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Chemical Characteristics and Essential Nutrients of Agroindustrial Effluents in Malaysia

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Abstract

Three major agroindustrial effluents, namely, palm oil mill effluent (POME), latex concentrate rubber effuent (LCRE) and standard Malaysian rubber effluent (SMRE), were analyzed for chemical characteristics and biochemical properties and their essential nutrients evaluated. All the three raw effluents were highly acidic and did not contain dissolved oxygen. The raw POME contained significantly ($p<0.05$) higher chemical oxygen demand (COD) than raw LCRE followed by SMRE, indicating a high load of organic nutrients in these effluents. Crude protein in raw SMRE and LCRE was significantly higher than that in raw POME. But crude lipids, carbohydrate and ash content of raw POME were found to differ significantly ($p<0.05$) from those of raw LCRE and SMRE. All the 11 essential amino acids were available in raw LCRE, SMRE and POME. The POME contained higher amounts of unsaturated fatty acids such as oleic acid (18: 1n-9), linoleic acid (18: 2n-6), linolenic acid (18: 3n-3) and arachidonic acid (20: 2n-6) than the other effluents. However, very little amounts of eicosapentaenoic acid (20:5n-3) were available in POME only. All together, 27 essential minerals were detected by scanning electron microscope and 22 essential minerals were quantified by Inductively Coupled Plasma Mass Spectrophotometer, among which, Fe, Zn, P, Mg, Mn, Ca, K, Cr, Co, Cu, S, Se, Al, Sn, B, Mo, V, Li and Pb were found to be significantly ($p<0.01$) higher in raw POME than in the other effluents.

Introduction

Malaysia's leading agro-industries include palm oil and rubber processing and sago manufacturing (Phang 1987; Phang 1990; Isa 1993; Yusoff and Chan 1997). Factories engaged in these industries discharged huge amounts of waste effluents, which are a major source of aquatic pollution in Malaysia (Phang and Ong 1988; Anton 1992). Palm oil mills discharge more than 15 million metric ton of raw palm oil mill effluent (POME) from almost 110 processing mills (Anton 1992 and Habib *et al.* 1997) while rubber mills discharge more than 36.5 million metric ton of raw rubber effluents, mainly standard Malaysian rubber effluent (SMRE) and latex concentrate rubber effluent (LCRE), from approximately 210 rubber processing mills (Isa 1993; Habib *et al.* 1997).

The discharge of LCRE, SMRE and POME create serious pollution in Malaysian waterways due to the high organic content and chemical oxygen demand of these effluents (Phang 1990; Isa 1993; Anton 1992; Habib *et al.* 1997; Yusoff *et al.* 1996; Yusoff and Chan 1997). On the other hand, all these effluents contain protein, carbohydrate, nitrogenous compounds, lipids, minerals and other essential nutrients (Okiy 1987; Phang 1990; Habib *et al.* 1997; Yusoff and Chan 1997). A case in point is POME, which has been used as poultry and cattle feed (Vadiveloo 1987) because it contains large amounts of nutrients such as crude protein and lipids, amino acids and fatty acids. POME's gross energy content is about 454 Kcal/100g , which is comparable to that of maize (500 Kcal/100g) (Okiy 1987). These nutritional aspects makes effluents potentially interesting in the culture of live fish foods and the preparation of fish feed.

However, little information is available regarding the nutritional aspects of these agroindustrial effluents. Therefore, the present work was undertaken to thoroughly investigate the nutritional values, including essential nutrients, namely, protein, lipids, carbohydrate, ash, total carotene, amino acids, fatty acids and minerals, of these effluents.

Materials and Methods

Raw LCRE from Atheston Estate rubber factory, Atheston, Negari Sembilan, raw SMRE from Kilang Getah MARDAC Serdad, Selangor, and raw POME from Golden Hope Mill, Carry Island, Selangor, Malaysia, were collected. The collected LCRE was screened through 250 mm net and 2% $CaCl_2$ was then added to it to accelerate precipitation of the colloidal particles. The upper portion was thrown away and the ppt. was centrifuged at 3000 rpm for five minutes. The supernatant was then thrown away and the ppt. was washed 4-5 times with deionized water through repeated centrifugation to remove $CaCl_2$. The residue was collected in plastic vial and kept at 0°C for two days and then at -80°C for two more days. The SMRE was screened and centrifuged and the ppt. was stored at 0°C for two days and then at -80°C for two more days. The POME was screened through 250 mm net and kept in 500-ml plastic bottle at 0°C for two days and then at -80°C for two more days. The frozen samples of LCRE, SMRE and POME were freeze-dried at -50°C under vacuum

pressure of 150 millitorr for three days and then kept at -80°C for chemical analyses.

The chemical properties of raw effluents such as pH, dissolved oxygen, chemical oxygen demand, total suspended solids, total solids, total nitrogen, ammoniacal nitrogen and ortho-phosphate were analyzed following the standard methods (Cleseri *et al.* 1989). Proximate composition and total carotene content of the freeze-dried effluents were done following the standard methods of Horwitz (1985). The amino acids of the samples were determined from the peaks that came up in HPLC 501 after injection of 10-ml prepared samples. The deproteinized effluent samples were hydrolized with 0.2% methanesulfonic acid containing 0.2% tryptamine (Millipore 1990).

The fatty acids of freeze-dried samples were analyzed from the peaks that came up in GC-14A after injection of 1.0 ml fatty acid methyl ester (FAME) prepared through the hydrolysis of the lipids of samples with 14% Boron trifluride with methanol. This follows the method of Folch *et al.* (1957) as modified by Benitez (1989), and Habib *et al.* (1997). Fatty acids were identified and quantified by Shimadzu GC-17A Mass Spectrophotometer (QP-5000) using a Omegawax 320 capillary column: 30 m x 0.32 mm ID, 0.25 mm film (Supelco). Minerals were detected by scanning electron microscope and then quantified by Inductively Coupled Plasma Mass Spectrophotometer after digestion of samples with 10 ml conc. HNO_3 with 1.0 ml H_2O_2 for 2 h. following Horwitz (1985). Sulphur was analyzed for S-column after the samples were burned at 670°C for two minutes using a CHNS Elemental Analyser (Perkin Elmer 1994).

Results and Discussion

The average chemical contents of raw LCRE, SMRE and POME are shown in Table 1. All the raw effluents were acidic and did not carry any dissolved oxygen. However, raw POME was found to have a significantly ($p< 0.05$) higher chemical oxygen demand (COD), an indication of a higher organic nutrient load in POME than in the other two effluents (Phang 1990; Isa 1993; Anton 1994). Again, POME contained significantly ($p<0.05$) higher amounts of total solids (TS), total suspended solids (TSS), total N and ortho-phosphate than raw LCRE and raw SMRE.

Table 1. Mean chemical contents ($mg.l^{-1}$ except pH) of raw latex concentrate rubber effluent (LCRE), standard Malaysian rubber effluent (SMRE) and palm oil mill effluent (POME).

Parameters	Raw LCRE	Raw SMRE	Raw POME
pH	4.45 ± 0.05^a	4.35 ± 0.03^a	3.91 ± 0.04^b
Dis. O_2	0	0	0
COD	7906.22 ± 12.88^b	3508.08 ± 8.66^c	83356.18 ± 212.52^a
TS	6862.35 ± 10.35^b	2860.35 ± 7.55^c	67932.12 ± 167.45^a
TSS	5945.22 ± 6.23^b	2550.15 ± 5.38^c	49233.57 ± 65.24^a
Total N	1205.15 ± 5.28^b	985.37 ± 3.66^c	1494.66 ± 10.46^a
NH_3-N	496.66 ± 2.44^a	296.15 ± 1.45^b	50.42 ± 1.28^c
PO_4-P	189.73 ± 1.68^b	125.08 ± 1.07^c	315.36 ± 1.55^a
C:N:P ratio	15.63:8.97:1.0	10.51:10.25:1.0	99.12:4.74:1.0

Means (± SE, n = 9) with different superscripts in each row indicate significant differences ($P < 0.05$, df. 23, 3).

Proximate composition analysis show that the crude lipids, carbohydrate and ash content of raw POME were significantly ($p<0.05$) higher than in raw LCRE and SMRE (Table 2). However, crude protein in raw LCRE (14.68±0.16) was found to be significantly ($p<0.05$) higher than that in raw SMRE (13.51± 0.18) and POME (9.07±0.15). This might be due to the use of ammoniacal nitrogen during transportation of raw rubber from garden to the mills (Phang 1987; Isa 1993). Total carotene content was also present in higher amounts in POME than in the other two effluents (Habib *et al.* 1997). The higher nutrient content of raw POME makes it a potential source of utilizable raw matter for animal feed as well as for the growth of aquatic microplants and organisms (Okiy 1987; Phang 1990; Yusoff *et al.* 1996).

However, all three effluents contained essential nutrients, viz., amino acids, fatty acids and minerals. All of the 11 essential amino acids and the other seven nonessential ones were present in raw LCRE, SMRE and POME (Table 3). The important amino acids, viz., phenylalanine, methionine, leucine and lysine were found to be significantly ($p<0.05$) higher in POME than in the other two effluents. Again, histidine and tyrosine in raw LCRE and SMRE were significantly ($p<0.05$) higher than those in raw POME. However, present results indicate that raw POME contained considerable amounts of amino acids (Okiy 1987). Raw LCRE and SMRE was found to contain very high amounts of isoleucine. The nonessential amino acids, such as aspartic acid, glutamic acid, serine, glycine and cystine were higher in raw POME than in the other two effluents. It was found that most of the unsaturated fatty acids, including oleic acid (18: 1n-9), linoleic acid (18: 2n-6), linolenic acid (18 3n-3), arachidonic acid (20: 4n-6) and eicosapentaenoic acid (20: 5n-3), were present in significant and higher amounts in raw POME than in raw LCRE and raw SMRE (Table 4). These findings again prove the suitability of raw POME as a good source of essential micronutrients (Okiy 1987).

The presence of the 27 essential minerals in raw LCRE, SMRE and POME were investigated by energy dispersive of X-ray analysis (EDAX) through scanning electron microscope (Table 5). Of the 27 minerals, 23 essential ones were detected and quantified by ICP Mass Spectrophotometer (Table 6). Minerals such as Fe, Zn, P, Mg, Mn, Ca, K, Cr, Co, Cu, Ni, S, Se, Si, Al, B, Mo, Sn, V, As, Li and Pb were significantly ($p<0.05$) higher in raw POME than in raw LCRE and SMRE (Phang 1987; Dolmat *et al.* 1987). Na proved

Table 2. Proximate composition (g/100g dry sample) of raw LCRE, SMRE and POME.

Proximate composition	Raw LCRE	Raw SMRE	Raw POME
Moisture	6.62 ± 0.03^a	6.69 ± 0.03^a	6.75 ± 0.03^a
Crude protein	14.68 ± 0.16^a	13.51 ± 0.18^b	9.07 ± 0.15^c
Crude lipid	6.15 ± 0.10^b	5.25 ± 0.09^c	13.21 ± 0.12^a
Carbohydrate	30.37 ± 0.12^b	30.15 ± 0.12^b	32.12 ± 0.12^a
Ash	18.08 ± 0.12^b	16.22 ± 0.11^c	20.55 ± 0.14^a
NFE	26.04 ± 0.10^b	28.13 ± 0.11^a	19.47 ± 0.10^c
Total carotene (mg/100g)	6.30 ± 0.05^b	5.95 ± 0.04^b	20.07 ± 0.11^a

Means (±SE, n =9) of withdifferent superscripts in each row indicate significant differences ($P < 0.05$, df. 23, 3).

Table 3. Amino acids (g/100g protein) of raw LCRE, SMRE and POME.

Amino acids	Raw LCRE	Raw SMRE	Raw POME
Aspartic acid	5.06 ± 0.11	6.49 ± 0.12	9.66 ± 0.19
Glutamic acid	6.87 ± 0.14	8.52 ± 0.18	10.88 ± 0.21
Serine	5.82 ± 0.12	5.35 ± 0.11	6.86 ± 0.15
Glycine	3.29 ± 0.08	3.62 ± 0.06	9.43 ± 0.17
Histidine*	2.48 ± 0.06^a	2.34 ± 0.05^a	1.43 ± 0.04^b
Arginine*	3.55 ± 0.09^b	4.08 ± 0.09^a	4.15 ± 0.10^a
Threonine*	7.88 ± 0.15^a	6.49 ± 0.12^b	2.58 ± 0.05^c
Alanine	8.71 ± 0.18	8.18 ± 0.16	7.70 ± 0.16
Proline	5.73 ± 0.13	6.25 ± 0.12	4.57 ± 0.10
Tyrosine*	2.79 ± 0.07^b	3.44 ± 0.09^a	3.26 ± 0.06^a
Phenylalanine*	1.98 ± 0.05^b	2.08 ± 0.05^b	3.20 ± 0.07^a
Valine*	3.18 ± 0.08^b	3.63 ± 0.09^a	3.56 ± 0.06^a
Methionine*	4.60 ± 0.10^b	4.76 ± 0.11^b	6.88 ± 0.15^a
Cystine	0.98 ± 0.04	0.88 ± 0.04	3.37 ± 0.06
Isoleucine*	15.26 ± 0.18^a	10.61 ± 0.22^b	4.53 ± 0.11^c
Leucine*	6.09 ± 0.11^b	5.80 ± 0.11^b	6.86 ± 0.15^a
Lysine*	2.34 ± 0.07^c	3.32 ± 0.08^b	5.66 ± 0.14^a
Tryptophan*	1.78 ± 0.06^a	1.74 ± 0.06^a	1.26 ± 0.05^b

Means (±SE, n=9) of*essential amino acid with different superscripts in each row indicate significant differences ($P<0.05$, df. 23, 3).

Table 4. Fatty acids (g/100g lipid) of raw LCRE , SMRE and POME.

Fatty acids	Raw LCRE	Raw SMRE	Raw POME
Capric acid (10:0)	4.10 ± 0.04	5.25 ± 0.05	4.29 ± 0.03
Lauric acid (12:0)	10.21 ± 0.05	12.41 ± 0.02	9.22 ± 0.03
Myristic acid (14:0)	21.59 ± 0.72	18.66 ± 0.14	12.66 ± 0.11
Palmitic acid (16:0)	5.28 ± 0.03	8.24 ± 0.07	14.45 ± 0.12
Heptadecanoic acid (17:0)	1.05 ± 0.04	1.08 ± 0.03	1.39 ± 0.02
10-heptadecanoic acid (17:01)*	1.55 ± 0.02^a	1.62 ± 0.03^a	1.12 ± 0.02^b
Stearic acid (18:0)	24.35 ± 1.63	21.15 ± 0.04	11.41 ± 0.08
Oleic acid (18:1n-9)*	0	0	8.54 ± 0.06
Linoleic acid (18:2n-6)*	0.68 ± 0.04^b	0.77 ± 0.04^b	9.53 ± 0.05^a
Linolenic acid (18:3n-3)**	0.42 ± 0.03^b	0.52 ± 0.02^b	4.72 ± 0.04^a
Arachidic acid (20:0)	15.85 ± 0.13	12.77 ± 0.11	7.56 ± 0.03
Eicosatrienoic acid (20:3n-6)**	1.52 ± 0.02^b	1.88 ± 0.02^a	1.49 ± 0.02^b
Arachidonic acid (20:4n-6)**	1.06 ± 0.03^a	0.32 ± 0.02^b	1.12 ± 0.03^a
Eicosapentaenoic acid (20:5n-3)**	0	0	0.36 ± 0.02
Behenic acid (22:0)	5.52 ± 0.10	4.44 ± 0.11	2.62 ± 0.03

Means (±SE, n = 9) of*unsaturated and **polyunsaturated fatty acids with different superscripts in each row indicate significant differences ($P<0.05$, df. 23, 3).

to be an exception. Some researchers have reported that raw LCRE and SMRE contain reasonable amounts of essential minerals that are very helpful for plant growth (Bakti and Karim 1989; Karim and Bachik 1989; Isa 1993). These essential minerals play crucial roles in maintaining proper physiological processes in plants and animals (Kamen 1987; McDowell 1992). Pb content, although higher in raw POME, was not beyond the toxic or sublital levels (James *et al.* 1996). However, the minerals were not present in their optimum levels. The present results show that these three agroindustrial effluents contain high amounts of organic as well as essential nutrients. These nutrients may be used to grow microalgae as natural food for aquatic organisms, and

Table 5. Mineral contents (weight %) of raw LCRE, SMRE and POME detected by energy dispersive of X-ray analysis (EDAX) through scanning electron microscope.

Minerals	Raw LCRE	Raw SMRE	Raw POME
Fe*	4.05 ± 0.03	3.22 ± 0.03	5.58 ± 0.03
Zn*	3.54 ± 0.03	2.91 ± 0.03	2.47 ± 0.01
P*	11.71 ± 0.12	10.60 ± 0.10	12.69 ± 0.13
Na*	7.42 ± 0.05	5.80 ± 0.05	6.25 ± 0.06
Mg*	1.22 ± 0.02	0.52 ± 0.02	0.79 ± 0.04
Mn*	1.55 ± 0.02	1.24 ± 0.02	1.29 ± 0.02
K*	16.35 ± 0.11	19.66 ± 0.14	22.27 ± 0.15
Ge*	0.38 ± 0.01	0.45 ± 0.03	0.14 ± 0.01
Ca*	9.96 ± 0.08	9.47 ± 0.07	7.64 ± 0.05
Cl*	12.79 ± 0.12	15.33 ± 0.12	17.68 ± 0.12
I*	3.89 ± 0.03	4.65 ± 0.04	3.29 ± 0.03
Co*	0.44 ± 0.02	0.55 ± 0.02	1.21 ± 0.02
Cr*	2.16 ± 0.03	2.14 ± 0.02	2.36 ± 0.03
Cu*	1.66 ± 0.02	0.48 ± 0.06	1.38 ± 0.02
Ni*	3.27 ± 0.03	3.89 ± 0.03	1.36 ± 0.02
S*	2.75 ± 0.03	2.66 ± 0.03	4.50 ± 0.04
Se*	0.38 ± 0.01	0.33 ± 0.04	0.42 ± 0.02
Si*	9.33 ± 0.06	10.05 ± 0.12	5.99 ± 0.05
Sn*	0.88 ± 0.01	0.65 ± 0.02	0.42 ± 0.02
Al*	3.45 ± 0.03	3.55 ± 0.05	4.50 ± 0.03
Mo*	1.27 ± 0.02	1.66 ± 0.04	0.27 ± 0.02
B*	0.14 ± 0.02	0.16 ± 0.03	0.12 ± 0.02
Br*	0.36 ± 0.02	0.22 ± 0.03	0.18 ± 0.02
V*	0.22 ± 0.02	0.16 ± 0.02	0.15 ± 0.01
As*	0.54 ± 0.03	0.22 ± 0.02	0.45 ± 0.03
Li*	0.22 ± 0.02	0.20 ± 0.02	0.28 ± 0.03
Pb*	3.72 ± 0.04	2.24 ± 0.04	1.55 ± 0.03
Hg	0.22 ± 0.02	0.18 ± 0.02	0.15 ± 0.01
Cd	0.52 ± 0.04	0.44 ± 0.03	0.32 ± 0.03

*Essential mineral

Table 6. Mineral content (mm/g dry sample) of raw SMRE, LCRE and POME detected and quantified by inductively-coupled plasma mass spectrophotometer.

Minerals	Raw LCRE	Raw SMRE	Raw POME
Fe*	186.61 ± 1.84^{b}	168.25 ± 1.74^{c}	1311.25 ± 6.22^{a}
Zn*	4.72 ± 0.12^{b}	2.95 ± 0.05^{c}	17.58 ± 0.32^{a}
P*	646.63 ± 4.19^{b}	436.24 ± 2.44^{c}	14524.52 ± 55.12^{a}
Na*	42.39 ± 0.58^{b}	105.22 ± 1.16^{a}	102.97 ± 1.05^{a}
Mg*	24.35 ± 0.57^{c}	39.12 ± 0.75^{b}	$132.94 \pm 1..44^{a}$
Mn*	1.46 ± 0.08^{b}	6.95 ± 0.07^{b}	27.92 ± 0.22^{a}
Ca*	143.55 ± 1.16^{b}	64.20 ± 1.15^{c}	1600.59 ± 4.65^{a}
K*	39.52 ± 0.45^{d}	105.25 ± 1.18^{c}	1951.55 ± 5.52^{a}
Co*	0.05 ± 0.002^{b}	0.09 ± 0.005^{b}	0.25 ± 0.02^{a}
Cr*	0.79 ± 0.02^{b}	0.64 ± 0.03^{b}	4.02 ± 0.10^{a}
Cu*	1.58 ± 0.03^{b}	3.15 ± 0.04^{b}	11.08 ± 0.36^{a}
Ni*	0.24 ± 0.004^{b}	0.45 ± 0.03^{b}	2.46 ± 0.05^{a}
S*	7.02 ± 0.11^{b}	8.50 ± 0.16^{b}	14.50 ± 0.49^{a}
Se*	1.59 ± 0.09^{b}	1.35 ± 0.03^{b}	12.32 ± 0.27^{a}
Si*	15.16 ± 0.29^{a}	6.95 ± 0.27^{c}	9.62 ± 0.15^{b}
Al*	97.95 ± 0.48^{c}	112.45 ± 1.22^{b}	447.01 ± 2.18^{a}
B*	2.21 ± 0.06^{b}	1.96 ± 0.02^{b}	15.77 ± 0.58^{a}
Mo*	0.46 ± 0.03^{b}	0.62 ± 0.04^{b}	6.07 ± 0.11^{a}
Sn*	12.42 ± 0.11^{b}	10.42 ± 0.32^{b}	33.80 ± 0.74^{a}
V*	0.32 ± 0.01^{c}	1.42 ± 0.05^{b}	3.08 ± 0.06^{a}
As*	0.74 ± 0.02^{b}	0.96 ± 0.03^{b}	6.09 ± 0.11^{a}
Li*	0.55 ± 0.04^{b}	0.60 ± 0.04^{b}	1.44 ± 0.05^{a}
Pb	3.45 ± 0.11^{b}	3.25 ± 0.05^{b}	5.15 ± 0.08^{a}
Cd	0.05 ± 0.002^{c}	0.18 ± 0.02^{b}	0.44 ± 0.03^{a}

Means (±SE, n=9) of* essential minerals with different superscripts in each row indicate significant differences ($P<0.05$, df. 23, 3).

allowing these organisms to recover nutrients that may ultimately prove useful in keeping the aquatic environment partially free from pollution.

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