Research Journal of
Seed Science

ISSN 1819-3552

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Seed Science

ISSN 1819-3552
DOI: 10.3923/rjss.2018.1.11

Research Article
Study on Biochemical Properties of *Hevea brasiliensis* Seeds Stored at Three Different Temperatures

[1]Nor Hassifi Shuib, [2]Aimi Izyana Ismail, [3]Azuan Adinan and [4]Shaikh Mohd Hizami Shaikh Abd Hadi

[1]Crop Management Programme, Production Development Division, Malaysian Rubber Board, 47000 Sungai Buloh, Selangor, Malaysia
[2]Centre of Excellent for Advanced Processing and Product Technology, Malaysian Rubber Board, 47000 Sungai Buloh, Selangor, Malaysia
[3]Lembaga Getah Malaysia Machang, Kelantan, Malaysia
[4]Lembaga Getah Malaysia Wilayah Utara, Ipoh, Perak, Malaysia

Abstract

Background and Objective: Malaysia needs approximately 200 t of seeds usually for the replanting programme in the rubber plantation. However, its behavior as recalcitrant seed has no dormancy characterization, short life span and the germination rate decreased over time have given a critical challenge to set up suitable storage condition. Improper storage condition affected the rootstock preparation for planting materials in rubber nursery and maintaining the biochemical nature of the seed in storage period is important to maintain seed longevity. Therefore, the study aim to determine the effect of storage temperature on the biochemical properties of *Hevea* seed and to evaluate the ability of the potential temperature condition to maintain the seed longevity. **Materials and Methods:** Fresh *Hevea* seeds were collected from RRIM Mini Station of Tok Dor. Three temperature conditions, i.e. 8, 13 and 27°C were applied as storage conditions. The data obtained were subjected to one-way analysis of variance (ANOVA) using statistics software Prism 5 and Excel. The level of significance of the differences between mean values was estimated by post-test of Newman-Keuls test. **Results:** The results showed that the temperature of 27°C was significantly affected the high loss of enzyme activity, high moisture decrease, high lipid and protein contents, high contamination in the seed, high nutrient loss and high ratio saturated to unsaturated fatty acid. Thus, it reflected the low germination rate and seed viability only maintained until 2 weeks of storage. Temperatures of 8 and 13°C can maintain the seed viability until 8 weeks storage but stored at 13°C produced the best results based on the higher germination, lower loss of enzyme activity, lower moisture decrease, low lipid and protein contents, no contamination in the seed, lower nutrient loss and lower ratio saturated to unsaturated fatty acid. **Conclusion:** The temperature of 13°C was the most suitable for storing the *Hevea* seeds.

Key words: *Hevea* seed, storage temperature, biochemical in *Hevea* seed, seed viability, fatty acid

Citation: Nor Hassifi Shuib, Aimi Izyana Ismail, Azuan Adinan and Shaikh Mohd Hizami Shaikh Abd Hadi, 2018. Study on biochemical properties of *Hevea brasiliensis* seeds stored at three different temperatures. Res. J. Seed Sci., 11: 1-11.

Corresponding Author: Nor Hassifi Shuib, Crop Management Programme, Production Development Division, Malaysian Rubber Board, 47000 Sungai Buloh, Selangor, Malaysia Tel: +603-61459561 Fax: +603-61565058

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

One of critical issues in *Hevea* seed is during post harvesting of the seed prior to germination. High rate of germination can only be observed from fresh seed and when seed is stored in proper seed storage. Malaysia needs approximately around 200 t of seeds usually for the replanting programme. On the average, three seeds were need to produce one planting material. However, the germination rate of *Hevea* seed is decreasing over time and this situation affected the rootstock preparation for planting materials at rubber nursery. According to Malaysian Rubber Board[1], the seed has a short life and the germination rates of the seed decreased significantly after 3-4 days exposure to the sun. In Malaysia, there are only two times the seedfall season every year, which are in March and August every year. Seedfall occurs approximately 5 months later. However, despite of weather conditions, times of seedfall has affected, sometimes early and lately.

Hevea seed belongs to recalcitrant seeds and its behavior pattern reported that the recalcitrant seeds had no dormancy, had a short life, having undergone drying when physiologically ripe, apart from the water content that relatively high which triggers the process of respiration and will lose its viability in a short time[2]. The seed has high moisture content with more than 30% at time of shedding from the mother plant[3,4] while Berjak and Pammenter[5] reported that the moisture content to be range between 30-70%. All these behaviors have caused the seed vulnerable to drying process and low freezing as well as high temperatures. For example, *Hevea* seed was eradicated when the seed moisture content was lower than 15-20% on a fresh kernel weight basis. Its viability was completely lost when it was kept at temperature lower than 0°C or at high temperature at 45°C[2,3]. According to Anonymous[6], *Hevea* seed was best stored at 7-10°C in moist sawdust. Meanwhile, there was an increase in free fatty acid and production of cyanogenic glycosides or hydrocyanic acid during storage and these conditions reduced the survival of rubber seed[7].

During storage, seeds will experience aging and deterioration. Seeds are retreating, the respiration rate increases, which causes a reduction in food reserves and can starve the meristem tissue. Seed deterioration can be observed from the decline of physiological seed quality such as loss of enzyme activity, reduced respiration, increase in free fatty acid content and increase in seed leachates which can lead to overall changes[8]. Charloq *et al.*[9] also stated that recalcitrant seeds cannot tolerate water loss, whereby, it cannot be stored using conventional seed bank conditions. Particularly with respect to storage, recalcitrant seeds do not undergo intracellular differentiation or any significant metabolic shut down. Setbacks indication such as seed moisture reduction, respiration rate, decreased food reserves, increased of seed conductivity value and germination decline was found after the storage period. The recalcitrant seed storage could not be dried to below 30% moisture content and would be damaged and not tolerant to low temperatures[5]. In these conditions, the metabolism still active and ongoing process towards germination even in the resting state. When the seeds were dried and the water content decreased, it resulting in deterioration of seed viability and no single technique was adequate for storing recalcitrant seeds for a long period[9]. The biochemical and physiological changes in the seed lead to reduced vigor, culminating in the loss of germination capacity[10].

Marques *et al.*[10] reported that in storage conditions, temperature and relative humidity are key factors in maintaining seed quality in rice, influencing the speed of the biochemical processes and interfering water content of seeds. It's because the biochemical and physiological changes after a storage period can cause an overall change in the content of seed and reduce the viability of seeds and have an impact on the next phase of life. A proper storage condition need to be developed in order to reduce the biochemical change and resulted to the survivability of *Hevea* seed. In order to do this, an understanding of the biochemical properties in the seed is essential. While several studies have been carried out on other recalcitrant crops such as cotton[11], comprehensive information on biochemical changes in *Hevea* seed owing to storage temperature is limited. Information on biochemical changes such as lipid, protein and nutrient contents are not well understood. In addition, information on chemical change affecting the declining of germination rate of *Hevea* seed still limited.

Therefore, the objective of this study was to determine the effect of storage temperature, i.e. 8, 13 and 27°C on the biochemical properties of *Hevea* seed and to evaluate the ability of the potential temperature condition to maintain the seed longevity.

MATERIALS AND METHODS

Fresh *Hevea* seeds were collected from RRIM Mini Station of Tok Dor, Terengganu during main seedfall in August, 2015. Weight of 3-4 g was selected to be used to avoid variations due to the weight of the seed. Then, the seeds were transferred and stored at temperature 8, 13 and 27°C. Temperature of 27°C was selected as a control treatment and

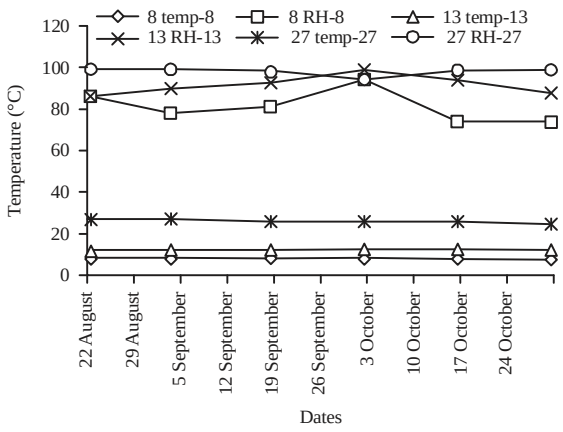

Fig. 1: Temperatures (°C) and relative humidity (RH) in storage environment

due to the conventional practice. Another temperature was chosen as a cold temperature in the study. During storage, the temperature and relative humidity of the environment were monitored (Fig. 1). Every 2 weeks, the seeds were harvested from storage rooms for the chemical analysis with three replications for each analysis.

Germination test: The germination test was being carried out at seedbed by sowing the seeds in sand and sawdust which was evaluated every day for 30 days after sowing. About 30 seeds/replication were used in the test.

Tetrazolium test: The tetrazolium test was used to estimate seed viability and loss of enzyme activity using the method proposed by Sakhibun[12]. *Hevea* seeds were shelled and then soaked in water for 4 h. The endosperms were bisected longitudinally to expose the cotyledons and the embryo axis were immersed in 2,3,5-Triphenyl tetrazolium chloride 1% at 40°C for 2 h. The results were based on red stained in the embryo and cotyledon (Fig. 2).

Moisture content: Moisture content was analyzed using the method described in ISTA[13]. The wet weights of the seeds were recorded. Then the seeds were dried in a ventilated oven at 103°C for 17 h. The dry weight was recorded and the percentage moisture (wet basis) was determined.

Determination of total lipid content: The total lipid content was carried out according to the method of Folch *et al.*[14] with minor modification. Minor modifications that had been done change the quantity of the sample to 1 g and reduce the extraction period to 1 h with moderate shaking. About 1 g of

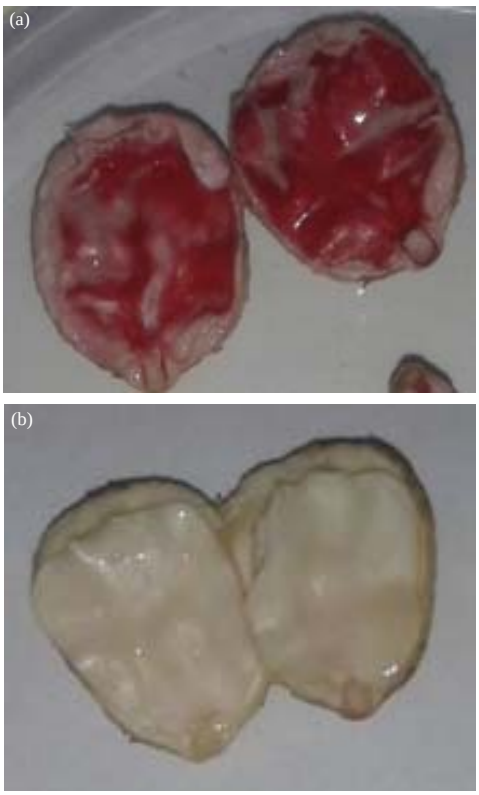

Fig. 2(a-b): Tetrazolium evaluation in seed (a) Viable seed and (b) Non-viable seed

endosperms was added to 40 mL of chloroform: methanol (2:1). The mixture was allowed to stand for 1 h and was filtered through a filter paper into a separating flask. Then, 12 mL of sodium chloride 0.9% was added and the mixture was shaken vigorously and was let stand until complete separation. Then the lower phase was collected and dried in a desiccator.

Determination of total protein: The dried sample of endosperms was ground into powder form before ready to be analyzed. These samples were analyzed using Kjeldhal method based on AOAC 988.05 17th edition.

Contamination in seed: The test was carried out according to the procedure of MS 320:2008[15] for seed health testing. *Hevea* seeds were shelled and bisected longitudinally to expose the cotyledons and the embryo axis. The endosperm halves containing the embryo axis and the cotyledons were visually examined under light microscope.

Determination of nutrients: The dried sample of endosperms was ground into powder form before ready to be analyzed. These samples were used for nutrient analysis of nitrogen (N),

phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), manganese (Mn), zinc (Zn), iron (Fe) and copper (Cu). N was determined by Kjeldhal digestion method while P was determined by Bray's method and both were quantified by a calorimetric autoanlyser. K was determined by using a flame photometer while Mg, Ca, Mn, Cu, Zn and Fe were determined by using an absorption spectrometer for specific wavelengths. The absorption wavelengths for Mg, Ca, Mn, Cu, Fe and Zn were 2025, 4226, 2794, 3247, 2483 and 2138 A°, respectively.

Fatty acid composition in seed: The test was carried out according to the procedure of Nielson[16]. The lipid content was converted to the fatty acid methyl esters (FAMEs) by transesterification using the sodium methoxide method. Then, the FAMEs were analyzed on a gas chromatograph equipped with a flame ionization detector (FID).

Statistical analysis: The data obtained were subjected to the one-way analysis of variance (ANOVA) using Statistics Software Prism 5 and Excel. The level of significance of the differences between mean values was estimated by post-test of Newman-Keuls test at a limit of 5%.

RESULTS

Seed viability: The evaluation was based on standard germination and tetrazolium test. The tetrazolium test also was carried out to measure loss of enzyme activity in order to confirm symptom of seed deterioration. Results from standard germination test stated that around 91% of seeds were germinated at the beginning of storage (Table 1). Seeds stored at 13°C gave the highest germination rate from 0-92% after 10 weeks of storage. The quite similar pattern also was observed at 8°C, where the germination rate from 0-89% after 10 weeks storage. However, no significant difference was observed between these treatments. Meanwhile, the germination rate of seeds stored at 27°C gave significantly the lowest germination after 2 weeks storage by 35% and decreased rapidly to 0%, after only 4 weeks storage.

In the tetrazolium test, 100% of seeds were viable at the beginning of the storage (Table 1). After 4 weeks, seeds stored at 27°C gave significantly the lowest colour stained and no longer viable after 6 weeks of storage. Seeds at 8 and 13°C maintained to be viable for up to 8 weeks and stored at 13°C able to produce highest stained at 33% compared to 8°C at 30% after 6 weeks storage. However, no significant difference was between both treatments.

Moisture content: Fresh seeds contain a very high moisture around 24% (Table 2). The moisture content in *Hevea* seeds was significantly decreased with increase in storage period. Seeds at 27°C significantly decreased after only 2 weeks storage and both at 8 and 13°C significantly reduced after 4 weeks storage. In addition, seeds stored at 27°C contain the lowest ($p<0.05$) moisture after 2 weeks storage by 20.18% and this pattern was continued until the end of the studies at 10.05%. This finding could support the germination rate whereafter 4 weeks, no germination had been detected at 27°C. Seeds at 13°C gave higher moisture content during 2-4 weeks of storage at 22.28 and 18.45% compared to 8°C at 22 and 16.46%. However, no differences were observed

Table 1: Germination test on *Hevea* seeds during storage under different temperatures

		Weeks after storage					
Parameter (%)	Temperature (°C)	0	2	4	6	8	10
Germination	8	89.0±11.50^{aA}	84±3.00^{aA}	62.0±12.00^{aB}	50±2.50^{aB}	7±6.67^{aC}	0±0.00^{aC}
	13	92.0±11.00^{aA}	85±3.00^{aB}	75.0±10.00^{aB}	60±4.00^{aB}	7±6.67^{aC}	0±0.00^{aC}
	27	92.0±11.00^{aA}	35±3.00^{bB}	0.0±0.00^{bC}	0±0.00^{bC}	0±0.00^{aC}	0±0.00^{aC}
Tetrazolium	8	100.0±0.00^{aA}	88±11.00^{aA}	67.0±13.00^{aB}	30±6.67^{aC}	10±6.67^{aD}	0±0.00^{aD}
	13	100.0±0.00^{aA}	88±11.00^{aA}	67.0±6.67^{aB}	33±6.67^{aC}	10±6.67^{aD}	0±0.00^{aD}
	27	100.0±0.00^{aA}	89±11.00^{aA}	20.0±11.00^{bB}	0±0.00^{bC}	0±0.00^{aC}	0±0.00^{aC}

Data are expressed as Mean±SE. Mean values by the same lowercase letter in the column and uppercase letter in the line are not significantly different ($p>0.05$)

Table 2: Moisture content of *Hevea* seeds during storage under different temperatures

		Weeks after storage					
Parameter (%)	Temperature (°C)	0	2	4	6	8	10
Moisture	8	24.20±0.59^{aA}	22.00±1.75^{aA}	16.46±1.00^{aB}	14.07±0.64^{aB}	11.96±0.42^{aC}	11.56±0.08^{aC}
	13	24.20±0.59^{aA}	22.28±0.59^{aA}	18.45±0.37^{aB}	13.80±0.50^{aB}	12.00±0.20^{aC}	10.97±0.32^{aC}
	27	24.20±0.59^{aA}	20.18±3.94^{bB}	14.92±0.84^{bB}	11.90±0.49^{bC}	10.30±0.24^{bC}	10.05±0.05^{bC}

Data are expressed as Mean±SE. Mean values by the same lowercase letter in the column and uppercase letter in the line are not significantly different ($p>0.05$)

Table 3: Total lipid content of *Hevea* seeds during storage under different temperatures

Parameter (%)	Temperature (°C)	Weeks after storage 0	2	4	6	8	10
Lipid	8	32.05±0.59^{aA}	33.50±0.29^{aA}	32.35±0.06^{aA}	43.50±9.70^{aB}	42.14±2.14^{aB}	40.17±0.86^{aB}
	13	32.05±0.59^{aA}	36.10±0.38^{bA}	34.11±0.26^{aA}	44.09±2.09^{aB}	42.55±1.16^{aB}	42.63±3.63^{aB}
	27	32.05±0.59^{aA}	39.81±0.74^{cB}	44.15±2.10^{B}	45.52±2.58^{B}	43.27±2.12^{B}	46.63±1.16^{aB}

Data are expressed as Mean±SE. Mean values by the same lowercase letter in the column and uppercase letter in the line are not significantly different (p>0.05)

Table 4: Protein content of *Hevea* seeds during storage under different temperatures

Parameter (%)	Temperature (°C)	Weeks after storage 0	2	4	6	8	10
Protein	8	11.50±0.59^{aA}	12.90±0.59^{aA}	13.20±0.37^{aA}	16.20±0.54^{aB}	17.40±0.42^{aB}	15.70±0.08^{aB}
	13	11.50±0.59^{aA}	14.10±0.59^{bB}	13.80±0.84^{aA}	16.20±0.50^{aB}	16.70±0.20^{bB}	16.70±0.32^{aB}
	27	11.50±0.59^{aA}	15.10±0.59^{bB}	16.50±0.37^{bB}	16.90±0.49^{aB}	17.30±0.20^{aB}	17.30±0.05^{aB}

Data are expressed as Mean±SE. Mean values by the same lowercase letter in the column and uppercase letter in the line are not significantly different (p>0.05)

Fig. 3(a-b): Contamination in seed, (a) Contaminated and (b) Non-contaminated

between both treatments. In overall, seeds at 8°C decreased from 24.2-11.56% and at 13°C decreased from 24.2-10.97%, during 0-10 weeks of storage.

Lipid content: At the beginning of storage, fresh seeds contain 32% total lipid and the content significantly increased with the increase of storage period (Table 3). Seeds at 27°C significantly increased after only 2 weeks storage and both at 8 and 13°C significantly increased after 6 weeks storage. Since *Hevea* seed is an oilseed species, it accumulates large quantities of total lipids during storage. Seeds stored at 27°C contain the highest ($p<0.05$) lipid after 2 weeks storage by 39.81% and this pattern was continued until 10 weeks by 46.63%. Meanwhile, seeds at 8°C increased from 32-40% and at 13°C increased from 32-42%, during 0-10 weeks of storage. However, no significant difference was between these treatments.

Protein content: The fresh seeds contain 11.50% total protein and the content significantly increased with the increase of storage period (Table 4). Seeds at 27°C significantly increased after only 2 weeks storage and both at 8 and 13°C significantly increased after 6 weeks storage. In addition, seeds stored at 27°C contain the highest ($p<0.05$) protein after 4 weeks storage by 16.50% and this pattern was continued until 10 weeks by 17.30%. Meanwhile, seeds at 8°C produced the increment from 11.50-15.70% and at 13°C increased from 11.50-16.70%, respectively. However, no significant difference was between these treatments.

Contamination in seed: The contamination evaluation was based on physical observation (Fig. 3). At the beginning of storage, no contaminated seeds had been detected (Table 5). This pattern was continued in seeds at 8 and 13°C until the end of the studies. However, seeds stored at 27°C gave highest ($p<0.05$) broken seeds after 4 weeks storage by 8% and significantly increased to 22% during 10 weeks storage.

Nutrient analysis: Analysis of nutrient content showed the mixed results (Table 6). The total N content showed the decreased pattern during the storage period and stored at 8°C gave the highest decrement from 3.03-2.62%. The K content showed the decreased pattern during 10 weeks of storage and stored at 8°C gave the highest decrement from 0.86-0.79%. The Mg and Ca contents not significantly reduced, although shown the reducing pattern during the storage period. Stored at 27°C contributed to the highest decrement for both contents from 0.27-0.23% in Mg and 0.08-0.05% in Ca content.

Table 5: Contamination in *Hevea* seeds during storage under different temperatures

	Temperature	Weeks after storage					
Parameter (%)	(°C)	0	2	4	6	8	10
Contamination	8	0^{aA}	0^{aA}	0^{aA}	0^{aA}	0^{aA}	0^{aA}
	13	0^{aA}	0^{aA}	0^{aA}	0^{aA}	0^{aA}	0^{aA}
	27	0^{aA}	0^{aA}	8^{bB}	8^{bB}	11^{bC}	22^{bD}

Data are expressed as Mean±SE. Mean values by the same lowercase letter in the column and uppercase letter in the line are not significantly different (p>0.05)

Table 6: Nutrient contents of *Hevea* seeds during storage under different temperatures

	Temperature	Weeks after storage				
Nutrients	(°C)	2	4	6	8	10
N (%)	8	3.03^{a}	3.01^{a}	2.60^{a}	2.62^{a}	2.62^{a}
	13	2.93^{b}	3.06^{a}	2.71^{a}	2.64^{a}	2.78^{b}
	27	3.08^{a}	3.01^{a}	2.84^{b}	2.90^{b}	2.87^{c}
P (%)	8	0.49^{a}	0.48^{a}	0.52^{a}	0.48^{a}	0.49^{a}
	13	0.49^{a}	0.45^{a}	0.51^{a}	0.45^{a}	0.50^{a}
	27	0.49^{a}	0.49^{a}	0.53^{a}	0.53^{b}	0.53^{a}
K (%)	8	0.86^{a}	0.89^{a}	0.82^{a}	0.79^{a}	0.79^{a}
	13	0.86^{a}	0.82^{a}	0.81^{a}	0.68^{a}	0.84^{a}
	27	0.83^{a}	0.91^{a}	0.93^{b}	0.90^{b}	0.81^{a}
Mg (%)	8	0.26^{a}	0.25^{a}	0.24^{a}	0.24^{a}	0.24^{a}
	13	0.26^{a}	0.24^{a}	0.26^{a}	0.23^{a}	0.23^{a}
	27	0.27^{a}	0.27^{a}	0.26^{a}	0.26^{a}	0.23^{a}
Ca (%)	8	0.06^{a}	0.06^{a}	0.06^{a}	0.04^{a}	0.05^{a}
	13	0.07^{a}	0.06^{a}	0.06^{a}	0.06^{a}	0.05^{a}
	27	0.08^{a}	0.09^{b}	0.05^{a}	0.05^{a}	0.05^{a}
Mn (mg kg^{-1})	8	13^{a}	12^{a}	2^{a}	nd	nd
	13	13^{a}	10^{a}	2^{a}	nd	nd
	27	12^{a}	15^{b}	3^{a}	nd	nd
Fe (mg kg^{-1})	8	56^{a}	68^{a}	62^{a}	62^{a}	55^{a}
	13	81^{b}	48^{b}	58^{b}	58^{b}	54^{a}
	27	49^{c}	84^{c}	68^{c}	57^{c}	55^{b}
Zn (mg kg^{-1})	8	54^{a}	51^{a}	58^{a}	62^{a}	57^{a}
	13	51^{b}	49^{b}	62^{b}	47^{b}	54^{b}
	27	48^{c}	53^{c}	64^{b}	56^{c}	60^{c}
Cu (mg kg^{-1})	8	12^{a}	15^{a}	32^{a}	29^{a}	25^{a}
	13	12^{a}	12^{b}	30^{a}	23^{b}	25^{b}
	27	13^{a}	16^{a}	30^{a}	27^{a}	27^{a}

nd: Not detected. Data are expressed as Mean±SE. Mean values in columns with same superscripts are not significantly different (p>0.05)

Manganese followed the path of the previous nutrients. The Mn content was reduced during the storage period and stored at 27°C contributed to the highest decrement. Meanwhile, stored at 27°C also contributed to the highest decrement in Fe content during the storage period.

Besides the decreased trend, nutrients of P, Zn and Cu showed an increased trend. It was further observed that the increase in these nutrients content was major to storage at 27°C. The Zn content increased throughout the storage period, especially at 27°C from 48-60 mg $kg^{-1}$and this could be related to the high protein content. Highest P increment from 0.49-0.53% and 13-27 mg kg^{-1} in Cu content in seeds stored at 27°C could due to the microbial contamination which degrades the storage seed.

Fatty acid composition in seed: Saturated fatty acids (SFA), i.e. pentadecanoic acid (C15:1), margaric acid (C17:0), stearic acid (C18:0), arachidic acid (C20:0), heneicosanoic acid (C21:0) and unsaturated fatty acid (UFA), i.e. oleic acid (C18:1) were detected during the studies (Table 7). The C15:1 content showed the decreased pattern during the storage period and stored at 13°C gave the highest decrement from 9.89 to 8.02 g/100 g during 2-10 weeks of storage. The C17:0 content also reduced during the storage period and stored at 13°C gave the highest decrement from 0.70 g/ 100 to 0.49 g/100 g during 6-8 week of storage. Stored at 8°C contributed to the highest decrement in C18:0 from 11.40 to 9.60 g/100 g despite this fatty acid reduced during the storage period in all the treatments. The C18:1 content showed the decreased trend and stored at 8°C gave the highest decrement from 30.45 to 24.91 g/100 g during 2-10 weeks of storage. Meanwhile, the C20:0 content showed the increased trend and stored at 8°C gave the highest increment from 38.06 to 44.13 g/100 g during 2-10 weeks of storage. The

Table 7: Fatty acids composition in *Hevea* seeds during storage under different temperatures

Weeks after storage	Temperature (°C)	Composition (g/100 g)							
		C15:1	C17:0	C18:0	C18.1	C20:0	C21:0	SFA	UFA
2	8	9.45[a]	nd	11.40[a]	30.45[a]	38.06[a]	10.63[a]	69.87	30.45
	13	9.89[a]	nd	12.68[a]	30.53[a]	36.62[b]	10.28[a]	69.54	30.53
	27	8.52[b]	nd	10.00[b]	30.14[a]	40.81[c]	10.54[a]	69.47	30.14
4	8	9.97[a]	nd	11.51[a]	27.68[a]	33.76[a]	12.08[a]	67.31	27.68
	13	8.86[b]	nd	9.03[b]	29.51[b]	40.77[b]	11.32[b]	59.98	29.51
	27	10.51[c]	0.84[a]	9.76[b]	28.48[c]	37.85[c]	12.57[a]	71.52	28.48
6	8	9.45[a]	0.79[a]	8.81[a]	27.79[a]	32.22[a]	10.93[a]	62.20	27.79
	13	10.33[b]	0.70[a]	11.42[b]	33.72[b]	34.75[b]	9.08[b]	66.28	33.72
	27	8.07[c]	nd	9.60[a]	27.82[a]	43.93[c]	11.12[a]	72.18	27.82
8	8	11.03[a]	0.50[a]	nd	31.71[a]	35.73[a]	11.78[a]	68.27	31.71
	13	11.73[a]	0.49[a]	12.88[a]	32.97[b]	30.63[b]	11.31[a]	67.04	32.97
	27	9.51[b]	0.82[b]	14.04[b]	31.31[a]	34.52[a]	9.79[b]	68.68	31.31
10	8	8.92[a]	nd	nd	24.91[a]	44.13[a]	15.13[a]	75.09	24.91
	13	8.02[a]	nd	9.88[a]	25.45[b]	39.55[b]	17.09[b]	74.54	25.45
	27	8.74[a]	nd	8.14[a]	24.53[b]	43.93[c]	15.66[a]	75.47	24.53

nd: Not detected, Data are expressed as Mean±SE. Mean values in columns with same superscripts are not significantly different (p>0.05)

C21:0 content also followed the path of the C20:0. This fatty acid was increased and stored at 13°C gave the highest increment from 10.28 to 17.09 g/100 g during the storage period. The results also showed that the total SFA content was increased from 69.47-69.87 to 75.09-75.47 g/100 g while UFA was decreased from 30.14-30.53g/100g to 24.91-25.53 during the storage period.

DISCUSSION

The viability test were based on the standard germination and tetrazolium test. Results showed that seeds stored at 8 and 13°C were able to germinate until 8 weeks storage and stored at 27°C only maintained until 2 weeks storage. Specifically, stored at 13°C gave the highest germination rate, followed by storing at 8°C and stored at 27°C. Meanwhile, results of the tetrazolium test were found to be similar to standard germination. The results also showed that the standard germination test was lower that the results given by the tetrazolium test. The differences between these tests could be due to some environmental effect during the test. It was supported by Sakhibun[12], who reported that the tetrazolium test gave higher values than standard germination, especially in seeds with high moisture content. Although the present results show a slight disparity between the two tests, the topographical of both tests were found to be similar on the seed viability.

Although no difference between stored at 8 and 13°C, the present results showed that stored at 13°C produced slightly better germination and tetrazolium test compared to 8°C. These conditions may suspect to some early symptom of seed deterioration at 8°C. *Hevea* seeds were chilling sensitive and store at too low temperature can lose its viability[17]. In addition, store at too low temperature can cause the formation of intracellular ice crystals which later contribute to seed deterioration. Husin *et al.*[18] reported that a little dehydration usually affects the viability of the seeds and it was also susceptible to chilling and microbial contamination.

In formulating the criteria of *Hevea* seed using tetrazolium test, the seeds were classified as viable when the embryonic axis and the cotyledons were stained red[12]. Moore[19] stated that healthy embryos were stained normal carmine red and should indicate seed viability and seedling vigour. If more than half of the cotyledon was unstained, the seed was considered non-viable since it will not have sufficient potential to mobilize its reserves for germination. The tetrazolium test distinguishes between viable and dead tissues on the basis of their respiration rate in the hydrated state. Although many enzymes were active during respiration, the test utilizes the activity of dehydrogenase enzymes as an indicator to the seed viability[9]. Thus, the enzymes could maintained longer in the seed stored at 8-13°C compared to 27°C.

This study shows that the fresh *Hevea* seeds contain a very high moisture around 24% and was confirmed by Sakhibun[12]. Other results showed that the moisture content decreased with increased in storage period. The decrease observation in *Hevea* seed also was reported by Duang-iat *et al.*[20]. The present study also showed that the seeds stored at 13°C gave highest moisture content, followed by storing at 8 and 27°C. Meanwhile, seeds stored at 27°C contain the lowest moisture after 2 weeks storage and this finding could support the germination rate where after 4 weeks, no germination had been detected at 27°C.

When the seeds of high moisture become dry, the membrane systems become irreversibly disrupted and the membrane systems were not capable of reinstating the original structure of cellular membranes to aid in compete reformation[4,21]. Thus, mechanical injury increases as the moisture content of the seed decreases. Therefore, too low moisture content will bring breakdown of membrane structure, hastens seed deterioration and this was proven by seeds stored at 27°C. Chin *et al.*[2] reported that *Hevea* seeds not germinated when the moisture content was lower than 15-20% on a fresh kernel weight basis.

The fresh *Hevea* seeds contain 32% total lipid and the content significantly increased with the increase of storage period. Since *Hevea* seed was an oilseed species, it accumulates large quantities of total lipids during storage. Besides that, the increased content of lipid could effected from the decrease of water content and the increase of free fatty acid in the seed. The present study showed that the seeds stored at 27°C gave highest lipid content and no significant difference between the temperatures of 8 and 27°C. Seeds stored at 27°C contain the highest lipid after 2 weeks storage and this finding could support the germination rate where after 4 weeks, no germination had been detected at 27°C.

According to Tappel[22], the lipid autoxidation accelerated at high temperature due to a drastic decrease of water content. As resulted in the present study, the seeds stored at 27°C drastically decrease water content and subsequently show lowest seed viability compared to store at 8 and 13°C. Tappel[22] reported that the free radicals of lipid peroxides damage cytochrome by changing its physical and catalytic properties. Lipids, which are a part of cell membranes, come into close contact with other constituents, including macromolecules or enzymes when seed is very dry. The products of lipid autoxidation are able to join the macromolecules and slowly destroy the functioning of a cell. It has been noted that lipid autoxidation occurs in all cells, but in fully imbibed cells where water acts as a buffer between the reactive compounds and the macromolecules, thus preventing enzyme inactivation[23-25]. This condition has been concluded as the main cause for seed deterioration and was directly linked to membrane integrity of the seed[5].

The fresh *Hevea* seeds contain 11.50% total protein and the content significantly increased with the increase of storage period. Seeds at 27°C significantly increased after only 2 weeks storage and no significant difference between the temperatures of 8 and 13°C.

The increase of protein content could due to decrease of water content which changed the structure of enzymes, structural protein and decreased in cell membrane integrity. In addition, increasing amount of protein considered as indication of stress tolerance to storage[9]. LeVan *et al.*[26] reported that high protein levels in soybean seeds were related to lower seed germination.

During the study, no contamination had been detected in seeds at 8 and 13°C. However, seeds stored at 27°C gave highest broken seeds after 4 weeks storage. This result was supported by Walters *et al.*[24], who reported that low temperature synergistically minimized aging reactions. The results also showed that the bucket of seeds without contamination stay germinated compared to the bucket of seeds with contamination. It seems that the contaminated seeds in future would affected other seeds in the area.

The contamination could due to the storage fungi or bacterial infection which had the capability to grow without free water. In addition, the contamination in seed could due to the infection from outside via some damage on the seed coat. It's also supported by Mamicpic and Caldwell[27] and Igeleke and Omorusi[28] which reported that mechanical damage promotes invasion by storage fungi, which can enter the seed through cracks in the seed coat and will increase the free fatty acids due to invasion of fungi. Several studies had reported that free fatty acids caused seed deterioration in cotton and *Hevea* seed[7,8]. During storage, the respiration process is still ongoing, so that the seeds had a fast metabolism and the heat generated makes the seeds moist, so that they very easily contaminated with microbes and experiencing faster deterioration if no precaution from the seed coat. The presence of fungus attacks was also found in cotton seeds (recalcitrant)[11], in ebony seed storage[29] and reviewed by Igeleke and Omorusi[28].

Analysis of nutrient showed that the content of N, K, Mg, Ca, Mn and Fe were decreased during the storage period while the content of P, Zn and Cu gave an increased pattern. Specifically, stored at 8°C gave the highest decrement on N and K contents, while stored at 27°C produced the highest decrement on Mg, Ca, Mn and Fe contents. Seeds at 27°C also produced the highest content of P, Zn and Cu.

N is important to the developing seed through phloem in the form of amino acids or amides for the synthesis of storage proteins[30]. Pavithra *et al.*[30] reported that the K ions are involved in cell turgor pressure and in sucrose unloading from seed coat to the developing embryo. Mg plays a function in photosynthesis and contributes directly to transport of photoassimilates from source organs. In addition, the nutrient

was also required for the maintenance of growth rate of roots and young shoot[31]. Meanwhile, Ca is needed for the stability and functions of cell walls and biological membranes[8]. Mn is related to the protein storage. Fe is an important co-factor for many reactions involved in photosynthesis and respiration[30]. The present results showed that stored at 27°C contributed to the highest decrement of the nutrient and this finding may supported why the low germination rate observed at 27°C. Besides that, stored at 8°C also produced high decrement on several nutrient and this could due to the early symptom of seed deterioration, as *Hevea* seeds are chilling sensitive and store at too low temperature can lose its viability[17]. These decreased results in nutrient content are similar to the finding of Fagbohun and Lawal[32], who reported a decrease pattern in soybean during storage. The decreased of the nutrient could due to the high light intensity and heat stress during storage and becoming chlorotic and necrotic, probably due to extensive production of reactive oxygen species[31]. This decrease of the nutrient in future will affect many physiological processes like respiration, cell wall metabolism, photosynthesis and nitrogen fixation[33] and this situation would cause non-germination to the seed.

The increased pattern on Zn could be related to the high protein content and for P and Cu could due to the microbial contamination which degrades the storage seed. Many enzymes require Zn for their integrity and function because it was the most important nutrient affecting protein synthesis in plants. However, despite the Zn high, seem that the decreased activity of the enzyme in the seed could lower its respiratory potential, which in turn lowers both the energy and food supply. Bognar *et al.*[34] reported that the variation in the nutrient contents could due to redistribution of nutrient elements in seeds and microbial contamination. This contamination could be from the air, storage room or improper handling of the seeds. Smitt *et al.*[35] also reported that the variation in nutrient during storage period could be explained by the group fungi which degrade the stored seed and either liberates the nutrients or utilizes during the process.

During the storage period, saturated fatty acids (SFA), i.e. pentadecanoic acid (C15:1), margaric acid (C17:0), stearic acid (C18:0), arachidic acid (C20:0), heneicosanoic acid (C21:0) and unsaturated fatty acid (UFA), i.e. oleic acid (C18:1) were detected in the seed. The content of C15:1, C17:0, C18:0 and C18:1 gave the decrement pattern while C20:0 and C21:0 gave an increment pattern during the storage period. The results also showed that the total SFA content was increased while UFA was decreased during the storage period. The findings about the variation of fatty acid content was similar to Ghasemnezhad and Honermeier[36] and Dos Santos Oliveira[37].

Seeds stored at 27°C gave the highest SFA content and also contributed to the highest decrement to UFA content during the storage period. This reduction in UFA could be related to the conversion to other fatty acid and production of free fatty acid (FFA), especially in high temperature[23]. The decreased on UFA also reflected in the relative increased in another fatty acids such as C20:0 and C21:0. Fatty acids in free form are more susceptible to oxidation. Thus, the increase of the free form of UFA and its oxidation during the storage could be the reason why the content decreased. In future, the increased SFA and decreased UFA will cause to the lipid autoxidation and resorted to the inactive enzyme and membrane injury in seed[8]. Present results on rapid decreased UFA at 27°C was supported by Dos Santos Oliveira[37], who reported that the reduction in the fatty acids with the most marked decreased in unsaturated fatty acids such as oleic and linoleic acids was greater in seed stored at 25-30°C. According to Balesevic-Tubic *et al.*[38], seed that are high of UFA such as C18:1, C18:2 and C18:3, are more prone to deterioration than seed with the high level of SFA. This report was similar to the present study where fresh *Hevea* seed contains high C18:1 and decreased of it caused to seed deterioration especially stored at 27°C. Besides that, stored at 8°C also produced high decrement on UFA and this could due to the early symptom of seed deterioration, as reported that *Hevea* seed is sensitive to chilling condition and store at too low temperature can lose its viability[17].

CONCLUSION

The best way of seed storage is the one that causes smallest changes in the biochemical nature of the seed. The present study showed that the temperature of 27°C was significantly affected to the high loss of enzyme activity, high moisture decrease, high lipid and protein contents, high contamination in the seed, high nutrient loss and high ratio saturated to unsaturated fatty acid. Thus, it reflected in the low germination rate and seed viability and only maintain until 2 weeks of storage. Although temperature of 8 and 13°C can maintain the seed viability until 8 weeks storage, stored at 13°C produce the best results based on the higher germination, lower loss of enzyme activity, lower moisture decrease, low lipid and protein contents, no contamination in the seed, lower nutrient loss and lower ratio saturated to unsaturated fatty acid. In addition, stored below 8°C is not suggested cause suspect to early symptoms of seed deterioration due to *Hevea* seed is chilling sensitive and store at too low temperature can lose its viability. Therefore, the temperature of 13°C was the most suitable for storing the *Hevea* seeds.

Further study is required in *Hevea* seed to see whether the present storage temperature give any side effect to the seed vigour and seed quality for budding the rootstock to produce planting material.

SIGNIFICANCE STATEMENT

This study discovers that *Hevea* seed is sensitive to temperature. High temperature affected to the low seed viability due to the huge change in biochemical properties in the seed. Meanwhile, too low temperature is also not suitable due to moderate change in biochemical properties in the seed. Thus, moderate storage temperature is suitable to *Hevea* seed as one of tropical recalcitrant seed.

ACKNOWLEDGMENTS

We would like to thank the Director General of Malaysian Rubber Board for approval of funding for the research. Our thanks also to the Deputy Director General of Research and Innovation for his idea and criticism. The technical assistance by our supporting staff; Yusri, Arif, Hajar and Adilah were much appreciated.

REFERENCES

1. Malaysian Rubber Board, 2009. Development of Rubber Clones. In: Rubber Plantation and Processing Technologies, Malaysian Rubber Board (Ed.)., 1st Edn., Malaysian Rubber Board, Malaysia, pp: 48-49.
2. Chin, H.F., M. Aziz, B.B. Ang and S. Hamzah, 1981. The effect of moisture and temperature on the ultrastructure and viability of seeds of *Hevea brasiliensis*. Seed Sci. Technol., 9: 411-422.
3. Ang, B.B., 1977. Problem of Rubber Seed Storage. In: Seed Technology in the Tropics, Chin, H.F., I.C. Enoch and R.M.R. Harun (Eds.)., Universiti Pertanian Malaysia, Malaysia, pp: 117-122.
4. Pammenter, N.W. and P. Berjak, 2000. Aspects of recalcitrant seed physiology. Revista Brasileira Fisiologia Vegetal, 12: 56-69.
5. Berjak, P. and N.W. Pammenter, 2013. Implications of the lack of desiccation tolerance in recalcitrant seeds. Front. Plant Sci., Vol. 4. 10.3389/fpls.2013.00478.
6. Anonymous, 2009. Development of Rubber Clones. In: Rubber Plantation and Processing Technologies, Anonymous (Ed.)., Malaysian Rubber Board, Malaysia, pp: 21-38.
7. Iyayi, A.F., P.O. Akpaka and U. Ukpeoyibo, 2008. Rubber seed processing for value-added latex production in Nigeria. Afr. J. Agric. Res., 3: 505-509.
8. Copeland, L.O. and M.B. McDonald, 1985. Seed Longevity and Deterioration. In: Principles of Seed Science and Technology, Copeland, L.D. and M.B. McDonald (Eds.)., 2nd Eds., Macmillan Publishing Company, Minnesota, pp: 145-169.
9. Charloq, Z. Lubis, T.H. Siregar, S.B. Damanik, A. Yazid and M. Husni, 2016. Physiology changes of shelled rubber (*Hevea brasilliensis* Muell. Arg.) seed after 16 days storage with PEG 6000 30% coating to induce secondary dormancy. J. Agron., 15: 11-18.
10. Marques, E.R., R.F. Araujo, E.F. Araujo, S.M. Filho, P.C. Soares and E.G. Mendonca, 2014. Dormancy and enzymatic activity of rice cultivars seeds stored in different environments. Seed Sci., 36: 435-442.
11. Arief, R., E. Syamaun and S. Saenong, 2004. Evaluation of physical and physiological quality of seeds corn and seeds size CV. Lamuru of different shelf life. J. Sci. Technol., 4: 54-64.
12. Sakhibun, B.M.H., 1990. Hevea seed: Its characteristics, collection and germination. Planters' Bull. Rubber Res. Inst. Malaya, 202: 3-8.
13. ISTA., 1985. International rules for seed testing. Seed Sci. Technol., 13: 299-355.
14. Folch, J., M. Lees and G.H.S. Stanley, 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem., 226: 497-509.
15. Anonymous, 2008. Malaysian standards 320: Seeds-sampling and test methods. Malaysian Standards Institute, Malaysia, pp: 18-21.
16. Nielson, S.S., 2010. Preparation of Fatty Acid Methyl Esters (FAMEs) and Determination of Fatty Acid Profile of Oils by Gas Chromatography. In: Food Analysis Laboratory Manual, Nielson, S.S. (Ed.)., 2nd Edn., Springer, Germany, pp: 159-163.
17. Shuib, N.H., 2016. Biji getah bersifat rekalsitran. Buletin Sains and Teknologi LGM, Buletin Bil., No. 14, Lembaga Getah Malaysia, pp: 52-53.
18. Husin, S.B.M., H.F. Chin and Y.L. Hor, 1981. Viability test on *Hevea* seeds by the tetrazolium method. J. Rubb. Res. Inst. Malays, 29: 44-51.
19. Moore, R.P., 1969. History supporting tetrazolium seed testing. Proc. Int. Seed Test. Ass., 34: 233-242.
20. Duang-iat, W., V. Wongvarodom, W. Santipracha and S. Sdoodee, 2013. Physiological quality and desiccation sensitivity of rubber (*Hevea brasiliensis*) seeds during fruit maturation. Kasetsart J. (Nat. Sci.), 47: 818-827.
21. McKersie, B.D. and R.H. Stinson, 1980. Effect of dehydration on leakage and membrane structure in *Lotus corniculatus* L. seeds. Plant Physiol., 66: 316-320.
22. Tappel, A.L., 1962. Hematin compounds and lipoxidase as biocatalysts. Proceedings of the Symposium on Foods: Lipids and Their Oxidation, June 1961, Oregon State University, Corvallis, Oregon, pp: 122-138.
23. Harrington, J.F., 1973. Biochemical basis of seed longevity. Seed Sci. Technol., 1: 453-461.

24. Walters, C., L.M. Hill and L.J. Wheeler, 2005. Dying while dry: Kinetics and mechanisms of deterioration in desiccated organisms. Integrat. Comparat. Biol., 45: 751-758.
25. Bewley, J.D. and M. Black, 1994. Seeds: Physiology of Development and Germination. 2nd Edn., Plenum Press, London, ISBN: 10-0-306-44747-9, Pages: 445.
26. LeVan, N.A., A.S. Goggi and R. Mullen, 2008. Improving the reproducibility of soybean standard germination test. Crop Sci., 48: 1933-1940.
27. Mamicpic, N.G. and W.P. Caldwell, 1963. Effects of mechanical damage and moisture content upon viability of soybeans in sealed storage. Proc. Assoc. Official Seed Anal., 53: 215-220.
28. Igeleke, C.L. and V.I. Omorusi, 2007. Review of post-harvest deterioration of rubber seeds. J. Agric. Soc. Res., 7: 11-19.
29. Yuniarti, N., D. Syamsuwida and A. Aminah, 2008. Decrease the influence of moisture content of changes in physiology and biochemistry seed ebony (*Diospyros celebica* Bakh.). Seeding technology research center of Bogor. J. Penelit. Hutan Tanaman, 5: 191-198.
30. Pavithra, H.R., B. Gowda and M.B. Shivanna, 2014. Biochemical changes in the composition of developing seeds of *Pongamia pinnata* (L.) Pierre. Ind. Crops Prod., 53: 199-208.
31. Cakmak, I., 2014. Major function of calcium and magnesium in crop plants. Proceedings of the 16th World Fertilizer Congress of CIEC30, October 20-24, 2014, Portuguese, pp: 30-32.
32. Fagbohun, E.D. and O.U. Lawal, 2011. Effect of storage on nutrient composition and mycoflora of sundried soyabean (*Glycine max*). Afr. J. Food Sci., 5: 473-477.
33. Gao, S., R. Yan, M. Cao, W. Yang, S. Wang and F. Chen, 2008. Effects of copper on growth, antioxidant enzymes and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. seedling. Plant Soil Environ., 54: 117-122.
34. Bognar, A., H. Bohling and H. Fort, 1990. Nutrient Retention in Chilled Foods. In: Chilled Foods, Gormley, T.R. (Ed.)., Elsevier, London, pp: 305-336.
35. Smitt, J.P., A. Rinzema, J. Tramper, H.M. Van and W. Knol, 1996. Solid state fermentation of wheat bran by *Trichoderma ressei* QMQ 414. Applied Microbiol. Biotechnol., 46: 489-496.
36. Ghasemnezhad, A. and B. Honermeier, 2007. Influence of storage conditions on quality and viability of high and low oleic sunflower seeds. Int. J. Plant Prod., 3: 39-48.
37. Dos Santos Oliveira, A., M.L.M. de Carvalho, C. das Neves Vieira Barbara, R.M. Guimaraes, J.A. Oliveira and D. de Sousa Pereira, 2016. Biochemical changes in fiber naturally colored cottonseeds during storage. J. Seed Sci., 38: 101-109.
38. Balesevic-Tubic, S., M. Tatic, J. Miladinovic and M. Pucarevic, 2007. Changes of fatty acids content and vigour of sunflower seed during natural aging. Helia, 30: 61-67.