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Studies on physicochemical properties & proximate analysis of *Carica papaya* seed

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Abstract
The present study was carried out to examine the yield of crude oil extracted from the dried papaya seeds by solvent extraction method. The physicochemical properties, fatty acids, tocopherols and phenolic composition of crude oil extracted from papaya (*Carica papaya* Linn.) were assessed. From the present study carried out, the yield of the papaya seed oil is 25.6%. The physicochemical properties of Papaya seed oil were: acid value (2.805mg/g), saponification value(95.37mg KOH/g), iodine number(63.1), peroxide value(5.27mEq/kg), phenols(118.97µg/mL), tocopherols (51.77µg/g), Unsaponification value(1.318%), pH(5.6), density(0.94g/mL), moisture content of oil (0.210%), specific gravity(0.921). The values of proximate of dried papaya seed powder were: low moisture content(0.02%), high ash content(9%), high protein content(32.97g/kg), relatively high crude fibre content(18.83%), low carbohydrate content(895.485µg/g)and also the fatty acids present in the papaya seed oil were noticed.

Keywords: *Carica papaya* Linn, seeds, benzylisothiocynate, antihelminthic, papaya seed oil,

Introduction
Papaya (*Carica papaya* Linn) belongs to family Caricaceae is one of the important tropical plant cultivated in India, ranks first in its production. It is native to tropical America, the papaya has spread to several regions of the world, and its largest producers are India, Brazil, Mexico, Nigeria and well distributed in most of the tropical countries. It is generally found in tropical zone of planet as it thrives in hot, humid and frost – free climates. The internal cavity of the fruit contains numerous black seeds, edible, spicy flavour, coated with mucilaginous substance and it comprise about 15% of the wet weight of the fruit (Desai, Wagh, 1995).
The seed of papaya has antimicrobial activity against *Trichomonas vaginalis* trophozoites. Its seeds also have contraceptive effects in adult male Langur Monkeys, possibly in adult male humans (Lohiya *et al*., 2008; Oderinde *et al*., 2002). It could also be used in urinogenital disorder like trichomoniasis with care to avoid toxicity (Calzada *et al*., 2007). The seeds, irrespective of its fruit maturity stages have bacteriostatic activity on gram positive and negative organisms which could be useful in treating chronic skin ulcer. The papaya seed macerate has a clinical potential on conjugal R plasmid transfer from *Salmonella typhimurium* to *Escherichia coli in vitro* and in the digestive tract of gnotobiotic mice (Krishna *et al*., 2008) [9]. The seed being consumed offers a cheap, natural, harmless, readily available mono therapy and preventive strategy against intestinal Parasitosis (Okeniyi *et al*., 2007) [13].
Benzylisothiocynate is a bioactive substance present in the seeds as the chief or sole antihelminthic (Kermanshai *et al*., 2001). There is an increasing interest in the C. papaya seed due to its medicinal value. BITC applications ranged from vascular relaxation to inhibition of cancer proliferation. The seeds do not naturally contain BITC. The BITC production occurs when the benzyl glucosinolate, present in the interior of the seeds, contacts the myrosinase enzyme, present in the seed's surface. Papaya seed oil utilized in high amount such oils could leave to reduced risk of coronary heart disease. In addition, high oleic oil has sufficiently stability be used in demanding application such as frying. Papaya seed oil can be considered as high oleic oil and hence viewed as a healthy alternative to many other vegetable oil (Corbett, *et al* 2003).The seed has been shown to be a good source of oil (25.6%) that may be useful for medicinal, biofuel, and industrial purposes (Afolabi *et al*., 2011). The physicochemical properties of oils determine their quality and whether they are suitable for consumption (Fokou *et al*., 2009). Papaya seed is a source of protein, fibre and oil. Papaya seed oil is semi liquid with reddish yellow color. This oil is potential to be searched for edibility as it is rich in beneficial in triacylglycerols (i.e. triolein>37%) and mono unsaturated fatty acids (i.e oleic acid>70%). In addition, papaya seed oil is stable against oxidation with considerable

antioxidant activity. Papaya seed has as constituents fatty acids, crude proteins, crude fibre, papaya oil, carpaine, Benzylisothiocynate (BITC), Benzyl glucosinolate, Glucotropaeolin, Benzyl Thiourea, Hentriacontane, Caricin and an enzyme Nyrosin.
The composition of these seeds, compiled by Marfo, Oke and Afolabi 1986 and Puangsri, Abdulkarim and Ghazali 2005 [16], revealed that they are a rich source of protein (27.3- 28.3%), lipids (28.2-30.7%) and crude fibres (19.1-22.6%). Marfo, Oke and Afolabi 1986 found appreciable quantities of calcium and phosphorous in the seeds. Oleic, palmitic, linoleic, and stearic acids were the most abundant fatty acids found in the papaya seed oil (Marfo, Oke, Afolabi, 1986; Puangsri, Abdulkarim, Ghazali, 2005) [16]. Masson *et al*. (2008) determined the fatty acid composition and bioactive compounds of the oil extracted from seeds of papaya. Oil extraction from papaya seeds may add economic value to a large quantity of seeds that are generally discarded. The objectives of this research are: To examine the yield of papaya seed oil and to assess the physicochemical properties of papaya seed oil. To assess the proximate analysis of dried papaya seed powder and to examine the fatty acid composition of papaya seed oil.

Materials and Methods

This chapter deals with the details of materials procured and methods followed for the extraction of oil from dried papaya seeds and determination of physico-chemical properties & proximate analysis of them.

Raw Materials

The papaya was procured from the local market Bapatla

Fig 1: Papaya seeds

Papaya seed oil extraction: Papaya seed oil is extracted by solvent extraction method (AOAC, 1980). Before the extraction, the seeds were dried at 50 ºC for 20h and grind them into powder.

Procedure: Rinse all the beakers and place them in oven with the temperature about 100º C and also the samples. If all moistures were removed from the beakers, place them in desiccators about 5-10 min. to bring them into room temperature. Now weigh the empty beaker and let the weight be W_1. This is the Initial Beaker Weight (IBW).Now insert the thimble in the thimble holder and place it on the beaker. Weigh the samples and transfer them to the thimbles. Let the sample weight be SW. Sample weight may be 2-3g.Pour the solvent in the beaker. The volume may be 80-100 ml. Load the beakers in the system. Switch ON the system and set the boiling temperature as the solvent’s maximum boiling point. Leave the process about 60 minutes. After the process time, increase the temperature to recovery temperature should be the range of 160 ºC-190 ºC. Now do the rinsing about 2-3 times in order to collect the remaining fat that may present in the sample or in the thimble. Now take out the beakers from the system then remove all the thimbles from the beaker and put the beakers into a hot air oven at 100 ºC. After 20-30 minutes, take out the beakers and place them in desiccator about 10-15 minutes for cooling up to the room temperature. After cooling, weigh the beakers. This is the Final Weight of the Beaker (FBW). Let the weight be W_2.

$$\%\,FAT = \frac{W_2 - W_1}{SW} \times 100$$

Physicochemical properties of papaya seed oil

Acid value: A small quantity of free fatty acids is usually present in oils along the triglycerides. Free fatty acid content is known as acid number/ acid value. It increases during storage. Keeping quality of oil, therefore, release upon the free fatty acid content. Acid number was defined as mg of KOH required to neutralize the free fatty acids present in 1 g of sample (AOAC, 969.33)

Saponification value: Saponification is the process by which the fatty acids in the glycerides of the oil are hydrolysed by an alkali. Saponification value is the amount (mg) of alkali required to saponify a definite quantity (1 g) of oil. This value is useful for comparative study of the fatty acid chain length in oils (AOAC, 920.160)

Iodine value: Iodine value is a measure of the degree of unsaturation in oil. It is constant for particular oil. Iodine value is a useful parameter in studying oxidative rancidity of oils since higher the unsaturation the grater the possibility of oils to go rancid. Iodine value/ number is defined as the ‘g’ of iodine absorbed by 100 g of oil (AOAC, 920.159).

Peroxide Value: Peroxide value is a measure of the peroxides contained in the oil. The peroxides present are determined by titration against thiosulfate in the presence of KI. Starch is used as indicator. (AOAC, 990.05).

Phenols estimation: Phenols react with phosphomolybdic acid in Folin – Ciocalteu reagent in alkaline medium and produce blue colored complex (molybdenum blue). Sadasivam S and Manickam 2008 [17]

Tocopherols: Tocopherols can be estimated after Emmerie – Engel reaction. This is based on the reduction of ferric to ferrous ion, by tocopherols, which then forms a red color with 2,2,’dipyridyl reagent. Tocopherol is first extracted into xylene and read at 460 nm; a correction is made after adding ferric chloride and read at 520 nm (AOAC, 925.41).

Unsaponification Value

Unsaponifiable matter represents substances which are insoluble or are incapable of forming soaps with alkali. In edible oils, it is present up to 1-2%.Extraction of oil from foods by ether raises this value. For pure oil the amount is negligible and is entirely due to phytosterol, a characteristic plant steroid, distinct from cholesterol, the animal steroid. The crystals of unsaponifiable matter are observed for the presence of this steroid to know the animal or plant origin of the oil. In the method, the oil is saponified with alcoholic KOH and then extracted with petroleum ether. The extract is washed with alcohol and water, evaporated and weighed. Unsaponifiable matter is represented as difference between the weight of the residue and the fatty acids content of it (determined by NaoH titration in alcoholic medium). (AOAC, 933.08).

Moisture Content: The moisture content of papaya seed oil was determined by using oven drying method (FSSAI, 2012).

Specific gravity

Procedure: Sample was melted if necessary then filtered through a filter paper to remove any impurities and the last traces of moisture. Made sure the sample was completely dry. Cooled the sample to 27 ^{0}C or ambient temperature desired for determination. Specific gravity was measured by specific gravity or density bottles. Carefully cleaned specific gravity bottles by filling with chromic acid cleaning solution and letting stand several hours. Emptied the specific gravity bottles and rinsed thoroughly with water, filled with recently boiled water previously cooled to about 27 ^{0}C and placed in water bath at temperature held at 27 ^{0}C. After 30 minutes, adjust water level to proper point on specific gravity bottle put stopper, removed from bath, wiped dry with clean cloth or towel and weighed. Filled the dry specific gravity bottle prepared sample (at 27 ^{0}C) in such a manner to prevent entrapment of air bubbles. Inserted the stopper, immersed in water bath at 27 ^{0}C and held for 30 min. carefully wiped of any oil that had come out of the capillary opening. Removed the bottles from the bath, cleaned and dried it thoroughly and quickly weighed ensuring that the temperature did not fall below 27 ^{0}C

Calculation

Specific gravity of oil was calculated as

$$\text{Density of oil} = \frac{\text{weight of bottle with oil-Empty specific gravity weight}}{\text{Volume of specific gravity bottle at 27°C}}$$

$$\text{Specific gravity of oil} = \frac{\text{Density of oil at 27°C}}{\text{Density of water at 27°C}}$$

Kreis Test for Oxidative Spoilage

Take in a test tube 1 mL of melted fat sample. Add 1 mL of HCl. Add 1 mL of 1 % phloroglucinol solution in ether. Mix well and observe the color formed. The color intensity is proportional to the degree of oxidation. Pink color formation indicates slight oxidation, and red color indicates extensive oxidation.

Proximate analysis of dried papaya seed powder

Moisture Content

The moisture content of dried papaya seed powder was determined by oven drying method (AOAC, 1999).

Protein content: The nitrogen in protein or any other organic material is converted to ammonium sulphate by H_2SO_4 during digestion. This salt, on steam distillation, liberates ammonia which is collected in boric acid solution and titrated against standard acid. Since 1mL of 0.1N acid is equivalent to 1.401 mg N, calculation is made to arrive at the nitrogen content of the sample (AOAC, 950.48)

Crude fibre content: During the acid and subsequent alkali treatment, oxidative hydrolytic degradation of the native cellulose and considerable degradation of lignin occur. The residue obtained after final filtration is weighed, incinerated, cooled and weighed again. The loss in weight gives the crude fibre content (AOAC, 2000)

Results and Discussion

Present study included the extraction of oil from dried papaya seeds. In this chapter, results of the study were discussed keeping in view the various objectives stated earlier. Results of different physico-chemical & proximate analysis were also discussed in this chapter.

Yield of Papaya seed oil

The yield of papaya seed oil extracted by solvent extraction method was 25.6% by using petroleum ether as solvent.

Physicochemical properties of Papaya seed oil

The physicochemical properties of papaya seed oil were as shown in table 1.

Table 1: Physico-chemical properties of Papaya seed oil

Acid Value	2.805mg/g
Saponification Value	95.37mg KOH/g
Iodine Number	63.1
Peroxide Value	5.27mEq/kg
Phenols	114.54mg/100g
Tocopherols	51.77µg/g
Unsaponification Value	1.318%
PH	5.6
Density	0.94g/mL
Kries test	No colour change
Specific Gravity	0.921
Moisture Content	0.210%

Acid value: The acid value of papaya seed oil in the present study was close to the standard value (2.53±0.08mg KOH/g). The acidity of the oil suitable for edible purposes shall not exceed 4mg KOH/g (Amoo *et al*., 2004) thus the papaya oil may be suitable for consumption. Acid Value is 2.805mg/g

Saponification Value: The saponification value of papaya seed oil in the present study was relatively low comparative that of almond nut seed oil (163.39±15.80) and palm kernel seed oil having a value of 191.97±3.16 (Afolabi,2008). This result indicated that papaya seed oil contains high molecular weight fatty acids since the saponification values have been reported to be inversely related to the average molecular weight of fatty acids in oil fractions (Abayeh *et al*., 1998) [1]. Saponification value is 95.7mg ko/Hg

Iodine Value: Iodine value is the measure of degree of unsaturation of the oil. It is useful in studying oxidative rancidity of oil since higher the unsaturation the greater the possibility of the oils to go rancid. The papaya seed oil tested in the present study had a low iodine value which was confirmed as non-drying oil. Low iodine values for papaya seed oil (66.0-74.8) were also obtained by other authors (Marfo, Oke, Afolabi, 1986; Puangsri, Abdulkarim, Ghazali, 2005) [16]. Iodine Value is 63.1

Peroxide Value: The peroxide value of papaya seed oil obtained in the present study was nearer to the standard value (5.37±0.13mEq/Kg). This value was not considered high since crude vegetable oil consists of 10 mEq/Kg of peroxide value (HUI, 1996). Peroxide Value is 5.27mEq/Kg

Phenols: Phenols are said to offer resistance to diseases and pests in plants. The total phenolic content of papaya seed oil was 114.5497mg/100g. This Value was higher than the content obtained by Siger, Nogala- Kalucka and Lampart-Szczapa (2008) in cold pressing oil seeds from soya bean (1.48 mg/100g), sunflower (1.20mg/100g), corn (1.26mg/100g), rape seed (1.31mg/100g), and rice bran (1.44mg/100g).

Table 2: Values of standards and test sample for phenolic content

Concentration (µg/g)	Absorbance (650 nm)
200	0.64
400	0.76
600	0.83
800	0.93
1000	1.04
1145.497	1.106

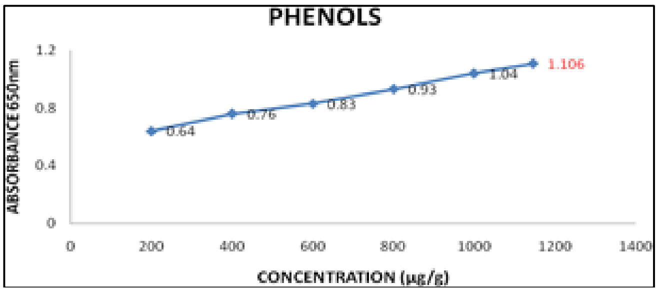

Fig 1: Variation of absorbance as a function of concentration of phenols in papaya seed oil (650 nm)

Tocopherol Content: The papaya seed oil in the present study had low content of tocopherol compared with other common oils such as soya bean (1797.6µg/g), maize (1618.4µg/g), and sunflower (634.4µg/g) oil (Tuberoso *et al*., 2007). Usually, high amounts of tocopherols are associated with polyunsaturated fatty acid content (Kamaleldin, Andersson, 1997; Masson, Camilo, Torija, 2008). Tocopherol Content is 51.77µg/g.

Unsaponification Value

The unsaponifiable matter percentage found in this study was similar to that reported for conventional vegetable oil (O'VRIEN, 2004) and also to that found in the literature regarding papaya seed oil: unsaponifiable matter of 1.39% (Puangsri, Abdulkarim, Ghazali, 2005) [16]. Unsaponifiable Matter is 1.318%

P^H value: The P^H value of papaya seed oil obtained in the present study was slightly low indicating the slightly acidic nature of the P^H value is 5.6

Test for Oxidative spoilage: When the oil was tested for oxidative spoilage, there was no color change which indicated absence of oxidative spoilage

Specific gravity: Specific gravity is important property that is always considered in oil which serves as feedstock for biodiesel. The specific gravity of the papaya seed oil was comparable with specific gravity reported for linseed oil and other seed oils that have been documented as good feedstock for biodiesel. The higher the value of specific gravity of the fuel is, the denser. This will affect a number of the fuel's properties, particularly the flow and the volatility. Specific Gravity is 0.921

Moisture Content: The moisture content of the oil was very low which a pointer to its storage stability was. Moisture Content is 0.210%

Proximate Analysis of Dried papaya seed powder

The proximate analysis of dried papaya seed powder was as shown in the table 3.

Table 3: Proximate analysis constituents of dried papaya seed powder

Moisture Content	0.02%
Ash Content	9%
Protein Content	32.97g/100g
Crude Fibre	18.83%
Carbohydrate Content	21.3%

Moisture & Ash Content: The dried papaya seed powder had a relatively low moisture content compared to the oil while it had relatively high ash content than the oil. Moisture content is 0.02% and Ash Content is 9%.

Protein Content & Crude Fibre: It can be observed that the dried papaya seed powder had a relatively higher protein and crude fibre contents than that of the oil. Hence, the dietary fibre in the dried papaya seed powder was high since crude fibre make up 50% of dietary fibre content (Cummings, 1981; Marria and Przemyslaw, 2006; Anderson *et al*., 2009). Therefore the dried papaya seeds will serve as a good source of dietary fibre, and protein for livestock and human consumption. Protein Content is 32.97 g/100g and crude fibre content is 18.83%

Carbohydrate Content: The carbohydrate content of dried papaya seed powder in the present study was low compared to that of the oil. Carbohydrate content is 895.4855µg/g.

Table: 4 Values of standard and sample for carbohydrate estimation

Concentration (µg/g)	Absorbance (630 nm)
200	0.41
400	0.52
600	0.78
800	0.821
1000	0.952
895.4855	0.911

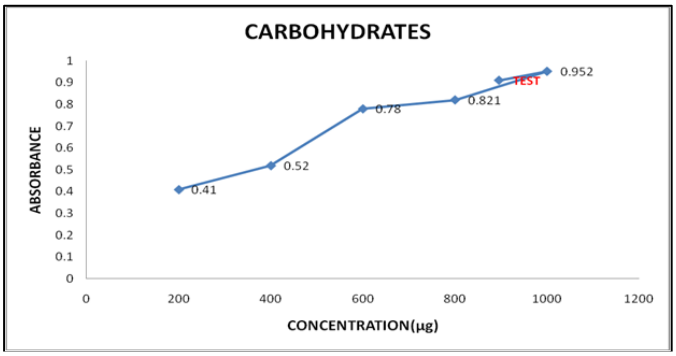

Fig 2: Variation of absorbance as a function of concentration of carbohydrates in dried papaya seed powder (630 nm)

Fatty acid Profile

In the present study, the fatty acid profile of papaya seed oil was observed from the chromatogram obtained by performing GC-MS analysis. The phytochemicals found in the papaya seed oil were listed in Table 5.

Phytochemicals found in the papaya seed oil have various applications such as a food additive, reduces the risk of heart diseases, adjunctive therapy in cancers, aids in the production of semi synthetic progesterone, stimulates proliferation of peripheral blood lymphocytes in humans, anti-obesity effect and aids in lowering the blood glucose levels in humans.

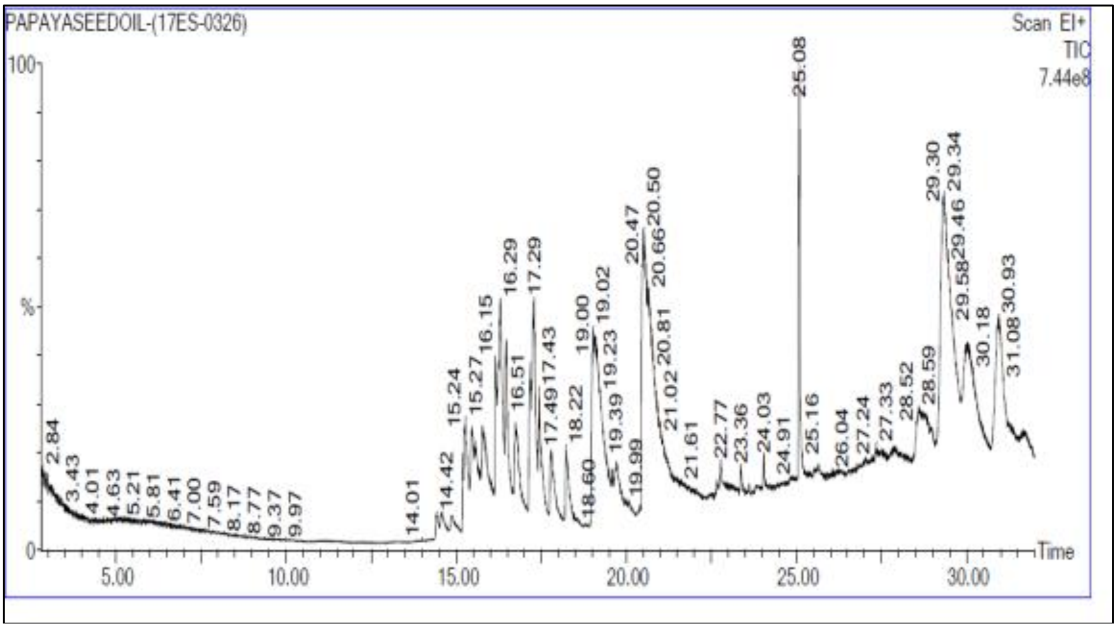

Fig 3: Chromatogram obtained for GC-MS analysis of Papaya seed oil

Table 5: List of phytochemicals and their activity

S. No	RT	Name	Area	Activity
1.	19.02	N-Hexadecanoic Acid	77,805,072.00	Food additive
2.	20.501	1,19- Eicosadiene	159,543,680.00	Reduces the risk of heart diseases, diabetes
3.	25.078	Squalene	28,545,458.00	Adjunctive therapy in cancers
4.	28.589	Stigmasterol	26,580,854.00	Precursor in the production of semisynthetic progesterone
5.	29.339	Gamma.-Sitosterol	136,190,352.00	Stimulates human peripheral blood lymphocyte proliferation
6.	30.005	Cholest-4-EN-3-One,26-(Acetyloxy)	49,018,372.00	Ant obesity effect
7.	30.935	Stigmast-4-EN-3-One	47,249,468.00	Lowering of blood glucose levels

Conclusion

In this present study, oil was extracted from dried papaya seeds using solvent extraction method. Oil produced was analysed for its physico-chemical properties & proximate analysis it was concluded that: Yield of papaya seed oil was 25.6%.Acid value for papaya seed oil ranged from 2.53±0.002 to 2.80±0.08 mg KOH/g which comparable to standard. Saponification value for papaya seed oil ranged from 95.32±0.01 to 97.01±0.02 mg KOH/g, those were nearly comparable to standard. Iodine value for papaya seed oil ranged from 63.1±0.01 to 65.2±0.01 which comparable to standard. Peroxide value for papaya seed oil ranged from 5.27±0.01 t0 5.37±0.13 m Eq/Kg which nearly comparable to standard. The concentration of phenols in papaya seed oil ranged from 112±0.25 to 114±0.55 mg/100g which comparable to standard. Tocopherol content of papaya seed oil (51.77µg/g) was low comparable with other common oils such as soybean, maize and sunflower oil. Unsaponifiable matter percentage was similar to that reported for conventional vegetable oil pH value of papaya seed oil was low indicating slightly acidic nature. Absence of oxidative spoilage in papaya seed oil. Specific gravity of papaya seed oil was comparable with that reported for linseed oil and other seed oils that have been documented as good feed stock for bio-diesel. Presence of low moisture in papaya seed oil. Presence of high content of protein and crude fibre in dried papaya seed powder. Papaya seed oil used in this study contains various phytochemicals like squalene, stigmasterol, sitosterol, palmitic acid and linoleic acid.

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