the difference or to the similarity of these values. For the similarity found by non-parametric Kruskal-Wallis test was put the same letter, and in the opposite case, different letters (according to the Mann-Whitney test on each time t_0, t_1, t_2 and t_3). The table announced on the myristic acid values no variance of every variety (the same letter “a” over time for the percentages) and on the palmitic values a significance (decreasing from 25.94 to 14.42 % until t_2, then increased to 19.59 %) for Soameva (different letters”a”, “b” or ”c” over time for the percentages). For MR10309, the two other saturated fatty acids C16:0 and C20:0 were respectively significantly different over storage time (p= 0.028 and 0.041) whereas they did not change for X1580 (p>0.050). Table 4 explained the invariability of oleic and linoleic acids for Soameva. The variation of linolenic acid of this variety, slightly significant, did not influence the propor-tion nearing the ideal one at t_0 and t_3. The oleic and linoleic acids were on the contrary significantly different for MR10309 and X1580 varieties. These two varieties got unstable mono-and polyunsaturated fatty acids over time.

Referring to Keys’s rule, the imaginary proportion (1/3, 1/3, 1/3) was acceptably verified at the times t_0 (14 days: 26.68-36.60-36.72%) and t_3 (one year: 21.45-39.07-39.48%) for Soameva and at t_0 for X1580 (Table 5) Soameva showed the ideal fatty acids value both in the early and the late storage period. X1580 was just on the limit (standard deviation = 3.5).

The three varieties (MR10309, Soameva and X1580) complied with the consumers’ taste because of their lipid

Table 5. Variation of fatty acids contents according to storage time on the basis of Keys's rule.

Variety	Time	SFA	MFA	PFA	Standard deviation
MR10309	t_0	20.68	40.29	39.02	3.71
	t_1	21.66	40.57	37.77	3.65
	t_2	19.74	45.03	35.23	6.28
	t_3	20.34	42.94	36.72	4.87
Soameva	t_0	26.68	36.60	36.72	**2.43**
	t_1	25.83	34.20	39.97	3.61
	t_2	16.71	55.97	27.32	8.96
	t_3	21.45	39.07	39.48	**3.43**
X1580	t_0	24.59	40.13	35.28	**3.50**
	t_1	21.89	31.13	46.97	6.07
	t_2	16.70	60.40	22.90	8.41
	t_3	19.35	35.20	45.45	6.52

Significant : standard deviation <3.5

content stability. The lipid stability is interesting, because it means that the quality of rice is maintained. It suggested that oxidation or hydrolysis phenomena were reduced and that the formation of free fatty acids decreased. As to Ramarathnam and Kulkarni (1983) for the Indian varieties, total lipid content of the brown and milled rice remained constant. However, a reduction in lipid content could be observed, related to hydrolysis or to oxidation phenomena, as mentioned by Nurunnabi et al. (1975) and by Zhou et al. (2002). Nurunnabi et al. (1975) found that lipid was slightly affected with a low decrease of 5.7% on raw rice (average after one year storage). Zhou et al. (2002) found also decreasing lipid content with storage time at 35°C. That is to say, both a warm climate and a long storage time might increase the oxidation phenomena, initiated by the action of lipases.

Storage time showed a significant effect on fatty acids contents. We globally noted a contrast on the two main fatty acids oleic and linoleic variation over time. There was a maximum at t_2 for C18:1 and a minimum at the same period for C18:2. However, the non glutinous Indica rice profile was characterised by a decrease of oleic acid and an increase of linoleic acid (Ramarathnam and Kulkarni, 1983). As discovered by Goffman et al. (2003) in rice bran, fat and fatty acids evolution in our Madagascan rice grain may be involved by a genetic cause. The variety Soameva was not much affected by storage disease. It was particularly a variety of medium size characterised by a smooth peel. So water could not well penetrate and oxidation phenomena have hardly happened; or physical injury could hardly have hit it.

Stored rice had increased activities of proteases, lipases and lipoxygenases. An accumulation of oleic acid was occurring under proteases and air action (from 14 days to three months). But when the lipid cells of the grain were saturated in oleic acids, oleic acids decreased and appeared more linoleic acids. The assumption is that there was an internal permutation of fatty parts (non starch lipids) in the grain. It was proved that linoleic acid was preferentially released by lipase (Zhou et al., 2002). Lipase inside the fatty part of the rice grain caused an oxidation phenomenon and transformed the oleic acids into linoleic ones. According to Mano et al. (1999), the oleic acid was subsequently transported to cytoplasm to be incorporated into the phosphatidylcholine, which is the substrate (phospholipase) for the further unsaturation to form C18:2 and C18:3. Another possibility might be an induction phenomenon produced by degradation of triacylgycerides. As described by Adrian et al. (1998), it brought on free radicals of hydrogen, from monounsaturated fatty acid molecules of C18:1, catalysed by light or by lipase. The linoleic acid molecules were produced. It is well known in plant tissues that the lipid membrane is destroyed by phospholipase, physical injury or high temperature. The linoleic acid is increased in those circumstances, especially higher temperature with the decreasing level of oleic acid 18:1.

Keys's rule proportion found at t_0 (14 days), t_3 (1 year) for Soameva (Table 5) was formed in one side at an earlier time after harvest and in the other side a later time. Those periods might then match with favourable periods for the formation of ideal proportion. Soameva variety was found to be more consistent against a long storage time.

Conclusion

Keys's rule globally related to variety, to site and to storage time. In summary, mono- and polyunsaturated fatty

acid rice grain proportion (MFA and PFA) was the main parameters causing instability of the rice grain quality (Ramarathnam and Kulkarni, 1983). Stale odour could be attributed to the oxidation of free unsaturated fatty acids.

Soameva was the variety which satisfied most to the expected quality according to varieties and storage time parameters. Its lipid and fatty acids are not significantly influenced by a long storage time. The Tox V3 and Tox V4 then came: they can be suggested to be cultivated in Marovoay.

Those results can be used by FO.FI.FA and other organisms using these cultivars. They are data for seed before vulgarisation. Future experimentation now should be made in multiple locations.

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