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Estimate Assumed Lignin Content under Co-Existence with on-Lignin Polyphenolic Compounds in Cacao (*Theobroma cacao* L.) Hulls and Shells

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Objectives

The aim of this study is to estimate lignin content using various analyses such as alkaline nitrobenzene oxidation, methoxyl group determination, and ozonolysis in cacao by-products, which contain non-lignin polyphenolic compounds.

Materials and Methods

- o Cacao (*Theobroma cacao* L.) hulls processed as residues of the food industry were kindly provided by Lotte Co. Ltd (Japan). Fully matured cacao fruits were harvested from Rajamandala Cacao Plantation at Rajamandala in West Java (Indonesia).
- o After separation of hulls and shells from bean, samples were extracted soluble with 70% acetone (v/v) to remove phenolic compounds. The extract free residues were performed various chemical analyses such as Klason lignin, alkaline nitrobenzene oxidation (NBO), methoxyl group content, and ozonation to estimate lignin content.

Results and Discussion

- o No significant differences in the yields of both Klason residues (27.2-32.0%) and acid soluble phenolic compounds (2.7-3.3%) were found for commercial hulls, intact hulls and shells.
- o Methoxyl contents of commercial hulls, intact hulls and shells were 166., 613, and 672 mmol/kg of dry samples, respectively. Based on these values, the range of lignin content of commercial hulls, intact hulls and shells were assumed as 1.7-3.0, 6.4-11 and 7.1-12.1%.
- o Total yield of NBO of intact hulls and shells were 19.7 and 103.0 mmol/kg respectively, correspond to 0.7-0.8% and 3.7-4.3% of assumed lignin content.
- o The total yields of erythronic and threonic acids by ozonation were 3.5, 26.5, and 68.0 mmol/kg of commercial, intact hulls and shells respectively. The range of assumed lignin content can be estimated as 0.3% in commercial hulls, 1.9-2.2% in intact hulls, and 4.9-5.7% in intact shells.

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Table 1. Yield of Klason residue and acid soluble phenolic compounds in hulls as residues from food industry, intact hulls and shells

	residues from food industry hulls	Intact	
		hulls	shells
Klason residues (% ODM ^a)	32.0	27.2	30.8
Acid-soluble phenolic compounds (% ODM)	3.3	3.2	2.7
Total polyphenolic compounds (% ODM)	35.3	30.4	33.5
Nitrogen in Klason residues (% KR)	2.5	2.2	2.8
Neutral sugar in Klason residues (% KR)	0.1	0.1	0.5

^a ODM: Oven dry material

Table 2. Content of methoxyl group, products of alkaline nitrobenzene oxidation and ozonation in hulls as residues from food industry, intact hulls and shells

(mmol/kg)		residues from food industry hulls	Intact	
			hulls	shells
Methoxyl group		166	613	672
Alkaline Nitrobenzene oxidation products	4-Hydroxybenzaldehyde	4.1	ND ^a	ND
	Vanillin	ND	18.4	79.6
	Syringaldehyde	ND	1.3	23.1
	4-Hydroxybenzoic acid	ND	ND	ND
	Vanillic acid	ND	ND	10.1
	Syringic acid	ND	1.5	2.5
	Total yield	- ^b	19.7	103
	S/V molar ratio	-	0.07	0.29
Ozonation products	Erythronic acid	2.2	15.5	41.7
	Threonic acid	1.3	11.0	26.3
	Total yield	3.5	26.5	68.0
	E/T molar ratio	1.7	1.4	1.6

^a Not detected, ^b Cannot be calculated

Table 3. Lignin content in hulls as residues from food industry, intact hulls and shells estimated from results for methoxyl content, products of alkaline nitrobenzene oxidation and ozonation

Indication	lignin (%)					
	residues from food industry hulls		intact			
			hulls		shells	
	M.W. ^c 180	M.W. 210	M.W. 180	M.W. 210	M.W. 180	M.W. 210
-OCH ₃ ^a	3.0	1.7	11.0	6.4	12.1	7.1
ANBO ^b	- ^d	-	0.7	0.8	3.7	4.3
Ozonation	0.3	0.3	1.9	2.2	4.9	5.7

^a Methoxyl group, ^b Alkaline nitrobenzene oxidation, ^c Molecular weight, ^d Cannot be calculated