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# Genotype and age of industrial plant *Jatropha curcas* L. affect physico-chemical properties of seed oil

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*Jatropha curcas*, has been established as a plant whose seeds contain a relatively high percentage of oil that is suitable for biodiesel production, among other industrial applications. Seed oil properties of *J. curcas* may vary depending on soils, age of plant, genotype of the plant, and agro-climatic conditions of a specific geographical region. Studies under such conditions have not yet been conducted. The present study was aimed at investigating the effects of genotype and age of the plant on the physicochemical properties of its seed oil. The seed oil was extracted using n-hexane as a solvent in the Soxhlet extraction apparatus from seed kernel collected from different genotypes and plants of different ages. Oil content in the kernel ranged from 49.78–53.75% (w/w) among the seed samples depending on genetic materials and plant age. The oil content showed very little but significant differences in density and specific gravity among the samples. The highest free fatty acid (FAA) was produced by the kernel oil of 3-year-old plants (7.78%), and 7- and 10-year-old plants gave the lowest FAA (1.26 and 1.31%, respectively). The kernel oil of 7-year-old plants produced the highest iodine value (93.60 mg/g oil) and the kernel oil of 3-year-old plants produced the lowest iodine value (61.10 mg/g oil). Kernel oil of 10-year-old plants gave the lowest (172.98 mg KOH/g) saponification value and 4-year-old Hybrid-3 gave the maximum value (209.97 mg KOH/g oil).

The seed oil of *Jatropha* accessions showed very little difference (39.88–40.85 MJ/kg) for high heating values irrespective of their age. On the other hand, the cetane number varied from 55.32 to 59.58 in the oil samples. The age of plant and seed sources had a significant effect on seed oil content and the physio-chemical properties of *Jatropha*.

#### KEYWORDS

*jatropha*, seed kernel, oil content, age of plant, hybrids, renewable energy, biofuel

## 1 Introduction

Recent studies have indicated that fossil fuel as a petrochemical energy stock is non-renewable and can be exhausted with time as a result of continuous and various utilizations (Demirbas, 2009; Oyekunle et al., 2019). Over 90% of the world population directly or indirectly depends on fossil fuel for their source of energy supply. To solve the problem, biofuel is being believed to be a good alternative to fossil fuels and is produced from vegetable oil and animal fats. Biofuel causes less environmental pollution (lower greenhouse effect) due to its low Sulphur content compared to fossil fuel (Wang et al., 2006). On the other hand, biofuel production from edible vegetable oils (rapeseed, corn, palm oil, soyabean etc.) creates a burden on the world food supply. Augmented natural apprehension and expected attenuation of fossil fuel reserves are the principal cause of searching for non-edible sources as an alternative feedstock for the production of biofuels (Charlene et al., 2004). In recent times, the utilization of non-edible oil such as *jatropha* as feedstock for biodiesel production has received more attention and is anticipated to play down the crisis for vegetable oil (FAO, 2009). Since non-edible oil is underutilized it can be a potential feedstock for biodiesel by reducing the dependency on crude oil import.

The plant parts (leaves, seeds etc.) of *J. curcas* are toxic to humans and animals. The toxicity of seeds is largely due to the existence of diterpine and curcun (Openshaw, 2000; Kureel, 2006; Kaushik et al., 2007; Achten et al., 2010). The seed oil of *Jatropha* comprises a high proportion (78–84%) of fatty acids which contains one or more carbon-carbon double/triple bonds (Heller, 1996). The properties of kernel oil of *Jatropha* obtained from parental accessions, their hybrids, and different age level are still scarce (Sirisomboon et al., 2007; Salimon and Abdullah, 2008; Sirisomboon and Kitchaiya, 2009). The properties of kernel oil are specific gravity (SG), density, refractive index (RI), viscosity, FFA content, iodine value (IV), acid value (AV), saponification value (SV), high heating value (HHV) and cetane number (CN). The biodiesel properties produced through transesterification are also affected by the seed oil properties of *Jatropha* (Emil et al., 2010; Kumar et al., 2011). The constituents of seed oil vary to a great extent with the genotype, climatic condition, soil, and management practices (irrigation, weeding, use of fertilizer, crop density, use of

pesticide, and inter-cropping). Variation also occurs when marginal farming is changed to commercial farming with irrigation facilities (Kumar et al., 2011). Seed oil properties are important attributes for biodiesel production, which affects several parameters of biodiesel from *Jatropha*. The seed oil content of *Jatropha* and its physio-chemical properties are considerably influenced by the type of genetic materials and age of the plant. Therefore, the present study aims to estimate kernel oil content, and physical and chemical characteristics of kernel oil collected from plants of different genotypes and ages.

## 2 Materials and methods

### 2.1 Collection and preparation of seed sample for oil extraction

Ten seed samples (Table 1) were collected from the *Jatropha* genotypes/accessions (parents, hybrids, or accessions) planted at Biofuel Research Station, National University of Malaysia (UKM), Malaysia. The experimental area was under tropical climate which is mostly experienced in Malaysia (McGinley and Clough, 2010) and the soil belongs to marginal land previously under tropical rain forest. Seeds were collected from yellow to dark brown fruits of healthy plants and de-hulled after drying under sunlight to lessen the amount of moisture. To get kernels, the fruits were de-shelled followed by de-hulled the seeds of the parents and hybrids. Seed kernels were dried at 100–105°C for 30 min in the oven drier to reduce moisture content following Sirisomboon et al. (2007) before oil extraction (Sirisomboon and Kitchaiya, 2009) as the seed kernels of *Jatropha* contain a high amount (34%) of moisture (Emil et al., 2010).

### 2.2 Extraction of seed oil

*Jatropha* seed kernels were ground to powder prior to oil extraction by solvent extraction method using n-hexane (Sayyar et al., 2009). Analytical grade chemicals were used in the extraction process without additional refinement. Solvent extraction with n-hexane (b.p. 60–70°C, 6 h by gentle heating) was used to extract oil from the samples of known weight without any interruption. The oil sample extracted using n-hexane was

TABLE 1 Seed sample with their source, origin and age of the plant from which seeds were collected.

Seed sample	Genotypes	Origin	Age of the plant
Sample-1	Parent 1	Malaysia	2 Years
Sample-2	Parent 1	Malaysian	3 Years
Sample-3	Parent 1	Malaysia	4 Years
Sample-4	Hybrid 1	Malaysia x India	4 Years
Sample-5	Hybrid 2	Malaysia x Indonesia	4 Years
Sample-6	Hybrid 3	Malaysia x Malaysia	4 Years
Sample-7	UKM Accession	Malaysian	5 Years
Sample-8	UKM Accession	Malaysian	6 Years
Sample-9	UKM Accession	Malaysian	7 Years
Sample-10	UKM Accession	Malaysian	10 Years

evaporated on a rotary water bath evaporator until hexane remain. The amount of extracted oil was calculated as the percent of the kernel powder used.

## 2.3 Percentage of oil extracted

A total of 120 ml n-hexane was used as solvent to extract oil from a 10 g sample placed in the Soxhlet extraction apparatus (Sayyar et al., 2009). The seed kernel powder was placed in the thimble before setting in the Soxhlet extraction apparatus. The remaining n-hexane from extracted oil was removed at 40°C by using a rotary water bath evaporator. Finally, the amount of extracted oil was determined as the percentage of oil in seed kernel powder of *Jatropha curcas*. The oil sample was then stored in the refrigerator at -2°C for further analysis of oil properties.

## 2.4 Physical properties

### 2.4.1 Density and specific gravity

The oil density and specific gravity were determined by Anton-Paar DMA 4500 density meter at 20 C in the laboratory.

### 2.4.2 Refractive index

The refractive index (RI) of the seed oil was determined by the transfer of a few drops of oil into the prism of the refractometer and determination was done at 40°C. RI determination was technically equivalent to ISO 6320:1995.

## 2.5 Chemical properties

Several chemical properties of kernel oil such as % free fatty acid (FAA) content, iodine value, acid value, and saponification value were estimated by following Standard Tentative Methods of Analysis (AOCS, 1991).

## 2.6 Fuel properties

### 2.6.1 High heating value

The gross calorific or gross energy value known as high heating value (HHV) represents the amount of heat released by the oxidation of oil in the air as fuel. HHV is the amount of energy generated by the full burning of a unit amount of oil. The high heating value of kernel oil of *Jatropha* was calculated using an equation adopted from Demirbas (1998) using the derived amount of IV and SV:

$$HHV = 49.43 - (0.041 \times SV) - (0.015 \times IV)$$

SV = Saponification value.

IV = iodine value.

### 2.6.2 Cetane number

CN of the seed oil was estimated by using the equation suggested by Bose (2009).

$$CN = 46.3 + \frac{5458}{sv} - 0.225 \times IV$$

SV = Saponification value.

IV = Iodine value.

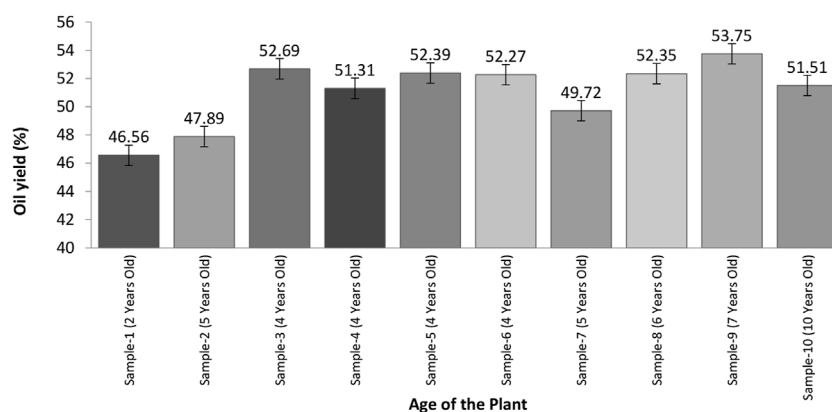
## 2.7 Statistical analysis

Data were collected for each sample and replicated thrice for all parameters. SAS 9.2, a standard statistical package was used to analyze the data (SAS, 2010).

## 3 Results

### 3.1 Seed oil content

The percentage of seed kernel oil was extracted using the solvent extraction method from 10 different samples and the results are



**FIGURE 1**  
Effect of plant age on seed oil content (%) of jatropha genotypes and hybrids.

**TABLE 2** Effect of plant age on physical properties of seed oil of jatropha.

Seed samples	Density (20°C)	Specific gravity (20°C)	Refractive index
Sample-1	0.898	0.900	1.46
Sample-2	0.926	0.928	1.46
Sample-3	0.913	0.914	1.46
Sample-4	0.941	0.943	1.46
Sample-5	0.938	0.941	1.47
Sample-6	0.935	0.936	1.46
Sample-7	0.938	0.940	1.46
Sample-8	0.945	0.946	1.46
Sample-9	0.906	0.908	1.46
Sample-10	0.912	0.914	1.46
Max	0.945	0.946	1.47
Min	0.906	0.908	1.46
Avg	0.928	0.930	1.46
Level Sig	**	**	ns
CV (%)	0.07	0.08	0.98
R <sup>2</sup>	1.00	1.00	1.00
MSD (5%)	0.001	0.001	0.001

\*\*, Level of significance at 1%; MSD, mean significant difference; Mean values are the average of triplicate determinations, Level of significance determined by Tukey's test and means with same rare not significant at  $p < 0.001$ .

presented in Figure 1. Kernel oil extracted from different seed samples through n-hexane solvent extraction varied from 46.56 to 53.75 by %w.

The highest kernel oil content (53.75%) was obtained from the seed sample-9 of seven-year-old plants originating from Malaysia and the lowest (46.56%) in the seed sample-1 (2 years old plant) from Malaysia. The oil extracted from the seeds kernel of plants aged 4 years onward yielded maximum kernel oil content of >50% (Figure 1). On the other hand, hybrids produced higher amounts of seed oil compared to parental genotypes.

### 3.2 Physical properties

Irrespective of plant age, density of seed oil, SG, and RI index revealed non-significant variances among the samples (Table 2). Light yellow color of oil was observed for all the extracted samples. Seed oil density ranged from 0.898 to 0.945 amongst all seed samples. The seed sample-7 showed the highest oil density and seed sample-1 showed the lowest oil density.

TABLE 3 Effect of plant age on biochemical characteristics of seed oil of *Jatropha*.

Seed samples	%FFA	AV	IV	SV
Sample-1	1.68	3.34	104.45	185.38
Sample-2	2.93	4.17	90.68	185.74
Sample-3	4.17	6.07	64.04	198.14
Sample-4	4.69	6.63	69.71	196.19
Sample-5	6.34	8.89	62.73	209.97
Sample-6	7.78	11.06	62.10	201.88
Sample-7	6.00	8.52	65.62	194.65
Sample-8	1.26	1.78	86.50	180.67
Sample-9	1.31	1.84	89.70	172.98
Sample-10	2.96	4.21	93.60	174.73
Maximum	7.78	11.06	104.45	209.97
Minimum	1.26	1.78	62.10	172.98
Average	3.91	5.65	78.91	190.03
F-value	**	**	**	**
CV (%)	3.89	4.07	0.15	0.29
R <sup>2</sup>	1.00	1.00	1.00	1.00
MSD (0.05)	0.47	0.70	0.32	1.61

\*\* , Level of significance at 1%; MSD , mean significant difference; Mean values are the average of triplicate determinations, Level of significance determined by Tukey's test and means with same rare not significant at  $p < 0.001$ . AV , Acid Value (mgNaOH/g oil), IV , Iodine Value (mg/g oil), SV , Saponification value (mgKOH/g oil).

### 3.3 Chemical properties

The suitability of oil as feedstock for biodiesel production depends on the biochemical characteristics of seed oil of *J. curcas*. Pre-treatment of seed oil samples before alkaline transesterification is determined by biochemical characteristics of seed oil. FFA value, AV, IV SV of ten seed samples are presented in Table 3. Biochemical characteristics of the seed kernel oil of *Jatropha* varied significantly irrespective of the age of the plant. Seed oil from sample-2 contained higher FFA content (7.78%) followed by sample-6 (6.34%) and sample-8 (6.00%) than other samples with FFA content between 1.26 and 4.69% (Table 3). None of the seed samples had FFA content <1% and only three seed samples (sample-1, sample-9, sample-10) obtained FFA content below 2% (1.68, 1.26, 1.31%).

The highest IV (104.45 mg/g oil) was observed in the kernel oil of sample-1 (2-year-old) and the lowest IV (99.10 mg/g oil) was found in sample 2 (3-year-old) (Table 3). Iodine value represents the content of unsaturated fatty acid.

The saponification value of seed oil among seed samples varied from 172.98 (sample-10) to 209.97 (sample-6) mg KOH/g and the average value for this study was 190.55 mg KOH/g (Table 3).

### 3.4 Fuel properties

HHV and CN ranged significantly among the seed samples (Figures 2, 3). The high heating value varied (39.88–40.99 MJ/kg) among the oils of the seed samples depending on the genetic material and age of the plant (Figure 2). The seed sample-7 (5-year-old plant) stored at room temperature 6 months after harvest gave the highest HHV (40.99 MJ/kg) and the seed sample-6 (4-year-old hybrid 3) gave the minimum HHV (39.88). Most of the oil crops showed similar HHVs (39–40 MJ/kg) except castor oil (37.27 MJ/kg).

The seed oil sample-8 (6-year-old plant) gave the highest CN (59.58) followed by the sample-5 (59.41), sample-2 (59.36) and the lowest (52.24) by the sample-1 (Figure 3).

## 4 Discussion

The results obtained from seed kernel of *Jatropha curcas* in the current research follow the array of the oil percentage of 46–54% by weight found in the report (Pramanik, 2003; Azam et al., 2005). EL Kinawy (2010) also reported 43% oil in the seed kernel of *Jatropha*. Punia (2007) reported that seed oil content is expected to be maximum in 6-year-old plants. *Jatropha* seed kernel contains higher oil than the kernel of linseed, soybean, and palm oil (Gunstone, 2004). A significant increase in seed oil content was noted in *Jatropha* plants at the age above 4 years depending on genetic materials. Selected genotypes (parents and hybrids) contained a higher percentage of oil in their kernel compared to wild sources (Islam, 2011; Islam et al., 2012). Storage time also affects the kernel oil content in *Jatropha* seed and may be due to loss of water and physiological changes that occurs in the seed kernel during storage. The higher amount of seed kernel oil of *Jatropha* indicated its suitability as non-edible vegetable oil as feedstock for biodiesel production and other oleo-chemical industries. *Jatropha curcas* plantation yields 2000 L of oil per annum per hectare of land (Azam et al., 2005).

More energy is released from the oil with high density compared to oil with low density. The density of a material is defined as the measurement of its mass per unit volume (e.g., in g/ml). The density of vegetable oil is lower than water and the differences between vegetable oils are quite small, particularly among the common vegetable oils. Generally, the density of oil decreases with molecular weight, yet increases with unsaturation level (Gunstone, 2004). Specific gravity varied from 0.900 (sample-1) - 0.956 (sample-7) amongst the seed oil samples. Density and specific gravity are the most important factors in the injection systems of diesel fuel. For complete combustion, optimum or tolerable limits needs to be maintained to allow ideal air-to-fuel ratio. A high flow of air may lead to partial combustion of biodiesel fuel or its blend (Azam et al., 2005).

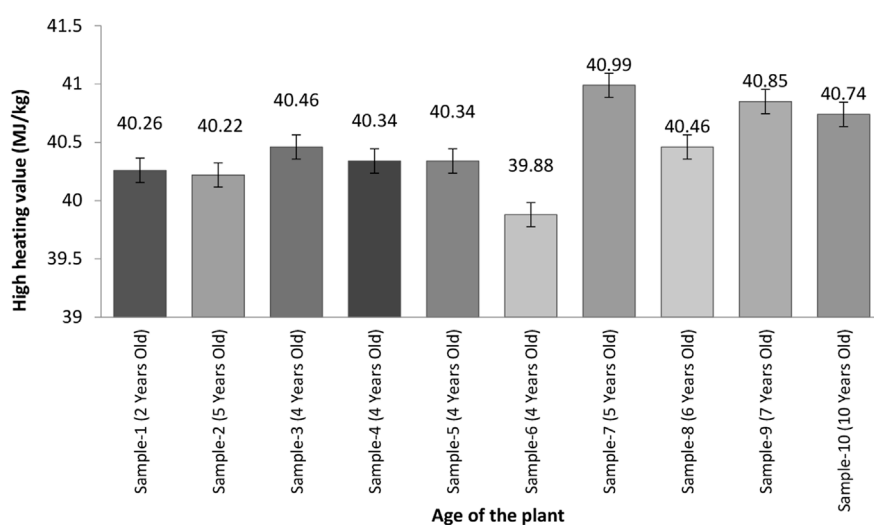


FIGURE 2

Effect of age of the plant and *Jatropha* genotype on high heating value (HHV) of the kernel oil sample.

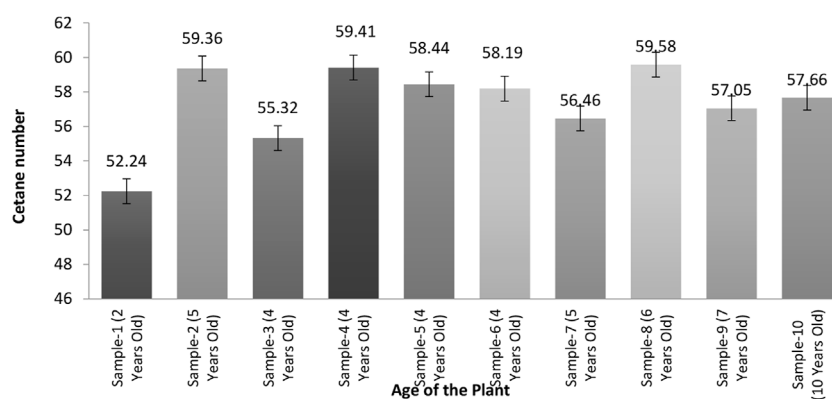


FIGURE 3

Effect of age of the plant and *Jatropha* genotype on CN of the kernel oil sample.

Free fatty acid (FFA) content is one of the most essential characteristics that represent oil quality. A higher FFA value exhibited a higher acid value in the oil sample. The suitability of *Jatropha* seed oil as feedstock for biodiesel production depends on its FFA content and high FFA content makes *Jatropha* oil unsuitable as feedstock. The transesterification process becomes complex if the oil sample contains a high amount of FFA due to the formation of soap with an alkaline catalyst (Canakci, 2007; Tiwari et al., 2007). This soap inhibits the separation process of biodiesel from glycerin (Demirbas, 2003). Canakci (2001) and Tiwari et al. (2007) recommended base transesterification to overcome bubbles (soap) formation from the oil with less (<1%) FFA content. Pretreatment with

acid before transesterification was also suggested for feedstock with high FFA (>1%) values (Canakci and Gerpen, 2001; Dorado et al., 2002). The esterification reaction of the oil with an acid catalyst is an alternative (Crabbe et al., 2001), but it is 4,000 times slower than the transesterification with an alkaline catalyst. The acid-catalyzed process does not have the same acceptability as alkaline transesterification in industrial applications due to slow transesterification. Two-step esterification was also reported as another alternative process for the feedstock having high FFA content (Ghadge and Raheman, 2005; Veljkovic et al., 2006).

The age and quality of seed oil depend on acid value. The acid value of the present study ranged from 1.78 to 11.06 mg



NaOH/g which indicated high FFA content but the value was relatively low compared to previous results obtained by other researchers (Emil et al., 2009; Belewu et al., 2010). The maximum acid value was observed in seed sample-2 (3-years-old) and minimum in seed sample-9 (7-years-old). The results indicated that oil properties are not affected by the age of the *Jatropha* plant but rather by genotype.

A higher amount of unsaturated fatty acid has been reflected by a higher iodine number in the oil sample (Knothe, 2005). *Jatropha* seed oil contains high iodine which is due to its high content of unsaturated fatty acids such as oleic and linoleic acid. Emil et al. (2009) reported 78.5% unsaturated fatty acid in the seed oil of *Jatropha curcas*. The high iodine value of *Jatropha* seed oil showed its suitability in the production of alkyl resin, shoe polish, and varnishes (Akintayo, 2004).

*Jatropha* seed oil has a high saponification value (Emil et al., 2009; Maricela Rodriguez et al., 2010; Antony Raja, 2011). A high saponification value indicates that *Jatropha curcas* seed oil contains typical triglycerides (Emil et al., 2009). The oil with a high saponification value required more base catalyst to neutralize existing FFA in the oil samples. The oils with high saponification value contain normal triglycerides which can be used in the shampoo and liquid soap industries.

High saponification values indicate the existence of a high proportion of FFA in the oil sample. The oil with a high saponification value infers the possibility of the formation of soap and problems in the separation of glycerin products during biodiesel production. This condition leads to a very low yield of biodiesel (methyl ester) using the oil sample with a high saponification value. Salimon and Abdullah (2008) described a similar characteristic of *Jatropha* seed oil of Malaysian origin.

Fatty acid methyl ester composition considerably influenced the cetane number (CN) of biodiesel fuels (Geller and Goodrum, 2004). Biodiesel fuel quality, especially ignition quality depends on CN of *Jatropha* seed oil (Bamgboye and Hansen, 2008). Cetane number quantifies the suitability of the oil to automobile ignition after injection into the engine (Knothe, 2005). The cetane number of the biodiesel derived from soybean oil varied from 45 to 60 whereas biodiesel derived from canola oil varied from 48 to 61.2 (Demirbas, 2003; Bamgboye and Hansen, 2008). High cetane number accompanied by complete ignition and low cetane number were related to a reduced ignition value of the oil sample.

## 5 Conclusion

The current research was carried out to evaluate how genotype and plant age of an industrial plant, *Jatropha curcas*, affect the physico-chemical properties of seed kernel

oil. It was observed that seed oil content varied among the genotype and is influenced by the age of the plant. The fatty acid content was observed higher in the plants with younger age compared to older ones, which affects the transesterification process during biodiesel production. The highest iodine value was found in the seed oil of a seven-year-old plant and the lowest in a three-year-old plant. The seed oil of a 10-year-old plant gave the lowest saponification value and Hybrid-3 (four-year-old) plants gave the highest value. It could be concluded based on the above findings that the physico-chemical properties are greatly influenced by the age and genotype of the *Jatropha curcas* plant.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding authors.

## Author contributions

All authors contribute: Conception or design of the work Data collection Data analysis and interpretation Drafting the article Critical revision of the article Final approval of the version to be published.

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