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A rapid turbidity procedure for estimation of oil content in copra, fresh coconut endosperm and allied tissue materials

The need for a fast and reliable method of determination of oil content in copra capable of dealing with numerous samples often presented during nutritional and genetical investigations on coconut has recently led workers to adopt cold percolation method (CPM) developed by Kartha and Sethi[1] as standard technique.[2] This note describes a rapid turbidity procedure (TP) for estimation of fat or oil in different tissue materials of coconut in their dry and even fresh forms.

Experimental

Principle : Turbidity of the emulsion formed by addition of water to fats or oils in a mixture of CCl_3-COOH in $(CH_3)_2$ C=O can be compared and measured quantitatively.[3]

Instrument: Spectronics—20 set at 530 nm[4] was used to take transmittance (T) readings on turbidity to convert them to extinction (E) values[5] correct to 2 decimals (—log T=E).

Extractant : 5% m/V solution of CCl_3-COOH-$(CH_3)_2$ C=O (Analar grade).

Standard coconut oil solution 1mg/ml : 1% m/V solution of pure coconut oil-extractant was diluted to 10% V/V solution using extractant.

Calibration graph : Turbidity of emulsion developed (made up and shaken for homogeneity) for a range of 1-20 mg of oil, by addition of glass-distilled water to 1-20 ml standard solution taken in 100 ml calibrated flasks were measured (as mentioned above) to construct a graph on the basis of linear equation derived to show Y (oil concentration) = $-0.3549+31.97 \times$ (extinction) (Fig. 1). R^2 value was found to be 96.55%.

Procedure : *Oil content in copra* : Soaked for about 5 minutes in 5 ml extractant exactly 20 mg of oven-dry sliced copra sample (taken in a 10 ml beaker) and gently

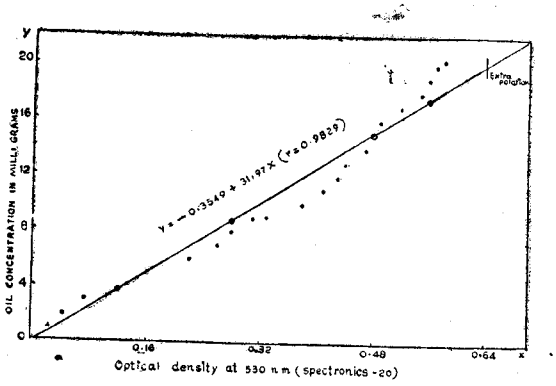

Fig. 1

squeezed the sample with a clean and dry glass rod to release oil. The clear extract was then transferred (without errors) to 100 ml calibrated flask. This step was completed with further 10 ml (5+5) portion of extractant, making the sample and beaker free of any traces of oil. The extract was emulsified, made up to volume, mixed thorougly and read for oil content as mentioned, in the calibration procedure. The read value × 5, would be the estimated percentage oil content of the sample on dry weight basis.

To try a more simplified procedure the sample was introduced directly into a 100 ml graduated measuring cylinder with stopper and adding the extractant (15 ml), sqeezed with a sufficiently long clean glass rod to release oil. The content as such was emulsified and read for oil content as said above. The interference of the partly disintegrated tissue particles of the sample would be negligible.

Oil content in fresh coconut endosperm : The procedure was similar to that described above, but to account for moisture content exactly 50 mg of sample was used and on moisture-free basis oil content was calculated (on evaluation of moisture content separately).

In case of difficulty of weighing exactly

20 or 50 mg of the samples, slightly more or less that much quantity of them can be finely sliced, weighed exactly and used for oil determination. Besides the graph, linear equation can also be used to calculate oil content when the extinction value of the turbidity is found.

Results and Discussion

There seems to be a reasonable comparison between the results obtained by the techniques followed and therefore, the turbidity procedure appears to be simple and reliable for routine work and capable of giving comparable results (Tables 1—5 and Fig. 1).

Fresh coconut endosperm or kernel when dried is called copra and it is rightly made from fully ripe nuts. Therefore, a direct estimation of oil content in the former is inferential of its oil content in the latter on the basis of arithmetic conversion to moisture-free form. Such an approach can have the advantage to skip-over, wherever imperative, certain time consuming preliminaries to process out fresh endosperm to copra for oil estimation. In the process of making copra in general and its samples in particular a notable difficulty observed is occurrence of rancidity in them. Rancidity would vitiate largely results on copra's oil content. For instance, from one and the same sample of fresh endosperm from a fully ripe nut, one piece cut out was allowed to undergo prebiodeterioration to render the copra made rancid and other was oven-dried direct in good condition to obtain non-rancid copra for tests. The rancid material gave abnormally high values (Table 3) and this instance may raise a point of interest to extend studies on the probable risk of reporting unrealistic values on oil content in case of testing copra deteriorated due to oil or oil bearing tissue spoilage. The technique is adequately simple to follow, precise and sensitive to quantify even minute quantities of fat or oil in test materials such as coconut embryo (Table 4), nut water, oil cake etc. for biochemical and related studies too. For example, a nut water sample (130 ml) from a ripe nut was extracted with petroleum ether and the extract was evaporated to dryness; by treating the residue with extractant and developing and measuring turbidity as mentioned above it was found to contain 13.7 mg of fat or oil/100 ml (of nut water). So also typical samples of coconut oil cake (oven-dried) were found to contain 10-11.8% of oil as determined by the simplified procedure described for oil content in copra.

Procedural precautions to be stressed are: (1) fresh extractant (prepared just before use) and glass-distilled water and meticulously cleaned (ungreasy) glass-ware are to be preferred for use, (2) slight heat persisting in the emulsion (developed during emulsifying extract) has to be brought down to room temperature (28°C) before it is made up to mark and read for turbidity, (3) as found

TABLE 1: Oil content of copra: values obtained by cold percolation method (CPM) and turbidity procedure (TP) compared.

Sample		% oil content by		remarks
		CPM	TP	
KID	5 (1)	65.0	64.0	
,,	33 (1)	69.0	67.0	
,,	103 (1)	68.0	68.5	
,,	115 (1)	70.0	70.0	The difference between
,,	99 (1)	72.0	70.0	CPM and TP is not
,,	43 (1)	70.0	68.5	significant and it lies
,,	108 (1)	70.0	68.5	between ± 5.3% at 5%
,,	101 (1)	60.0	63.5	interval.
,,	20 (1)	67.0	73.0	

TABLE 2: Oil content of coconut endosperm (kernal) on fresh and dry weight basis.

fresh weight mg.	oil content mg.	% moisture content	% oil content on the basis of fresh weigh	% oil content on the basis of oven dry weight	
				by calculation	done separately (20 mg of oven dry sample)
50.0	15.7	50.6	31.4	63.6	64.0
50.0	15.7	50.6	31.4	63.6	64.0
50.0	15.9	50.6	31.8	64.4	64.0

TABLE 3: Oil content of rancid-free and rancid copra

factors*	copra sample	
	rancid-free	made rancid
oil content (%) †	69.0	88.0
acid value (No.)	0.42	8.70
F. F. A. (%) (as lauric acid)	0.15	3.10

* value of each factor is the mean of three estimations.
† as estimated by turbidity procedure.

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2 Manual of Technical procedure for Coconut and Arecanut, C. P. C. R. I., Kasaragod, 1971.

TABLE 4: Oil content of coconut embryo

sample	weight of embryo (mg)		% moisture content	% oil content on the basis of	
	fresh	dry		fresh weight	dry weight
1	123.4	27.6	77.634	3.404	15.217
2	101.0	26.0	74.254	2.376	9.230

TABLE 5: Precision of the turbidity procedure

sample	replication	weight of sample (W)* mg.	transmittance (T) T%	extinction (E)-log T	oil content (%) †	
					Individual value	mean
N. W. 48-9	3	28.00	26.5	0.5768	64.59	64.20
		29.00	26.0	0.5850	63.27	
		26.00	2.90	0.5376	64.73	
N. W. 48-7	4	30.00	26.5	0.5768	60.28	60.22
		32.00	24.5	0.6108	59.91	
		28.00	36.0	0.4437	60.31	
		27.00	30.0	0.5227	60.57	
N. W. 48-10	4	35.00	21.0	0.6778**	60.90**	61.11
		33.65	22.0	0.6576**	61.42**	
		32.00	24.0	0.6198	60.81	
		25.95	31.0	0.5086	61.29	

† calculation of % of oil content as done by applying E values in the linear equation.

$$Y \text{ (oil concentration)} = -0.3549 + 31.97 \times \text{(extinction)}$$

$$\therefore \%\text{ of oil content} = 31.97 \times E - 0.3549 \times \frac{100}{W^*}$$

** calculated from extrapolated extinction and oil concentration values as per the equation given above.

for the present, the safer experimental time-limit to measure the turbidity developed would be between 10-45 minutes.

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