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A New Pollen Preparation Technique Using Technical Chemicals

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Abstract - The widespread use of pollen analysis in various fields in Indonesia is hampered mainly by the application of pro-analyst chemicals in standard pollen preparation. To solve this problem, a new pollen preparation technique using technical chemicals was developed. The results of comparison between this new technique with the standard technique used before showed that the pollen-spore yields, the quality of pollen slides, and the slide visibility resulted from both techniques is comparable. Furthermore, the new technique reduced the chemical cost of the standard technique by IDR. 87,125.00 per sample.

Keywords: Palynology, preparation technique, technical chemicals, safer and cheaper procedure

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Introduction

Pollen study in Indonesia was pioneered by Polak (1933) in the Lakbok-Ciamis swamp area, West Java. Subsequently, pollen study in Indonesia and the surrounding area continued, albeit with a very low frequency. In the 1960s-1970s, several palynologists published several papers including van Veen (1958), Muller (1961, 1964, 1966, 1968, 1972), Hopping (1967), Germeraad *et al*. (1968), Powell (1970), Walker (1970), Flenley (1972, 1973), Sowmi (1973), Anderson and Muller (1975).

In the decade of the 70s, several Indonesian palynologists began to cultivate this discipline. However, the development and utilization of pollen study in Indonesia is still limited. One of the reasons is the high cost of preparation and the difficulty in obtaining the chemicals used in today's standard preparations. The high cost of preparation is due to the variety of chemicals used in standard preparation techniques. The standard uses pro-analyst chemicals (p.a) which are quite expensive. The high cost of preparation not only hinders the development of palynology at universities, but also disturb the widespread use of pollen analysis in the world of oil and coal exploration, archaeology, agricultural industries such as the honey industry, environmental health, and forensics. The use of pro-analyst chemicals in pollen preparations also requires higher safety standards due to the high concentration of pro-analyst chemicals. This condition also himpeds the widespread use of pollen analysis, especially in universities in Indonesia.

In addition, certain types of chemicals have become more difficult to obtain in recent times for crime prevention reasons. The difficulty of obtaining acetic anhydride is an example. This acid

has been abused by criminals for extracting illegal drugs namely ecstasy. As a result, the police have tightened regulations on the sale of acetic anhydride. The purchasing procedure becomes more convoluted. The purchase quantity is also limited.

To accelerate the development of palynology, the difficulties associated with this pollen preparation need to be addressed immediately. Preparation techniques that are much cheaper and affordable especially for students and other users of pollen analysis need to be developed. This paper discusses several modifications to the standard pollen preparation technique. This modification is expected to result in a cheaper and safer preparation technique.

Methods and Materials

Methods

The development of cheaper and safer preparation techniques was carried out empirically. For this purpose, modifications to the preparation technique were designed and piloted. The trial results will be compared with the results of the standard preparation techniques used at the Palynology Laboratory, Center for Geological Survey (PSG) (Figure 1).

The standard pollen preparation technique uses pro-analyst chemicals (p.a). In the modified preparation technique, pro-analyst chemicals such as hydrochloric acid (HCl), fluoride origin (HF), sulfuric acid (H_2SO_4), zinc chloride ($ZnCl_2$), and potassium hydroxide (KOH) are replaced by technical chemicals, which is cheaper and safer because of its lower concentration. The acetic anhydride $\{(CH_3CO)_2O\}$ and glacial acetic acid ($C_2H_4O_2$) used were of the p.a type. The concentration of pro-analyst and technical chemicals were 99.95% and 37-40%, respectively. Hypothetically substituting pro-analyst chemicals with technical chemicals would not affect the preparation results. This is because the chemicals in pollen preparations are used to remove minerals, not to determine the concentration of these minerals. Any other physical or chemical separation method

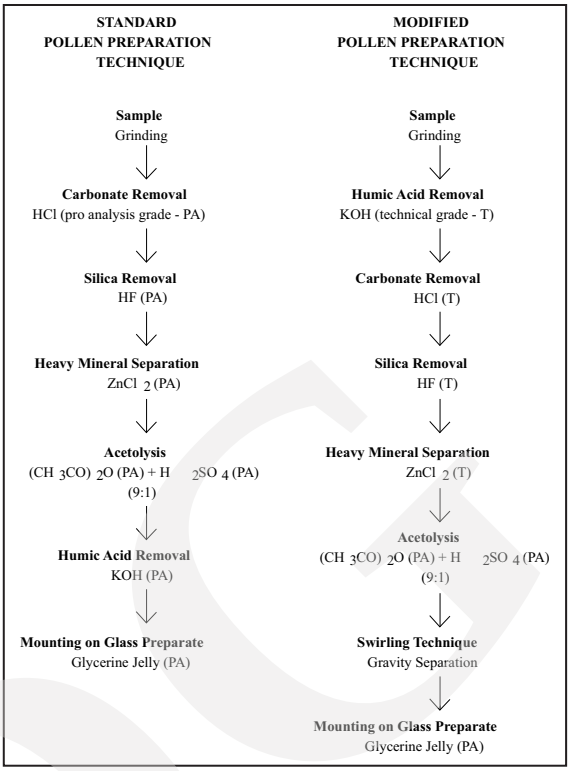

Figure 1. Comparison of pollen preparation steps between standard preparation techniques (Erdtman, 1972; Faegri and Iversen, 1975) used at the Palynology Laboratory, Center for Geological Survey (PSG) and modified preparation techniques.

can be used for the preparation of pollen and other microfossils, regardless their effectiveness. Even cleaning materials for everyday households such as various types of detergents and floor cleaners which generally contain oxidative/corrosive chemicals can also be used for the preparation of pollen and other microfossils.

The sequence of preparation steps is modified to make technical chemicals more effective, while also reducing the risk of accidents. KOH treatment is carried out at the beginning of process, because usually sediment samples rich in organic matter, especially peat, often containing bitumen. Bitumen causes the sample to become sticky and binds silica which is usually the main mineral in sediments. The KOH treatment will clean this bitumen thereby separating the mineral grains. The removal of carbonates (using HCl) and silica (using HF) in the next step becomes more effective for separating pollen and spores from their binding minerals. The separation of pollen grains and spores from

minerals will facilitate the separation of heavy minerals by gravity method using $ZnCl_2$. Filtration with a 105-micron sieve was carried out before the acetolysis treatment. The swirling technique that is also applied to separate fine mineral impurities, was performed after acetolysis.

The effectiveness of the modified pollen preparation technique was evaluated qualitatively based on three indicators *i.e*., the amount of pollen grain-spore yield, the quality of the preparations and the cost of the chemicals used. The three indicators of this modified pollen preparation technique were compared with the standard pollen preparation technique that has been used at the Palynology Laboratory, Center for Geological Survey.

The yield of pollen-spore grains was evaluated based on the results of counting all pollen-spores present in one pollen slide of each technique. Since the preparation was not carried out quantitatively by calculating the concentration of pollen-spore per unit volume or sample weight, the evaluation of the effectiveness of the preparation technique was carried out based on the deviation of the proportion of pollen-spore produced by the two preparation techniques. The preparation technique is considered to be effective, if the value of the proportion of pollen and spores is the same, comparable or has a small deviation of the proportion.

The quality of the preparations was evaluated qualitatively based on the presence of non-pollen concentration in the slide. To get better photos, observations were made under the Olympus BX53 with magnifications of 100x, 200x, 400x and 1000x. The population density of pollen, spores, organic matter, and minerals in one slide was used as an indicator of the quality of the slide. This density value is obtained by comparing with a chart to estimate the volume percent of minerals. Chemical costs were calculated based on the volume of chemical required for one sample. The cost per one sample is calculated based on the purchase price.

Materials and Studied Sites

The effectiveness of the modified palynological preparation technique was tested by carrying out the technique on five samples of organic clayey sediments. These sediments were collected from a coastal swamp deposit that is predicted to have been deposited at the end of Holocene, in Cilacap, Central Java (Figure 2). This sample was taken using a hand drill. Based on Simanjuntak and Surono, 1992), the drill points are located in a coastal alluvial deposits. This coastal deposit occupying the southern part of the studied area comprises fine-medium and very loose sand which shows the impression of layering (Herman, 2005). Those alluvial deposits overly Miocene and Pliocene formations that fill the Banyumas Basin, *i.e*. Halang, Kalipucang, and Gabon Formations. The Halang Formation is composed of alternating sandstone, claystone, marl, and tuff with breccia inserts as subsea fan deposits. The Breccia Member of this formation is made up of andesite, basalt, and limestone components, the base mass of coarse tuffaceous sandstone, sandstone intercalations and basalt lava. The Kalipucang Formation consists of reef limestones, locally clastic limestones, and at the bottom bituminous shale. The Gabon Formation comprises breccias with andesite components, with a bottom mass of tuff and coarse sandstone, localized lapilli tuff, lava, and lahar deposits, generally altered.

Results

Pollen and Spore Grains Yield

The yield of pollen and spore grains and the value of the pollen-spore proportion of the five analyzed samples are shown in Table 1. The total pollen grains and spores in one slide is quite abundant. The standard pollen preparation technique of five samples yielded total pollen and spore grains per slide at a range of 370-577 and 11-154, respectively. Meanwhile, the modified pollen preparation technique of five samples resulted in total pollen and spore grains per slide at a range of 263 - 559 and 20 -145, respectively. The deviation of pollen-spore proportions for both techniques ranged from 0 - 6%. The deviation

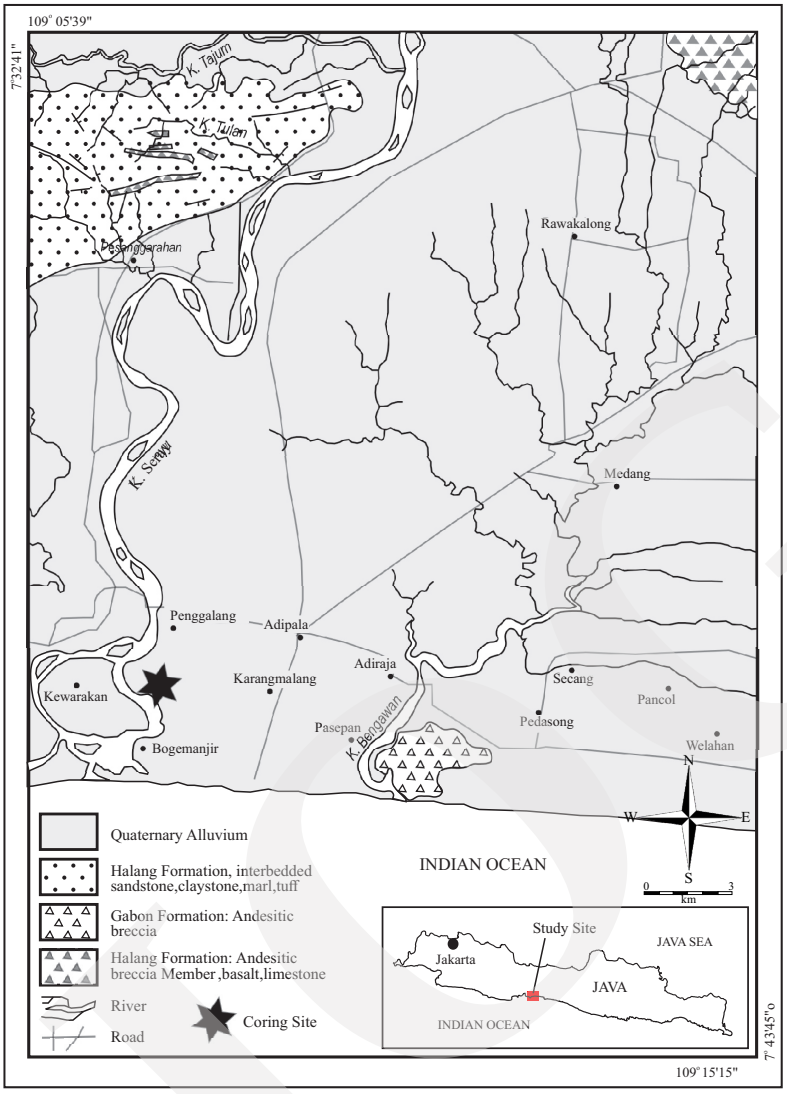

Figure 2. Geological map of the research area. The drill site is on the southern coastal plain of Cilacap which is composed at the top by coastal alluvium deposit. This alluvium covers the Miocene-Pliocene age formations that fill the Banyumas Basin, *i.e.* Halang , Kalipucang, and Gabon Formations.

values of pollen proportion obtained using the Traverse's diagram (Traverse, 1988) (Figure 3). It shows a comparable range between standard and modified preparation techniques (Table 1). Overlapping of the range of pollen proportion of the two techniques occurs in CLP-1, CLP-3, CLP-4 and CLP-5. In the CLP-2 sample, there is no overlapping values of pollen proportion of those two techniques.

Slide Quality

A comparison of photomicrograph slides between the standard and modified pollen preparation techniques are shown in Figures 4, 5, 6, 7, and 8. Percentage of the residue in the pollen slide is estimated using mineral percentage estimation chart (Terry and Chiligar, 1955). The results of the estimated residual percentage are shown in Table 2. Contrast differences of visual cleanness of slides of the two techniques is presented in Figure 9.

Based on the percentage estimation of on-slide residue of the five samples (Table 2), the modified pollen preparation technique shows a comparable quality with the standard pollen preparation technique. The visibility of the pollen-spores was also comparable, showing that the pollen-spore specimens were clean without impurities. However,

Table 1. Comparison of the Total Yield, Pollen-Spore Proportion, and the Range of Pollen Proportion Values of Standard and Modified Pollen Preparation Techniques

SAMPLE	DEPTH (cm)	POLLEN				SPORES				TOTAL POLLEN-SPORES (grain)		RANGE OF PROPORTION (%)		DEVIATION OF POLLEN-SPORE PROPORTION (%)
		YIELD (grain)		PROPORTION (%)		YIELD (grain)		PROPORTION (%)				POLEN		
		Standard	Modified	Standard	Modified	Standard	Modified	Standard	Modified	Standard	Modified	Standard	Modified	
CLP-1	130-132	384	368	71	71	154	145	29	29	538	513	68,87 – 73,13	68,73 – 73,27	0
CLP-2	140-142	370	263	81	75	85	87	19	25	455	350	78,97 – 83,03	72,60 – 77,40	6
CLP-3	150-152	394	401	97	95	11	20	3	5	405	421	95,30 – 98,7	93,29 – 96,71	2
CLP-4	160-152	387	376	94	94	23	23	6	6	410	396	92,21 – 95,79	92,21 – 95,79	0
CLP-5	170-172	577	559	85	86	100	90	15	14	677	649	83,47 – 86,53	84,45 – 87,55	1

at close-up appearances the modified technique seems to produce more cleanness of slide than the standard technique as shown in Figure 9.

Chemical Cost

Chemical cost was calculated per volumetric usage for one sediment sample. The calculation was based on the price list for the procurement of chemicals in 2021 at the Center for Geological Survey, Geological Agency.

The total cost of chemicals for the standard pollen preparation technique was IDR 123.210,00 per sample. The modified pollen preparation technique required a chemical cost of IDR. 36.085,00. Sulfuric acid (H_2SO_4), acetic anhydride ($(CH_3CO)_2O$), acetic acid ($C_2H_4O_2$) and glycerin jelly in both techniques used pro-analyst materials.

Duration of Preparation

Both preparation methods consist of the same steps (although in different order) except the swirling technique. Evaporating dish and pipette for the swirling technique step were used. The sample residue was placed in the evaporating dish and diluted using aquadest. The dish was rotated slowly by hand to separate the fine-sized floating organic material from the coarser sample fragments that settle to the bottom of the dish. Floating fine organic material was carefully sucked up using a pipette and collected in another dish before centrifuged. This step was repeated for several times until no more floating fine organic materials from the residue. The collected fine organic material was then wet sieved using 5 µm aperture filter cloth before another centrifuging.

Such swirling technique step makes the modified preparation method require a longer preparation time than the standard one. The time duration required for the swirling technique step depends on the concentration of fine grains and organic materials that make up the sample. The higher the concentration of fine grains and organic materials, the longer the duration of swirling technique step. For a sample that produce a concentrated residue such as high bituminous samples, the swirling tech-

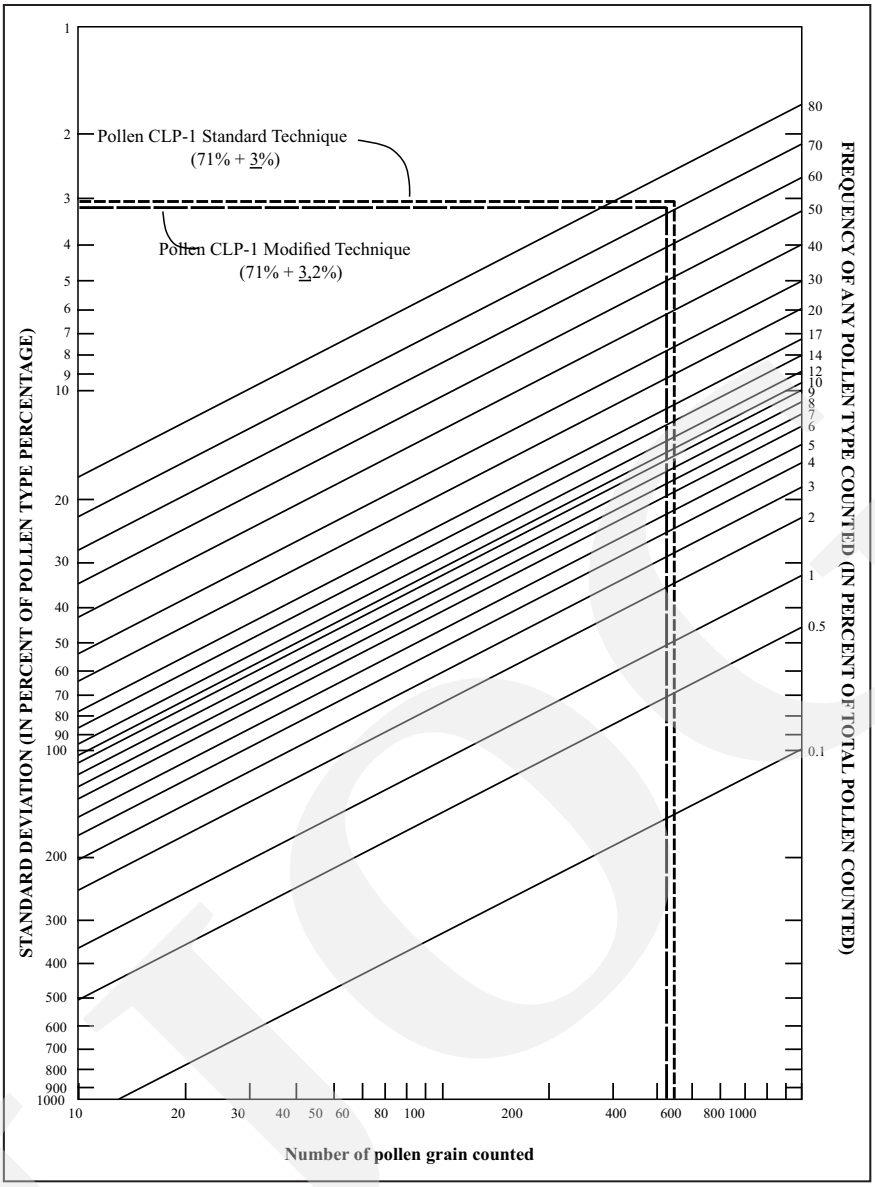

Figure 3. Traverse (1988) diagram, used to determine the deviation based on the proportion of pollen-spores, and total pollen-spore yields. Proportion of pollen in the CLP-1 was used as an example. The range of pollen proportions for CLP-1 using standard and modified preparation techniques, each gave results in almost the same range of 68.87 - 73.13% and 68.73 - 73.27% respectively.

nique may be repeated for 6 times and take up to 60 minutes. Consequently, the swirling technique step may add up to 60 minutes longer of duration of pollen preparation per sample.

Discussion

Effectivity

The pollen slides produced by the modified preparation technique had a quality comparable to that of the standard preparation technique in terms of the yield of pollen-spores. The modified technique even shows better visibility in term of cleanness compared to the standard technique, most probably due to application of the swirling technique step. The swirling technique followed by very-fine grains sieving, may be capable in removing more non-pollen materials. This leads to a cleaner residue and a better visibility of pollen in slide. However, several notes need to be given to this comparison method.

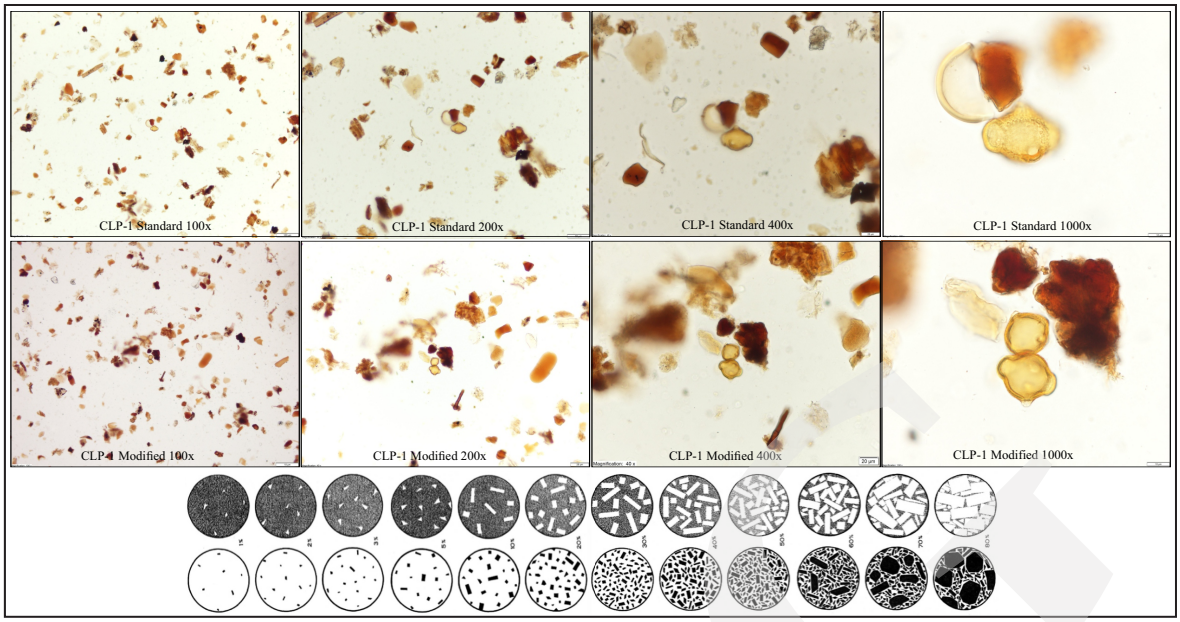

Figure 4. Photomicrographs of a qualitative comparison of visibility between the results of the standard pollen preparation technique (lower row) and the modified technique for CLP-1 sample (upper row). Circles are percentage comparator.

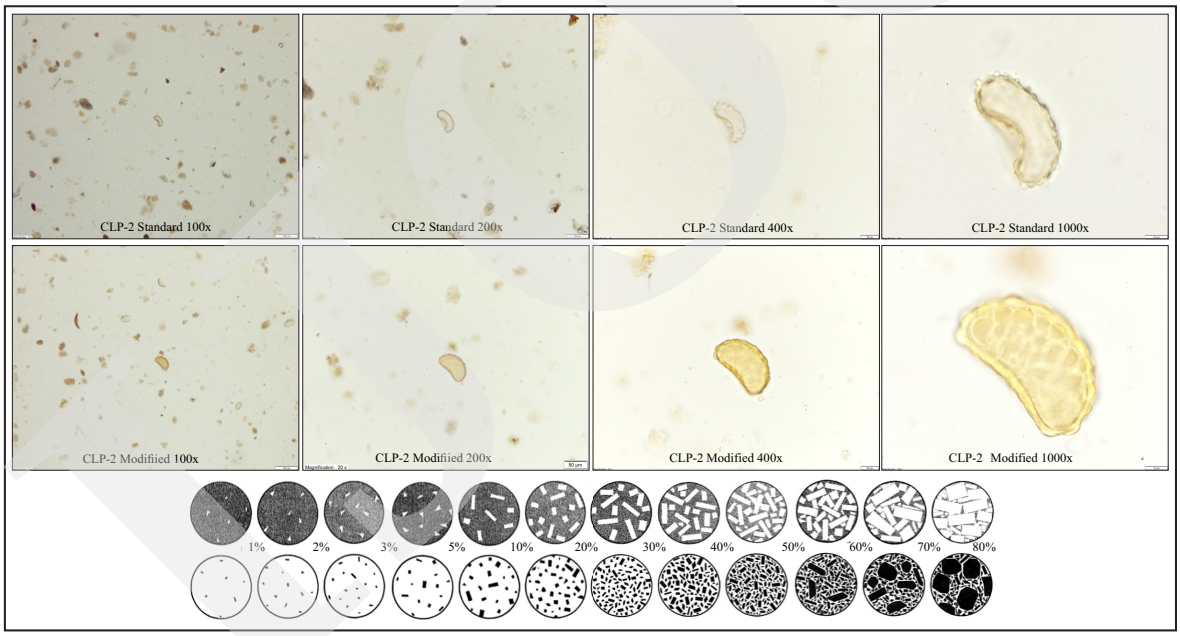

Figure 5. Photomicrographs of a qualitative comparison of visibility between the results of the standard pollen preparation technique (lower row) and the modified technique for CLP-2 sample (upper row). Circles are percentage comparator.

First, this comparison method is a qualitative one. The qualitative comparison (which are merely based on the similarity/comparability of pollen-spore proportions and/or proportion of vegetation groups and/or proportion of pollen and spore types) would highly depends on the counts of pollen-spores. The more counts, the more reliable the comparison results. Second, the comparability of pollen and spore proportion was merely assessed based on the magnitude of overlapping of the proportion after considering the deviation. The greater the overlapping, the more comparable the proportions.

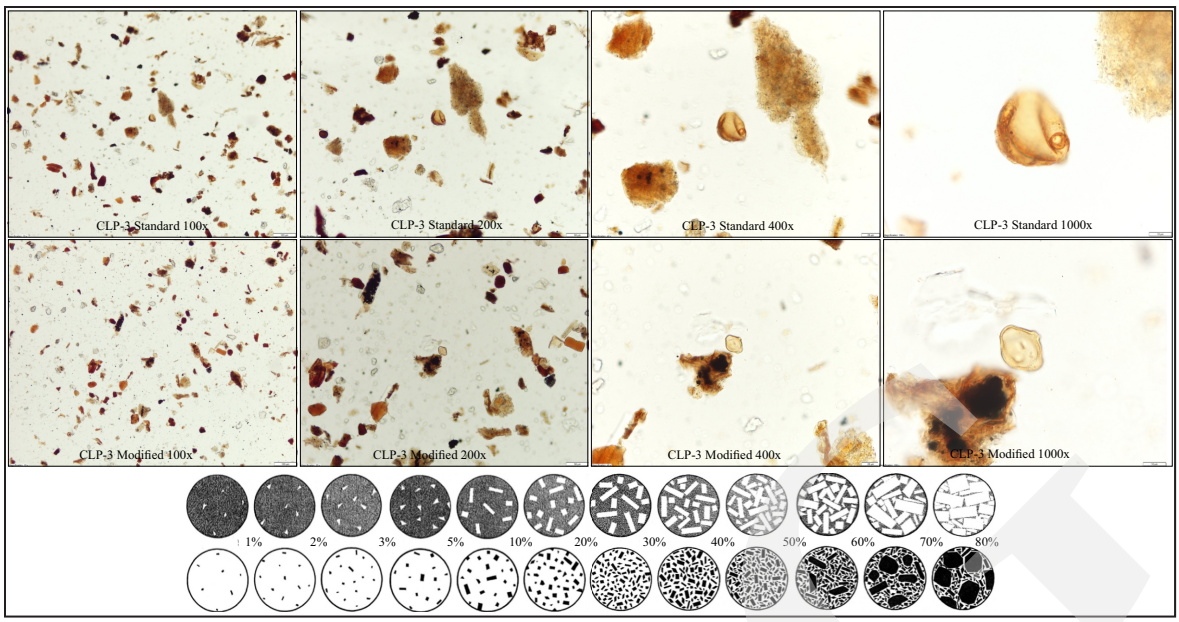

Figure 6. Photomicrographs of a qualitative comparison of visibility between the results of the standard pollen preparation technique (lower row) and the modified technique for CLP-3 sample (upper row). Circles are percentage comparator.

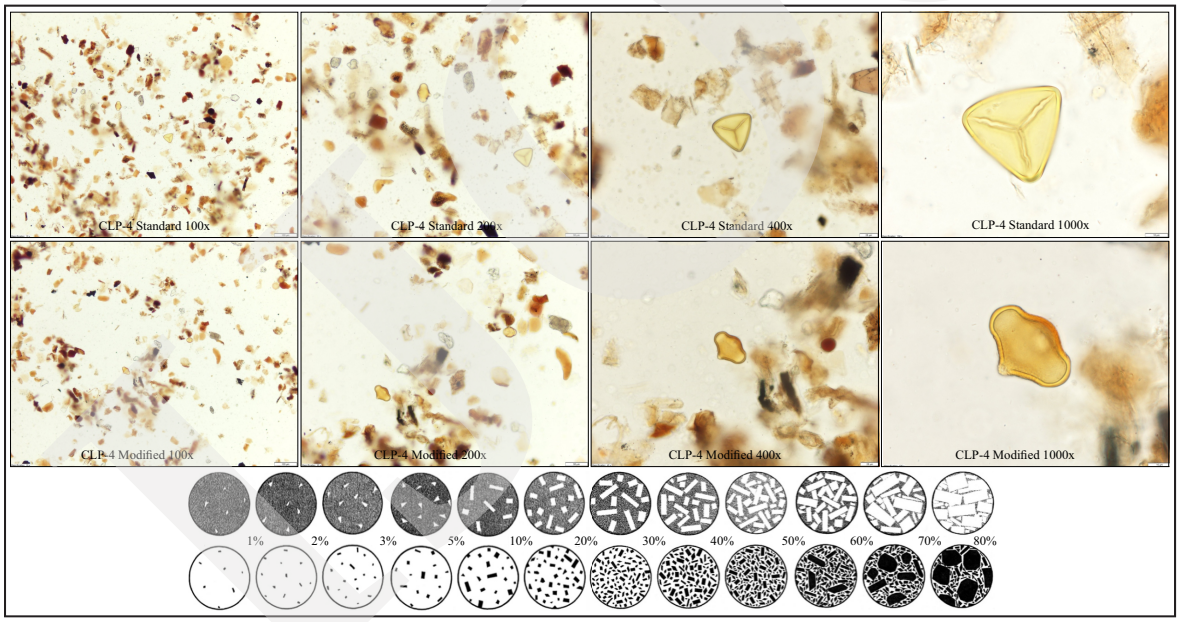

Figure 7. Photomicrographs of a qualitative comparison of visibility between the results of the standard pollen preparation technique (lower row) and the modified technique for CLP-4 sample(upper row). Circles are percentage comparator.

Efficiency

The modified pollen preparation technique evidently to be more efficient in a chemical budget than the standard preparation technique. This efficiency will increase, if the sample volume are reduced, leading to a decrease the chemical cost by half.

Laboratory Work Safety

The modified pollen preparation technique uses several chemicals with low concentrations. This reduces the risk of work accidents as well as the risk of contamination to the environment due to chemical waste disposal. However, standard chemical laboratory facilities are prerequisite for

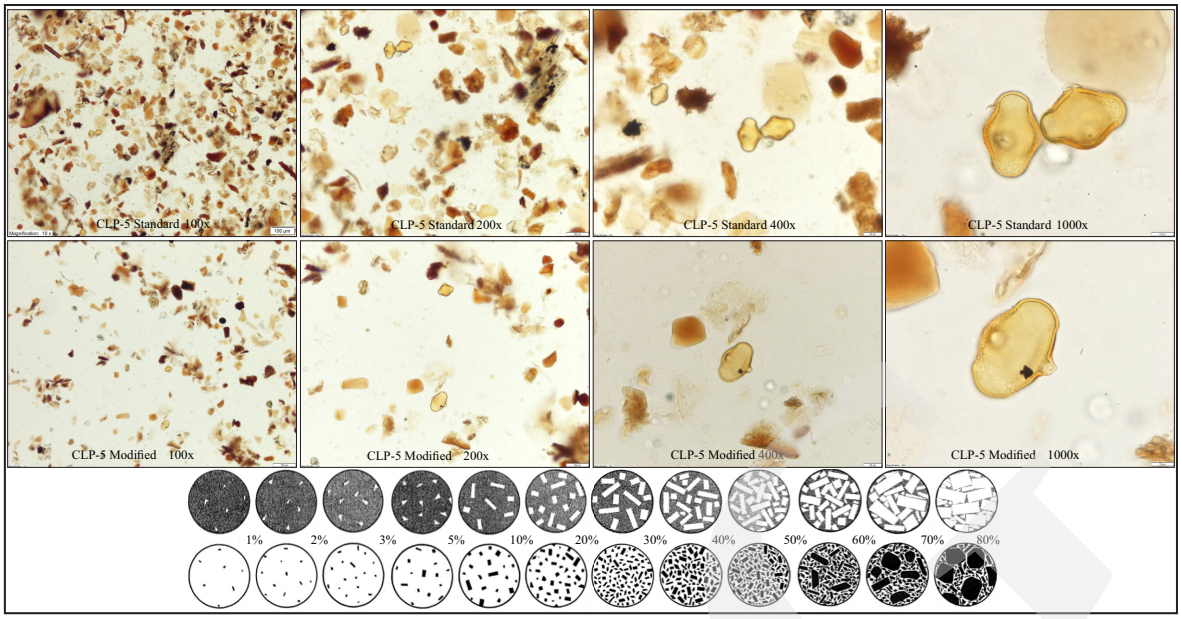

Figure 8. Photomicrographs of a qualitative comparison of visibility between the results of the standard pollen preparation technique (lower row) and the modified technique for CLP-5 sample (upper row). Circles are percentage comparator.

Table 2. Comparison of percentage estimates of the residue on slides under a microscope at 100x, 200x and 400x magnification. The visibility of the pollen-spores was determined based on the appearance of the pollen-spores at 1000x magnification.

SAMPLE	DEPTH (cm)	PERCENTAGE ESTIMATES OF RESIDU (%)						VISIBILITY OF POLLEN-SPORES	
		Standard			Modified			Standard	Modified
		100x	200x	400x	100x	200x	400x		
CLP-1	130-132	10-20	10-20	10-20	10-20	10-20	10-20	clear	clear
CLP-2	140-142	3-5	2-3	1-2	3-5	2-3	1-2	clear	clear
CLP-3	150-152	5-10	5-10	5-10	5-10	5-10	5-10	clear	clear
CLP-4	160-152	20-30	20-30	20	20-30	20-30	20	clear	clear
CLP-5	170-172	30-40	30-40	30-40	10-20	10-20	10-20	clear	clear

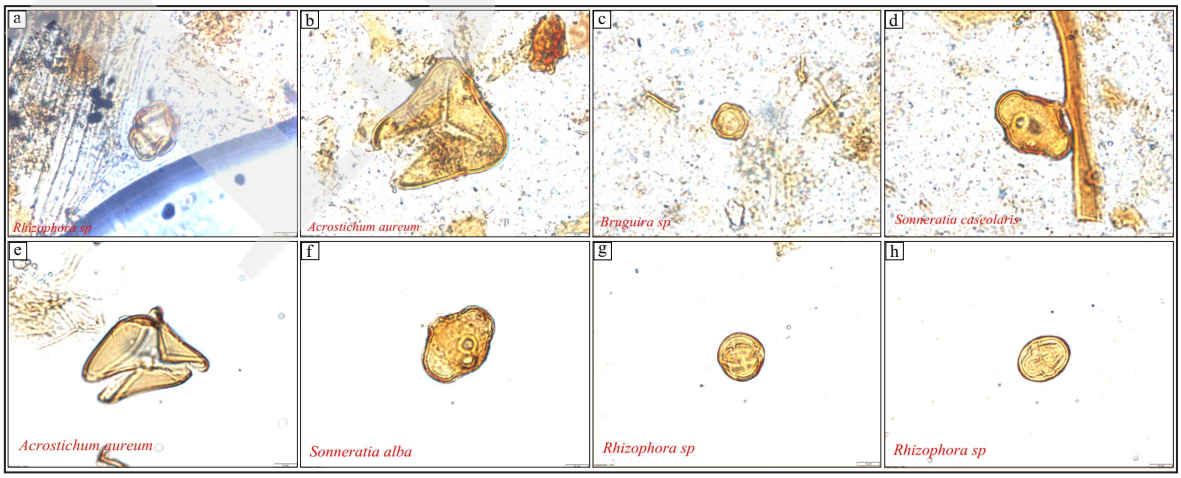

Figure 9. Photomicrographs of a comparison of visual cleanness of slides between the standard pollen preparation technique (a-d) and the modified technique (e-h) at the same magnification (the scale at right-bottom corner is 10 µm) for CLP-1 sample of several mangrove pollens. Slide of the standard technique shows more impurities of fine grains. Application of swirling technique removed impurities of fine grains lead to cleaner slide as shown in figures e-h.

Table 3. Comparison of Total Cost of Chemicals between Standard and Modified Pollen Preparation Techniques

	CHEMICAL	UNIT (lt/kg)	UNIT PRICE (IDR thousand)		PRICE PER SAMPLE (IDR thousand)	
			p.a	Tec.	standard	modified
1	Fluoric Acid (HF)	1 lt	942	30	23.55 p.a	0.75
2	Chloric Acid (HCl)	1 lt	490	35	12.25 p.a	0.875
3	Sulfuric Acid (H_2SO_4)	1 lt	273		2.73 p.a	2.73 p.a
4	Zink Chloride ($ZnCl_2$)	1 lt	1,842	65	46.05 p.a	1.625
5	Potassium Hydroxide (KOH)	1 kg	391	50	9.775 p.a	1.25
6	Acetic Anhydride ($(CH_3CO)_2O$)	1 lt	2,280		20.52 p.a	20.52 p.a
7	Acetic Acid ($C_2H_4O_2$)	1 lt	613		15.325 p.a	15.325 p.a
8	Glycerine Jelly	1 lt	400		0.01 p.a	0.01 p.a
	Total cost				123.210	36.085

Figure 10. Swirling technique using evaporating dish and pipette applied in the modified preparation technique (left) prior to sieving using 5 µm filter cloth (right).

this technique to be applied, so that they cannot be widely applied, especially in universities that are not equipped with adequate chemistry laboratories.

Conclusions

The development of pollen preparation techniques has been carried out by replacing several pro-analyst chemicals with technical chemicals and changing the treatment sequence from the standard pollen preparation technique (Erdtman, 1972) used in the Center for Geological Survey, Geological Agency. Evaluation of the results of the two techniques is as follows:

1. The replacement of pro-analyst with technical chemicals improves work safety in the palynology laboratory.
2. The modified pollen preparation technique can produce pollen slide with comparable quality to Erdtman's (1972) standard pollen preparation technique both in terms of pollen and spores yield and slide visibility. The application of swirling technique step in the modified technique seems to produce better quality of slide cleanness.
3. Cost of chemicals for pollen preparation of a sample using standard preparation techniques in the Palynology Laboratory of Center is IDR. 123.210,00. The replacement of p.a. chemicals of HF, HCl, KOH, $ZnCl_2$ with technical chemicals save the cost of chemicals by IDR. 87.125,00.

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