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THE QUALITY OF PHYSIC NUT (*JATROPHA CURCAS* L.) SEEDS PACKED IN PLASTIC MATERIAL DURING STORAGE

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ABSTRACT

The effect of storage duration on fungal population, moisture content, lipid and free fatty acid contents, lipase activity, viability and vigor of physic nut seeds was investigated. Physic nut seeds with initial moisture content of 7.9% were stored in plastic bags under warehouse conditions. Samples of physic nut were collected before storage, and subsequently after one to six months of storage. The results showed that the moisture contents increased after one month of storage, and became relatively constant up to six months of storage. The range of moisture contents (7.9 – 8.4%) was safe for storage of physic nut seeds. Sixteen fungal species were isolated from physic nut seeds during six months of storage. Fungal population decreased with the increase of storage duration. At the beginning of storage, most of the fungi that infected the seeds were classified as field fungi, such as *Colletotrichum* sp., *Cladosporium* spp., and *Fusarium* spp.. Their populations decreased with the increase of storage duration. After three months of storage, the existence of field fungi was generally replaced by storage fungi, such as *Aspergillus* spp., and *Penicillium* spp. dominate the population. Lipid contents, viabilities and vigors decreased with the increase of storage duration, while free fatty acids and lipase activities increased. Under uncontrolled conditions, physic nut seeds packed in plastic material can be stored up to one month for seeds to be planted, while it can be stored up to five months for producing oil.

Key words : storage duration, fungi, lipid, free fatty acid, lipase, viability, vigor, physic nut, *Jatropha curcas* L.

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INTRODUCTION

With the increasing demand of fuel products, and the decreasing of fossil fuel sources, the world prices of petrol and diesel are increasing, including in Indonesia. It is assumed, that the fossil fuel reserve will be exhausted within 10 - 15 years in Indonesia. Hence alternative sources of energy for running our generators, automobiles, etc are being considered (Hambali 2006). The possibility of obtaining oil from plant resources to be a supplement or replacement of fossil fuel has aroused a great interest. In several countries including in Indonesia, non-edible vegetable oil can be used as biodiesel, which is a nontoxic and biodegradable oil.

Physic nut (*Jatropha curcas* L.) seeds is known to be a good source for biodiesel. The oil contents in the seeds and in the kernels are 25 – 30% and 50 – 60%, respectively. The oil contains 21% saturated fatty acids and 79% unsaturated fatty acids (Gubitz *et al.* 1997). Other uses of physic nut extracted oil are for making soap, biopesticide, and medicinal substance for skin disease treatment. The physic nut oil has the requisite potential of providing a promising and commercially viable alternative to diesel oil, since it has desirable physicochemical and performance characteristics comparable to diesel. Cars could be run with physic nut without requiring much change in design (Hambali *et al*. 2006; Warsiki *et al*. 2007)

Physic nut seeds can be used as seeds and oil producer. According to Adikadarsih and Hartono (2006) the use of physic nut seeds for seedlings should derive from the fruits which skin is yellow up to blackish yellow in colour, because they have high percentages of viability and vigor, i.e. 89 and 81 %, respectively. Wanita and Hartono (2006) reported that seeds originated from physic nut fruits which skin is yellow up to blackish yellow in colour produce seeds with highest oil content, i.e. 28- 30 %.

To obtain good quality of physic nut seeds, some factors should be taken into consideration, i.e. the degree of fruit maturity, safe seed moisture content, appropriate container and storage condition, duration of storage, good viability and vigor of seeds. Consequently, appropriate postharvest handling is needed.

Christensen and Kaufmann (1969) reported that during storage seeds or grains could be infected by fungi which cause a decrease in viability, discolouration, various biochemical changes, heating and mustiness, loss in weight, and production of toxins when it is consumed may be injurious to human and domestic animals. In many cases, fungi infecting seeds are seed-borne pathogens. They play an important role in the transmission of numerous pathogenic species to seedlings as well as to the soil (Chelkowski 1991).

The objective of this study was to determine the effects of storage duration on fungal population, moisture content, lipid and free fatty acid contents, lipase activity, viability and vigor of physic nut seeds.

MATERIALS AND METHODS

Origin of physic nut seeds

Physic nut seeds were obtained from fresh harvested fruits which fruit skin was yellow up to blackish yellow in colour. The fruits were obtained from plants (Lampung accession) cultivated in Loyang Village, Cikedung Subdistrict, Indramayu Regency, West Java in May 2007.

Fruit peeling and seed drying

The fruit skins were peeled using a knife, then the seeds were air-dried on the selves in a shaded place up to moisture contents of about 8 %.

Packaging and storing of physic nut

Prior to packaging, damaged seeds were hand picked from the batch. Sound seeds were packed in plastic packaging materials (2 kg/bag) under normal oxygen concentration. They were then placed randomly on wooden shelves and stored for one, two, three, four, five and six months (June – November 2007) under warehouse conditions. The composition of the plastic packaging material was NY15/PE15/LLDPE70. According to Dharmaputra *et al*. (2007) total fungal population of peanut kernels packed in three different types of plastic packaging materials (OPP30/PE15/LLDPE80, NY15/PE15/LLDPE80, and NY15/PE15/LLDPE70) under low oxygen concentration were not significantly different. The characteristic of plastic packaging material is presented in Table 1. The plastic packaging bag was produced by PT Interkemas Flexipack in Tangerang, West Java.

Table 1. The characteristics of plastic packaging material

Name of product	Code	Compositition	Thickness (mm)	WVTR (Water Vapour Transmission Rate) (g/m^2/24 Hrs)	O_2TR (Oxygen Transmission Rate) (cc/m^2/24 Hrs)
TRL.VACUUM BAG 03	NY70	NY 15/PE 15/LLDPE 70	0.0974	0.6340	1.8734

Note : Analyzed by Test and Calibration Laboratory, Institute for Chemical and Packaging, Jakarta, Indonesia
OPP : Oriented Polypropilene
LLDPE : Linear Low Density Polyethylene

The experiment was arranged in completely randomized design. Three replications (= bags) were used for each storage duration (including at the beginning of storage). Each bag was used to pack physic nut seeds with different storage duration and replications. The ambient temperature and relative humidity of the storage room were recorded using a tinytag data logger.

Sampling method

Samples of physic nut seeds were collected from each bag before storage (at the beginning of storage) and subsequently every month thereafter until 6 months of storage. Each sample was divided three times using a box divider to obtain working samples for moisture content, fungal population, lipid and free fatty acid contens, lipase activity, viability and vigor of seeds, and reserved sample.

Determination of moisture content, fungal population, lipid and free fatty acid contens, lipase activity, viability and vigor

Moisture contents of seeds (based on wet basis) were determined using oven method (ISTA 1999, with a slight modification). Two replicates were used for each sample.

Fungi from each sample was isolated and enumerated using serial dilution method followed by pour plate method on Dichloran 18% Glycerol Agar (DG18) (Hocking and Pitt 1980; Pitt *et al*. 1992). Fungal identification was conducted based on Samson *et al*. (1996), Pitt and Hocking (1997) using Czapek Yeast Extract Agar (CYA) and CYA containing 20% sucrose (CY20S). Lipid and free fatty acid contents were determined based on Soxhlet and titration methods, respectively (AOAC 1999). Lipase activity was determined based on Moore (1973, with a slight modification).

Viability (percentage of germination) of seeds from each sample was determined by growing 25 seeds in rectangular plastic containers containing sand (5 kg/container) 7 and 10 days after planting (DAP) under green house conditions (Prihandana and Hendroko 2006). Normal germination was observed 7 DAP, while normal and abnormal germination were observed 10 DAP. Determination of seed vigor from each sample was conducted using rice straw paper method (Sadjad 1994). Vigor, less vigor, non-vigor, and death were observed 7 DAP.

Statistical analysis

Standard ANOVAs were used to analyse the results followed by Duncan's multiple range test at the 5% probability level.

RESULTS AND DISCUSSION

Moisture contents

Moisture content is the most important environmental factor that influence fungal growth in stored grains (Christensen *et al*. 1992). Storage duration gave very significant differences on the moisture contents of physic nut seeds. The moisture contents of physic nut after one month of storage (8.41%) were higher and significantly different from those at the beginning of storage (7.89%). The moisture contents decreased after two, three, four, five, and six months of storage, i.e. 8.14, 8.12, 8.14, 8.06 and 8.16%, respectively (Table 2). According to the Directorate General of Plantation Crop (2006) the safe moisture content for storage of physic nut seeds was 7 – 9 %. The moisture content of grains is in equilibrium with the relative humidity of the storage. Range of temperature and relative humidity are presented in Table 3. The moisture content of the seeds was correlated with the relative humidity of the storage.

The type of plastic packaging material also affects the moisture contents of physic nut seeds. Plastic packaging bags used in this study are made from good material for storing peanut kernels (Dharmaputra *et al.* 2007). According to Warsiki *et al.* (2007) the moisture contents of physic nut seeds packed in bags were higher than those packed in polypropylene bags.

Table 2. Moisture content, total fungal population, lipid and free fatty acid contents, lipase activity, viability and vigor of physic nut seeds during storage

Duration of storage (month)	Moisture content (% wet basis)	Total fungal population (cfu/g dry basis)	Lipid content (% dry basis)	Free fatty acid content (% dry basis)	Lipase activity (U/ml)	Viability (%)	Vigor (%)
0	7.89 a ± 0.07	8589.0 a ± 1359.3	41.2 a ± 0.34	0.10 a ± 0.01	0.23 a ± 0.08	89.33 a ± 8.33	83.33 a ± 5.77
1	8.41 d ± 0.02	6519.6 a ± 3224.5	37.9 b ± 0.69	0.60 b ± 0.01	0.35 ab ± 0.03	74.67 b ± 4.62	70.00 b ± 0.00
2	8.14 c ± 0.04	5917.7 a ± 1026.8	37.2 bc ± 0.66	0.62 b ± 0.01	0.42 bc ± 0.04	71.33 bc ± 1.15	63.33 bc ± 5.77
3	8.12 bc ± 0.02	1405.4 b ± 1196.7	35.9 cd ± 0.56	0.67 bc ± 0.03	0.51 cd ± 0.05	68.00 bc ± 2.00	56.67 c ± 5.77
4	8.14 c ± 0.03	1297.3 b ± 377.2	35.1 de ± 0.54	0.79 c ± 0.06	0.58 d ± 0.01	64.67 cd ± 2.31	36.67 d ± 5.77
5	8.06 b ± 0.02	1145.6 b ± 761.9	33.5 ef ± 0.86	1.00 d ± 0.09	0.71 e ± 0.07	60.00 de ± 3.46	33.33 de ± 5.77
6	8.16 c ± 0.03	225.4 b ± 28.4	31.7 f ± 2.06	1.10 d ± 0.14	0.93 f ± 0.15	53.33 e ± 2.31	26.67 e ± 5.77

Note : Means followed by the same letter in the same column are not significantly different according to Duncan's Multiple Range Test at the 5% level ($P > 0.05$).

Table 3. Range of temperature and relative humidity during storage

Duration of storage (month)	Temperature (°C)		Relative humidity (%)	
	Range	Mean	Range	Mean
1	24.4 - 28.3	26.3	55.2 – 82.8	74.1
2	25.1 -28.7	26.5	48.2 -70.2	70.2
3	25.1 – 29.1	26.5	55.2 – 78.5	69.4
4	25.1 – 28.3	26.7	46.0 – 80.7	68.1
5	25.1 – 28.7	26.7	55.6 – 82.4	73.1
6	24.4 – 28.3	26.1	65.6 – 85.4	76.5

According to Desai (2004) modern packaging materials and methods maintain seeds at their original quality from the time of their packaging to the time they are used for planting. The best way to maintain the viability and vigor of many kinds of seeds is to store them in a dry and cold place. Packages designed to protect most physical qualities of seeds, such as weight, size, colour, moisture content, and purity (freedom from weeds, inert matter, disease organisms and damage), as well as their physiological aspects, like viability, vigor and dormancy, are made up of materials that have sufficient tensile strength, bursting strength, and tearing resistance to withstand normal pressures and handling procedures. Polyethylene is the most extensively used thermoplastic film. Conventional low-density films are generally used in seed packages. This packaging material is ideal for providing seale-storage conditions in a humid climate, giving protection against high humidity in a seed store.

Fungal population

Sixteen fungal spesies were isolated during six months of storage (Table 4). They were *Aspergillus flavus, A. niger, A. ochraceus, A. penicillioides, A. restrictus, A. tamarii, A.wentii, Cladosporium* sp., *C. cladosporioides, Colletotricum* sp., *Eurotium chevalieri, E .rubrum, Fusarium moniliforme, F. semitectum, Penicillium citrinum,* and *P. oxalicum*. *Aspergillus flavus, A. niger, Cladosporium* sp., *F. moniliforme, F. semitectum,* and *P. citrinum* were always isolated during storage.

Table 4. Fungal population of physic nut seeds during storage

No.	Fungi	Fungal population (cfu/g dry basis)						
		Duration of storage (month)						
		0	1	2	3	4	5	6
1	*Aspergillus flavus*	6.5	1.1	6.5	88.2	8.7	17.4	9.8
2	*A. niger*	3.3	2.2	4.4	3.3	1.1	2.2	3.3
3	*A. ochraceus*	0.0	0.0	1.1	0.0	0.0	0.0	0.0
4	*A. penicillioides*	0.0	0.0	0.0	0.0	0.0	730.9	92.6
5	*A. restrictus*	0.0	50.2	61.0	0.0	695.6	0.0	0.0

Table 4. Continued

No.	Fungi	Fungal population (cfu/g dry basis) Duration of storage (month)						
		0	1	2	3	4	5	6
6	*A. tamarii*	2.2	1.1	2.2	0.0	1.1	1.1	1.1
7	*A. wentii*	1.1	1.1	2.2	4.4	2.2	0.0	0.0
8	*Cladosporium* sp.	427.7	462.9	90.4	37.0	20.7	44.6	17.4
9	*C. cladosporioides*	169.4	41.5	47.9	21.8	0.0	0.0	15.2
10	*Colletotrichum* sp.	5609.6	1880.1	1415.2	68.6	61.0	0.0	0.0
11	*Eurotium chevalieri*	0.0	0.0	0.0	1.1	0.0	0.0	2.2
12	*E. rubrum*	0.0	0.0	0.0	2.2	0.0	63.1	3.3
13	*Fusarium moniliforme*	1652.4	3703.5	580.2	1007.8	409.3	156.6	15.2
14	*F. semitectum*	247.5	10.9	209.0	14.1	3.3	6.5	3.3
15	*Penicillium citrinum*	276.8	291.5	3496.6	156.7	94.7	124.0	63.2
16	*P. oxalicum*	193.2	73.2	0.0	0.0	0.0	0.0	0.0
	Total	8589.7	6519.3	5916.6	1405.1	1297.6	1146.4	226.5

Duration of storage gave very significant differences on total fungal population. Total fungal population after one, two, three, four, five and six months of storage did not coincide with moisture contents at the same months (Table 2). Total fungal population decreased with the increase of storage duration. Total fungal population at the beginning of storage (8589.0 cfu/g.db) was higher and significantly different from that after six months of storage (225.4 cfu/g.db).

The highest total fungal population was found at the beginning of storage, because the high population of field fungi, such as *Cladosporium* spp., *Colletotricum* sp., *F. moniliforme,* and *F. semitectum* . *Cladosporium* spp. produces abundant conidia. Storage fungi found in physic nut seeds were species of *Aspergillus* and *Penicillium*. Based on their occurrence in the seeds, Christensen and Kaufmann (1969) divided two groups of fungi that cause deterioration of seeds, i.e. field fungi and storage fungi.

It was assumed that the decrease of fungal population during storage was due to the low of moisture contents in the seeds after one month up to six months of storage. In general the dominant fungal species during storage was field fungi. According to Sauer *et al.* (1992) fields fungi have high water requirements for their growth, compared to storage fungi.

It was also assumed that the decrease of total fungal population was due to the decrease of oxygen during storage. According to Moore-Landecker (1996) most fungi are obligate aerobes and require at least some free molecular oxygen in the atmosphere. Physic nut seeds also need O_2 found in plastic packaging bags for their life. Garraway and Evans (1984) concluded that the type of response by fungi to oxygen concentration depends on the species.

Lipid contents

Storage duration gave very significant differences on lipid contents of physic nut seeds. The lipid contents decreased with the increase of storage duration (Table 2). After one month of storage, lipid contents (37.9% db) were lower and significantly different from those at the beginning of storage (41.2% db). After six months of storage, lipid contents (31.7% db) were lower and significantly different from those at the beginning of storage (41.2% db), after one (37.9% db), two (37.2%db), three (35.9% db), and four (35.1% db) months of storage.

Physic nut seeds contain high lipid content. The lipid contents in the seeds and in the kernels are 25 – 30% and 50 – 60%, respectively. The lipid contains 21% saturated fatty acids and 79% unsaturated fatty acids (Gubitz *et al.* 1997). Lipid contents of physic nut used in this study were high (37.95%), because the seeds were derived from fruits which fruit skin is yellow up to blackish yellow. Although after five months of storage lipid contents (33.5% db) were significantly different from those at the beginning of storage (41.2% db), they were still able to be used for producing oil.

Lipid contents are affected by the degree of fruit maturities at the time of harvesting. Wanita and Hartono (2006) reported that the lowest (10.93 %) and the highest (29.38 %) lipid contents were found in physic nut seeds derived from fruits which fruit skins are green and yellow, respectively.

Free fatty acid contents and lipase activity

Due to hydrolysis process, fat and lipid in seeds are broken down into free fatty acids (FFA) and glycerol by lipases. Rapid increases in concentrations of FFA frequently are observed in deteriorating seeds, particularly when the temperature and moisture content are high. Many field and storage fungi produce lipases. Seeds also produce lipases, but these commonly are produced during germination, and are not present in stored seeds (Pomeranz 1992).

Storage duration gave very significant differences on FFA contents of physic nut seeds. FFA contents increased with the increase of storage duration (Table 2). After one month of storage, FFA contents (0.60% db) was higher and significantly different from those at the beginning of storage (0.10 db %). After five (1.00 % db) and six months of storage (1.10 % db) FFA contents were higher and significantly differents from those at the beginning of storage (0.10% db), after one (0.60% db), two (0.62 % db), three (0.67% db) , and four months of storage (0.79% db). According to St. Angelo and Ory (1983) lipase and lipoksigenase are important enzymes to degrade lipid in seeds.

Storage duration gave very significant differences on lipase activity of physic nut seeds. Lipase activity increased with the increase of storage duration (Table 2). After two months of storage, lipase activity (0.42 U/ml) was higher and significantly different from those at beginning of storage (0.23 U/ml). After five and six months of storage lipase activity (0.71 and 0.93 U/ml, respectively) were higher and significantly different from those at the beginning of storage. After six months of storage, lipase activity was higher and significantly different from those after five months of storage. As the moisture contents were considered low (Table 2) and the total fungal population decreased (Table 4) during storage, the lipase activity could be due to seed enzymes rather than fungal enzymes.

Seed viability and vigor

According to Pomeranz (1992) fungal infection can decrease seed viability and vigor of seeds during storage. Priestley (1986) reported that the most obvious, though not the most informative, indication of the quality of a seed lot is its germinability. The speed of germination, which has long been recognized as an indicator of seed vigor, is usually a more sensitive measure of seed deterioration than loss of viability.

Storage duration gave very significant differences on the percentages of germination and vigor. The percentages of germination and vigor decreased with the increase of storage duration (Table 2). The percentages of germination after one month of storage (74.67%) were lower and significantly different from those at the beginning of storage (89.33%). Directorate General of Estate Crops (2006) determined the germination of physic nut seeds to be planted should be more than 80%. After one month of storage, the percentage of germination (74.67%) was significantly different from those at the beginning of storage (89.33%). Nevertheless, based on statistical analyses, the percentage of germination after one month of storage was 74.67 ± 4.62%. Consequently, under uncontrolled conditions, physic nut seeds packed in plastic material can be stored up to one month for seeds to be planted. After six months of storage, the percentages of germination (53.33 %) were lower and significantly different from those at the beginning of storage (89.33). According to Copeland and Mc Donald (2001) seeds containing high lipid content decrease their viability faster compared to those containing high carbohydrate content. The seed germination percentage in this study was more than 80 %, because the seeds were derived from fruits which fruit skin is yellow up to blackish yellow in colour as required for good quality of physic nut seeds.

The percentages of vigor after one month of storage (70.00%) were lower and significantly different from those at the beginning of storage (83.33%). After six months of storage, the percentages of vigor (26.67%) were lower and significantly different from those at the beginning of storage (83.33 %), after one (70.00%), two (63.33 %), three (56.67%) and four months (36.67%) of storage.

According to Adikadarsih and Hartono (2006) the lowest percentages of germination (7 %) and vigor (4.33 %) were found in physic nut seeds derived from fruits which fruit skin is green in color ; while the highest percentage of germination (91.67 %) and vigor (84.67 %) were found in seeds derived from fruits with fruit skin is yellow in color.

Under conditions that favour them to grow, storage fungi invade the germs or embryos of seeds prefentially, and sometimes exclusively. Very often, and especially if the moisture content of the seeds is at or just slightly above the lower limit that allow a given species of storage fungi to grow, the germs may be invaded to the point of near decay, with no evidence of molding being evident outwardly, even with microscopic examination, and little or no invasion of the endosperm immediately adjacent to the germ. The first effect of this invasion is weakening of the germ, followed by death (Sauer *et al.* 1992).

CONCLUSIONS

Duration of storage affected the quality of physic nut seeds. After one month of storage, the moisture content of physic nut seeds increased, and became relatively constant up to six months of storage. During storage the moisture contents were still safe for storing physic nut seeds (7.89 – 8.41%).

Sixteen mould species were isolated from physic nut seeds during six months of storage. Fungal population decreased with the increase of storage duration. At the beginning of storage most of fungi infected the seeds were field fungi (*Colletotrichum* sp., *Cladosporium* spp., and *Fusarium* spp.). Their populations decreased with the increase of storage duration and replaced by storage fungi (*Penicillium* spp. and *Aspergillus* spp.).

Lipid contents, viabilities and vigors decreased with the increase of storage duration, while free fatty acids and lipase activities increased. Under uncontrolled conditions, physic nut seeds packed in plastic material can be stored up to one month for seeds to be planted, while for producing oil, they can be stored up to five months.

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