Article

Physicochemical Properties and Tissue Structure of High Kernel Elongation Rice (*Oryza sativa* L.) Varieties as Affected by Heat Treatment

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ABSTRACT: Rice varieties such as Mahsuri Mutan and Basmati 370 are categorized as specialty rice with kernel elongation ratio exceeding 1.6 and 2.0, respectively. Ageing treatment in rice could affect the physical and chemical properties of rice such as grain size and shape, quality and taste of the cooked rice. The main objective of this study was to determine the effects of ageing treatment on the physicochemical properties of Mahsuri Mutan, Basmati 370 and MR219 rice varieties. The three rice varieties were subjected to heat treatment at 90ºC using the oven for 3 hours. After the heat treatment, the samples were cooled at room temperature (25ºC) for 1 hour. Physicochemical properties such as alkali digestion value, water uptake ratio (WUR), solids in cooking water (SCW), high kernel elongation (HKE) ratio and amylose contents were determined. Data collected on physicochemical traits and ageing *vs* non-ageing treatments were subjected to analysis of variance (ANOVA) using SAS software version 9.4. From this study, Mahsuri Mutan and Basmati 370 showed superior HKE as compared to their respective rice progenies. This study also found that ageing (heat) treatment directly affected the increasing HKE for both populations. Phenotypic correlation co-efficient indicated that there was a high positive correlation between HKE and water uptake ratio, implying that selection for water uptake ratio would increase the HKE characteristic. The ageing treatment also showed significant difference in all the physicochemical traits of the varieties studied. Observation under electron microscope showed that the ageing samples had more cracks on the tissue structure compared to normal rice samples. The hexagon structure in Mahsuri Mutan gave more elongation effect on its kernel. The findings from this study could be useful to breeders in selection and development of new HKE rice variety.

Keywords: Artificial ageing; amylose; heat treatment; phenotypic correlation coefficients; rice samples; selection

INTRODUCTION

Generally, physicochemical changes are related to the alteration of different rice chemical components which may affect rice ageing pathway [1,2]. The process of inducing changes in rice in a short time to obtain desirable cooking properties, which resemble that of naturally aged rice, is referred to as accelerated or artificial ageing. It can be accomplished by heating rough or milled rice to high temperature which will deactivate enzyme lipase and thus can slow down the rate of lipid oxidation [3,4]. There are three major components that determine the quality of rice; amylose content, kernel elongation and aromatic quality. Cooking quality preferences vary depending on the culture of the country and location [5,6]. Consumers base their concept of quality on the grain appearance such as size and shape of the grain, the behaviour upon cooking, the taste, tenderness, milling quality, appearance and flavour of cooked rice [5]. However, in specialty dishes such as Biryani rice, the quality of cooked rice is important. Most of these dishes had high kernel elongation rice type which means the grain will elongate more after cooking and has a good texture than the conventional common rice. Thus, grain size and shape are the first criteria of rice quality that breeders should consider in developing new varieties for commercial production [7,8]. Length/breadth (L/B) filling between 2.5 mm to 3.0 mm and more than 6 mm length is considered widely acceptable [9].

Preferences of grain size and shape differed according to ethnic and geographical locations. Predominantly, long grain is preferred in the Indian sub-continent while South East Asia mostly consumes medium long rice. However, in temperate region, they prefer short grain varieties. Generally, long grain is mostly demanded in the international market [5,10]. The physical dimension of rice kernels is an important aspect to determine quality of grain in rice industry [11]. The physicochemical characteristics include grain length, breadth, L/B ratio, hulling and milling percentage. The cooking qualities are amylose content (AC), alkali spreading value (ASV), water uptake (WU), volume expansion ratio (VER) and kernel elongation ratio (KER) [12,13]. Grain quality is a very wide area encompassing diverse characters that are directly or indirectly related to exhibit one quality type [14,15]. Different cultivars showed significant variations in morphological, physicochemical and cooking properties [16,17]. The cooking quality is an important criterion for the determination of rice grade and market price. Cruz and Khush [5] noted that consumers' preference is based on the quality of grain such as size and shape, and the quality and taste of the cooked rice.

The genotype (rice variety) and environment (growth conditions) control starch and protein structures (including morphology) and that these structures control their properties of interest. Genotype x environment interaction analysis has been reported as major step necessary in developing improved varieties [18-21]. There are differences in yield and yield component traits such as starch and protein when genotypes or varieties are evaluated across environments. Whether the varieties are developed using conventional methods or marker-assisted approach [22-28], significant interaction is usually present between genotype and environment. Large GxE interaction effect has been reported to affect the progress of selection. Sar et al [29] studied the effects of variety and growth location on grain composition and starch structures were determined with the use of three rice varieties which had various compositions of amylose. The three rice varieties were planted at three varied agro-climatic regions of Cambodia. The result indicated increase in protein and decreased lipid contents of polished grains for rice planted in a region with above average temperature. Starch fine structures characterized by the chain-length distribution were significantly different among the cultivars [29]

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Rice ageing treatment is one of the factors that influence the physical and chemical characteristics of rice. Ageing treatment can improve rice cooking quality and increase kernel elongation rate [30,31]. Due to ageing treatment, significant changes occur in the physicochemical, sensory, cooking and pasting properties of rice [32]. According to Saikrishna et al. [32], aged rice had better commercial value with higher consumer preference in terms of cooked rice texture and flavor. The normal ageing takes more than six months to achieve good quality. However, Normand [33] recommended an artificial ageing for rice grain, where the rice grain was heated in 90ºC for 3 hours in closed container, which mimics the normal ageing treatment. Abdullah et al. [34] reported a significant change in tissue structure of Mahsuri, Mahsuri Mutant and Puteri varieties under light microscope after exposure to aged treatment. The present study was conducted to determine the ageing effects on physicochemical properties of three rice varieties namely; Mahsuri Mutant, MR219 and Basmati 370 and to compare the effects of ageing treatment on tissue structure between Mahsuri and Mahsuri Mutant.

MATERIAL AND METHODS

Source population and artificial ageing treatment

Two high kernel elongation rice varieties, Mahsuri Mutant and Basmati 370 were collected from Malaysia and India, respectively. Another variety (MR219) with characteristic normal kernel elongation was collected from the Malaysian Agricultural Research and Development Institute (MARDI). The samples were heated at 90ºC in the oven (Memmert,

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Germany) for 3 hours. After the heat treatment, the samples were cooled at room temperature (25°C) for 1 hour according to the method described by Faruq et al. **[**35**]**. Then the samples were cooked for determination of kernel elongation ratio.

Determination of physicochemical properties

Alkali digestion value

Ten milled kernels were placed in 10 ml of 1.7% potassium hydroxide (KOH) solutions in a petri dish and the grains were arranged in a manner that they did not touch each other. The grain samples were kept for 23 hours at 30 ^{0}C and the data on score spreading were recorded following the alkali spread value described by Oko et al. [36].

Water uptake ratio (WUR)

This was determined by cooking 2.0 g of whole rice kernels from each treatment in 20 ml distilled water for 20 minutes in a boiling water bath [36]. After that, superficial water was drained out from the cooked rice. The cooked rice samples were weighed, and the water uptake ratio was calculated as follows:

$$\text{Water uptake ratio} = \frac{\text{Weight of cooked rice}}{\text{Weight of uncooked rice sample}}$$

Solids in cooking water (SCW)

The method modified by Oko et al. [36] was used to determine the drying aliquot (excess cooked rice water). The empty Petri dish was weight (W_1), then aliquot samples were put into the Petri dish and weighed (W_2), the Petri dish with the aliquot was put in the oven at 40 ^{0}C for 1 hour to let the water evaporate. Finally, the weight of the Petri dish with dried aliquot was recorded (W_3). The solid content was determined with the formula:

$$\text{Solid in cooking water} = W3 - W1$$

High Kernel Elongation (HKE) ratio

The average lengths of ten milled rice of six generations in three replications were used for HKE. The rice kernel was soaked in tap water for 20 minutes in 20 ml test tube. Then, the test tubes containing the samples were put in boiling water for 30 minutes. The water in the test tube was drained out and cooked rice was placed on a glass sheet for few minutes to evaporate excess moisture and then measured the length of the cooked rice. Measurement was done using a digital Vernier caliper. Elongation ratio was measured according to the method described by Golam et al. **[**37**]**.

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$$\text{Elongation ratio} = \frac{\text{Average of cooked kernels}}{\text{Average length of raw kernels}}$$

Amylose content

Sample of ground rice (0.10 g) was put into 100 ml volumetric flask and 95% (v/v) 1 ml ethanol was added. The mixture was slightly shaken to ensure the entire sample was wet. Then, 9 ml of 1M sodium hydroxide (NaOH) was added and mixed thoroughly. After that, the volumetric flask was heated in hot water bath to dissolve the starch. Then, the mixture was cooled to room temperature before the addition of distilled water. The amylose assay was prepared by adding 0.1 ml (v/v) acetic acid to 5ml of distilled water, then 0.5 ml aliquot and 0.2 ml iodine solution was added before addition of distilled water for final volume (10 ml). Then, the assay was mixed using Vortex mixer (Velp Scientifica, Germany). The absorbance was measured at 720 nm against a blank solution by using spectrophotometer (Varian, USA). A calibration curve was plotted against the three tested varieties [38]. The samples were categorized as waxy, very low amylose, low, intermediate and high given 1–2, 2–9, 10–20, 20–25 and 25–30 percent amylose contents, respectively. The starch structure of milled rice (ageing and non-ageing) and cooked rice (ageing and non-ageing) of Mahsuri and Mahsuri Mutant were observed under Scanning Electron Microscope (SEM) at the Institute Bioscience, Universiti Putra Malaysia (UPM). The samples were viewed at 2000x magnification.

Statistical analysis

Data collected on physicochemical properties and ageing *vs* non-ageing treatments were subjected to analysis of variance (ANOVA) using SAS software version 9.4 [39]. More so, descriptive statistics such as mean, range, standard deviation and coefficient of variation (CV) were calculated for each trait. Mean comparisons were performed using least significant difference (LSD) test at 95% and 99% confidence level. Correlation coefficients were also analyzed using SAS Software programme version 9.4 to study the relationship between traits [39-41].

RESULTS AND DISCUSSION

Physicochemical properties of High Kernel Elongation Rice

The physicochemical characteristics such as water uptake ratio, alkali spread value (%), kernel elongation ratio and amylose content of the grain among the three sourced varieties with ageing treatment are as presented in Table 1. Highly significant differences were found among the three varieties and between ageing treatments on all the physicochemical characteristics. Basmati 370 had the highest solid cooking (quantity of water leached during cooking) percentage while MR219 had the lowest value which was not significantly different from the solid cooking percentage recorded in Mahsuri Mutant. For the kernel elongation ratio, Basmati 370 had the highest value of 2.71 followed by Mahsuri Mutant whereas MR219 had the lowest value of 1.14. Contrarily, Mahsuri Mutant had the highest value of 4.1 which is significantly higher than MR219 with 4.35. However, the lowest value of alkaline spreading was observed in Basmati 370. For amylose content, the highest value was observed in MR219 followed by Mahsuri Mutant and Basmati 370 at 24.63, 24.03, and 20.3 respectively. For ageing treatment, a higher value was observed for the entire traits in ageing treatment as compared with non-ageing. This result showed that Basmati 370 structure was softer and easier to reach out based on solid cooking parameter. Shamin et al. [42] reported higher cooking solid value in Basmati with shorter cooking time. The presence of interaction between varieties and ageing treatment for solid cooking, alkaline spreading value and water uptake ratio were presented in Tables 1 and 2. Based on the results, there was significant difference between ageing and non-ageing solid content of all the varieties except Basmati 370, whereas for alkali spreading value and water uptake ratio, all the varieties had significance difference between ageing and non-ageing treatments. Saikrishna et al. [32] reported that storage or ageing treatment increases chemical reaction activity during storage. Based on the study, amylose content was significantly affected by ageing period.

Table 1. Main and interaction effects among rice varieties and ageing effects on physicochemical properties

Factor	SC(g) (leached)	HKE	ASV	AC (mg/L)	WUR
Rice varieties (RV)					
MR219	0.07 b	1.44 c	4.35 b	24.63 a	9.35b
Mahsuri Mutant	0.08 b	2.15 b	5.10 a	24.03 b	7.45c
B370	0.17 a	2.71 a	3.30 c	20.03 c	10.7a
LSD Value	**0.0046**	**0.1065**	**0.07**	**0.5928**	**0.0719**
Ageing treatment					

(AT)					
Ageing	0.153 a	2.425 a	4.575 a	24.26 a	8.46a
Non-Aging	0.116 b	2.131 b	3.83 b	22.74 b	9.29b
LSD value	**0.0038**	**0.087**	**0.0572**	**0.484**	**0.0587**
RV	**0.021****	**3.266****	**6.540****	**50.148****	**21.311ns**
AT	**0.007****	**0.481****	**2.006****	**13.009****	**27.468ns**
RV × AT	**	**ns**	**	**Ns**	**

Note: Means within a factor and column followed by the same letter are not significantly different at P ≤ 0.05 by using LSD test. ** = Highly significant at P ≤ 0.01 ns = Not significant at P = 0.05.SC=Solid content; ASV= Alkali spread value and WUR=Water uptake ratio.

Table 2. Interaction between variety and ageing treatments on solid content, alkali spreading value and water uptake ratio

Factor	**SC (leached) g**		**ASV**		**WUR**	
Rice varieties (RV)	**A**	**NA**	**A**	**NA**	**A**	**NA**
MR219	0.069e	0.0825c	4.45c	4.25e	11.242e	7.4600b
Mahsuri Mutant	0.138b	0.02175d	5.4a	4.8b	8.603d	6.3000f
B370	0.1675a	0.16675a	4.35d	2.25f	12.163a	9.2398c

Note: Means within a factor and column followed by the same letter are not significantly different at P ≤ 0.05 by using LSD test. SC=Solid content; ASV= Alkali spread value and WUR=Water uptake ratio

Correlation among physicochemical characteristics

The correlation coefficients among the physicochemical traits are presented in Table 3. High kernel elongation had significant positive correlation with water uptake ratio and also positively correlated with solid content. However, HKE had non-significant correlation with amylose content and alkali spreading value (gelatinization). This finding showed that water uptake ratio during rice cooking was directly affected by the quality of rice elongation. The result showed that amylose content had negative correlation with WUR but positively correlated with ASV. This result was in contrast with the findings made by Saikrishna et al. [32] where the increase in water uptake value simultaneously increased the amylose content in cooked rice. Though, the ageing treatment may influence the result where the water uptake ratio was higher in ageing rice due to physical properties of the rice. After ageing, some cracking on the rice structure was developed due to a decrease in moisture content. This result gives a direct effect on the parameters; water uptake ratio and solid cooking leached upon cooking period. Aged milled rice has higher volume expansion and water absorption and less dissolved solid in cooking [43]. From this study, the grain elongation was positively correlated with solid content during cooking. However, Prodhan et al. [43] mentioned in his study that, the overall changes may depend on the rice variety, storage condition and further treatment. He also mentioned that the ageing process affected eating quality. Only water uptake ratio and solid content after cooking showed positive and highly significant correlation with high kernel elongation at r = 0.019 and r = 0.829, respectively. It was confirmed in Binoth et al. [44] that there was significant and positive correlation between water uptake ratio and expanded ratio which affected the kernel elongation. Same results were reported by Tomar and Nanda [45] and Deosarkar and Nerkar [46]. The non-significant correlation coefficient obtained in amylose content and high kernel elongation was in contrast with the findings made by Wu et al. [47] who reported that elongation ratio showed highly significant positive association with amylose content. However, rice variety could also influence the results. In the development of improved breeding lines having superior quality, the correlation among grain quality traits is useful in the choice of parents, screening and selection procedures for the segregating populations.

Table 3. Correlation co-efficients (r) of physicochemical properties of the rice varieties studied

Physiochemical characteristics	**WUR**	**SC**	**HKE**	**ASV**	**AC**
WUR	1.00	0.107^{ns}	0.019*	-0.153ns	-0.427**
SC		1.00	0.829**	-0.353*	0.026^{ns}
HKE			1.00	-0.264^{ns}	-0.206^{ns}
ASV				1.00	0.626**
AC					1.00

Note: *significant at 0.05 probability level, **highly significant at 0.01 probability level, WUR: Water uptake ratio, SC: Solid content, HKE: High Kernel elongation, ASV: Alkali spread value, AC: Amylose content.

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Examination of Kernel Structure through Electron Microscope (SEM)

The endosperm appearance of non-ageing milled and cooked rice of Mahsuri (Figure 1) was examined in comparison with non-ageing milled and cooked rice of Mahsuri Mutant (Figure 2). The endosperm of non-ageing milled Mahsuri is packed and granule. Meanwhile, the ageing Mahsuri is loosely packed and showed some cracking between the starch granule. The non-ageing starch granules are more compact than ageing milled rice of Mahsuri. The ageing effect causes the structure to form a crack between the starch granules because of hydration and lipid oxidation that occurred during the ageing process. The higher hardness and lower adhesiveness are likely to be associated with lower hydration process of starch granules in aged rice grains stored at a higher temperature [48]. According to Faruq et al. [30], internal anatomical structure of rice kernel, cell shape, and their arrangement might influence the water uptake and nature of swelling of kernel during cooking. Therefore, internal structure of rice grains could represent the effects of ageing for cooking evaluation. In the previous study of Faruq et al. [30], they observed that the internal cracks (vacuum-like structure) observed in aged rice kernel were higher than in non-aged kernels. The same situation also happens in non-ageing and ageing of milled rice of Mahsuri Mutant variety. However, the variety could also influence the number of cracking after ageing treatment [30]. With the comparison between Mahsuri as wild type variety and Mahsuri Mutant which is modified variety from Mahsuri, the cracking number is more in Mahsuri Mutant. These observations indicated that the internal structure of Mahsuri Mutant is loose compared to Mahsuri. Moreover, from the observation, the shape of internal structure between Mahsuri and Mahsuri Mutant were also quite different where the internal shape of Mahsuri Mutant is most likely round shape compared to Mahsuri which is more of flakiness shape. The shape of Mahsuri Mutant is similar to that of Basmati rice; a high kernel elongation type of rice [49]. Hormdok and Noomhorm [50] reported that the physicochemical properties changed during ageing process in rice.

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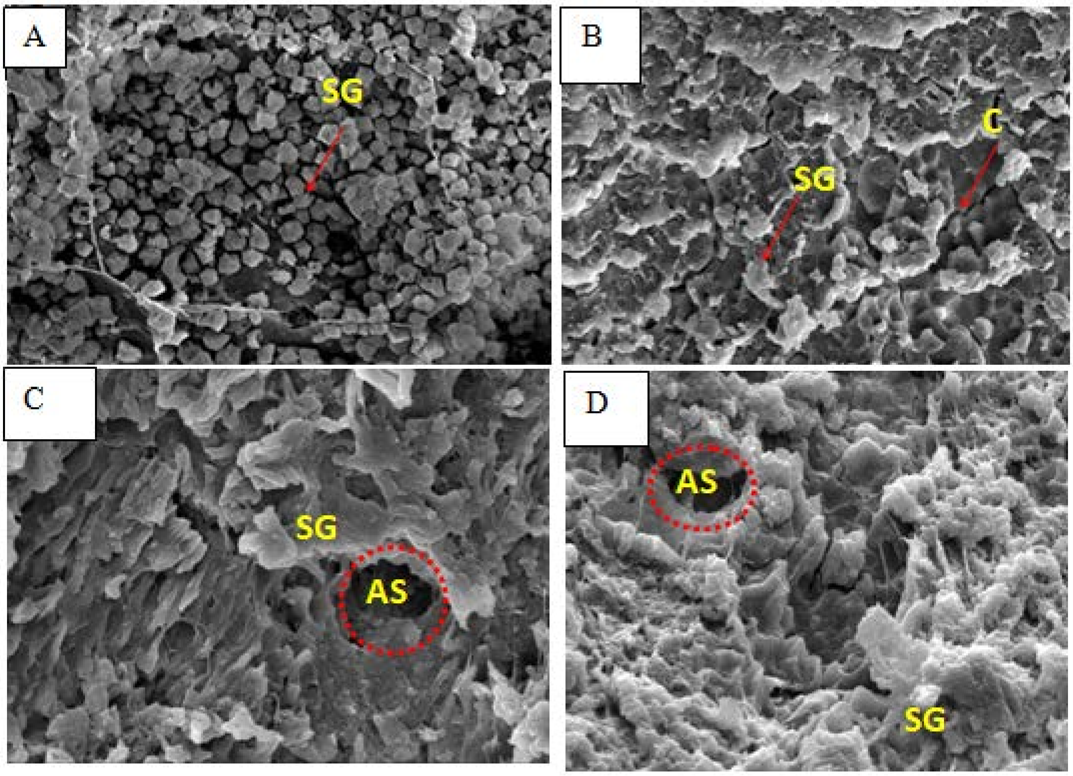

Figure 1: Endosperm morphology of Mahsuri variety. Non ageing milled rice (A), ageing milled rice (B), non-ageing cooked rice (C) and ageing cooked rice.C= Cracking between starch granule; SG= Starch granule; AS= Air space between structure

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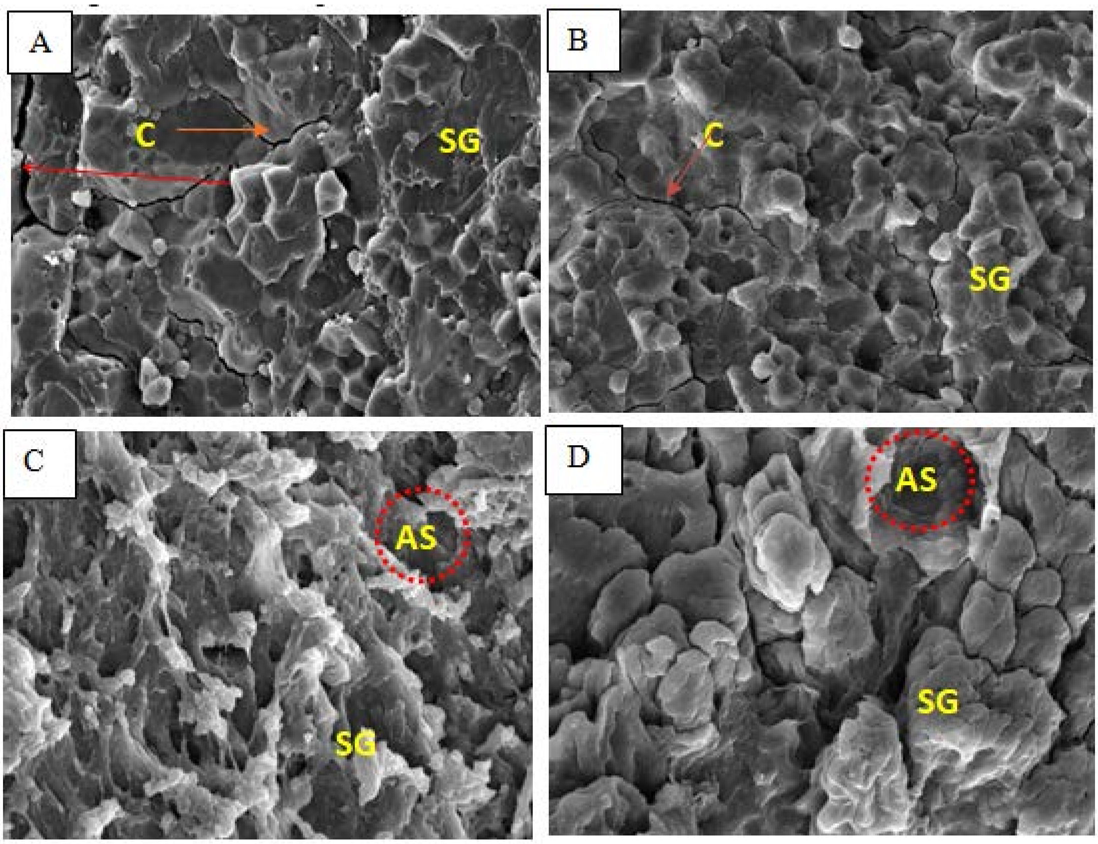

Figure 2: Endosperm morphology of Mahsuri Mutant variety. Non ageing milled rice (A), ageing milled rice (B), non-ageing cooked rice (C) and ageing cooked rice (D). C= Cracking between starch granule; SG= Starch granule; AS= Air space between structure

Besides the observation from SEM results on ageing and non-ageing milled rice, there were also different structural observations due to the physicochemical changes associated with ageing and non-ageing of cooked rice. The endosperm of elongating and non-elongating varieties showed differences in the shape and arrangement of cells. The structural arrangement of starch molecules within cells might influence the elongation pattern where the long belt or radially arranged cells in the non-elongating rice might restrict linear expansion of cells and allow the intermittent area to expand outwardly, resulting in the breadth-wise swelling. According to Kanlayakrit and Maweang [51], the elongating rice cells of uniform size and shape might influence lengthwise swelling. Following the study on Basmati variety's rice structure which was categorized as high kernel elongation variety; the tissue structure was nearly equidistant pentagonal or hexagonal cells arranged in a honeycomb pattern. Its difference in non-elongating variety, where the cells were arranged in a rectangular shape and radially in column extending from the center to the periphery in breadth-wise swelling type after cooked. Besides, there is the probability that lipoprotein matrix at sub-cellular level might influence the gelatinization and swelling of starch molecules in specific direction. The internal structure of cooking rice obviously changes after cooking for both varieties. The previous shape changes due to the physical and chemical changes during cooking activities. The shape looks flakiness and more air space formed. This happens before and after ageing treatment. However, the different situation happens in Mahsuri Mutant where after ageing, the internal granules were round shaped and arranged. Under the normal rice cooking condition, the SEM displayed uneven structure covered by the formed films.

Artificial ageing involved heat treatment on milled rice and the cracking structure formed between the starch granules. This could be as a result of hydration during the heat treatment. So, the hydration of the rice kernels was increased with temperature rise, which facilitated the leaching of carbohydrates. From the histological study of artificial ageing rice (heat treatment on rice grain) that was done by Tamura et al. [52], as the temperature increased, there was more cracking on the rice structure. The lines on the structure were observed between ventral and dorsal sides. As the temperature increased ≥80°C, the cracks spread, and some became voids and the cooked grain was swelling as the cracking fissure increased. Very interesting was the longitudinal grain swelling which contributed to more kernel elongation when cooked. This study showed that artificial ageing could give same results as traditional ageing (store for 3 to 4 month) as reported by Golam et al. [37].

The appearance of the hollows can be explained by the fact that during cooking, the starch located at the surface could leach out easily into water, resulting in high void density at the exterior surface [53,54]. Additionally, the internally stored starch absorbs water through voids and swells greatly, disrupting the cell walls beneath the surface [52]. The situation could affect the cell wall after ageing process. The morphological properties of cooked rice correspond to the texture properties. After the AM and AP diffuses out of the swollen granules and leached into the cooking water, the starch granules were disrupted and lose rigidity. The framework of rice grains loses its support, and the structure of rice grains become very loose, which results in the formation of a fluffy, soft and non-sticky texture of cooked rice [54]. The packed arrangement of starch granule shows the effect of expansion of the starch granule during cooking. The air space gives lengthwise effect during the cooking process where the expansion focused on the straight direction and less on breadth wise expansion. Meanwhile, under normal rice, the starch granule expansion during cooking focuses on breadth wise expansion and more air space between the starch granules. These actions affect the cooked rice physical properties and texture of the cooked rice. The lengthwise expansion gives fluffiness effect, just like the ageing effect. Ageing causes the physical and internal structure of the rice to change, leading to expansion of the rice. Besides, the strength of the wall structure gave an impact to the elongation in rice. According to Chandi and Sogi [55], high elongation and low solid loss in Pusa Basmati 1 rice may be attributed to the greater strength of cell wall line, which is able to hold the pressure until maximum elongation takes place without rupturing of the cell wall. This is why Mahsuri Mutant could elongate better than Mahsuri.

CONCLUSIONS

Based on the SEM observation, there were more cracking on the ageing tissue structure surface. This cracking can relate to water absorption and the ability of the kernel to absorb water and elongate during the cooking period. The reason for cracking phenomenon in ageing kernel is due to decrease in moisture content. Therefore, more space (vacuum) was created for the kernel to absorb more water and elongate. There was more cracking on aged Mahsuri Mutant compared to Mahsuri. This is one of the reasons Mahsuri Mutant's kernel can elongate better than Mahsuri. Besides, the shape of the tissue structure also gave main factor to the characteristic changes, where in Mahsuri Mutant, the starch granules shape was like that of Basmati 370. Ageing treatment had positive influence on major cooking quality traits such as kernel elongation, water absorption, alkali spreading value, and water uptake ratio. Besides, this ageing can improve the quality of rice and their marketability will be widened. The endosperm of non-ageing milled Mahsuri rice is packed and granule. Meanwhile, the ageing Mahsuri is loosely packed and had some cracking between the starch granule. The non-ageing starch granule was more compact than ageing milled rice of Mahsuri. The ageing effect causes the structure to form a crack between the starch granule because of hydration and lipid oxidation which occurred during the ageing process. The changes in internal structure of rice could induce the water absorption and lengthwise or breadth-wise effect could happen during cooking based on the rice structure of that variety. From this study, it was therefore concluded that ageing (heat) treatment has a direct effect of increasing HKE in the rice populations and could also amplify the expression of grain kernel

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characteristic. The current findings could be useful to breeders for specialty rice varietal development in the future.

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Conflicts of Interest: "The authors declare no conflict of interest."

Additional information

Supplementary data and information are available upon request from mrafii@upm.edu.my

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