論文の内容の要旨

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論文題目: **Developing Pattern of Secondary Cell Walls and Their Chemical Characteristics of Cacao (*Theobroma cacao* L.) Hull**

(和訳: カカオ(*Theobroma cacao* L.) 種子殻二次壁形成とその化学的性状)

Chapter 1: Introduction

A major cacao industry has emerged with the development of cocoa, cocoa butter, syrups, pastes and all kind of chocolates. Therefore, processing of cocoa leads to increasing disposal of wastes such as hulls, shells, and so on. Among the cacao by-products, cacao hull is regarded as potential and valuable sources of food industry, live stock feed, and is gaining considerable interest in developed countries. In order to improve its effective utilization, structural characterization of predominant polyphenolic compound, which is most important factor for its potential use, is prerequisite. However, there is a paucity of information on their structural features of predominant polyphenolic compounds. This investigation reported herein provides elucidative and valuable information for the above aims. In addition, the cacao hull has unusual developing pattern of secondary wall thickening such as tracheary elements (TEs). The morphological structure of TEs and their physico-chemical properties were also examined.

Chapter 2: Compositional Characterization of Cacao (*Theobroma cacao* L.) Hulls

Cacao (*Theobroma cacao* L.) hull is regarded as potential and valuable resources for the food industry and livestock feed, and is gaining considerable interest in developed countries. The chemical composition and structural characteristics of cacao (*Theobroma cacao* L.) hull were investigated in this Chapter. Holocellulose, Klason

residue, total amino acid, lipid and ash were quantified as 334 g kg^{-1}, 320 g kg^{-1}, 96 g kg^{-1}, 99 g kg^{-1} and 93 g kg^{-1} respectively. Neutral sugar composition of cell wall polysaccharides was also analysed. It was suggested by an alkaline nitrobenzene oxidation of cacao hull that the Klason residue was composed of structurally unknown polyphenols together with small amounts of lignin and tannin. The results for total amino acid (96 g kg^{-1}), and nitrogen content of the Klason residue (25 g kg^{-1}) and samples treated with various surfactants (21 – 27 g kg^{-1}), suggested the presence of polyphenol-protein complexes in cacao hull. In addition, relatively high amount of ash (potassium) and (essential) amino acid would be interesting in their potential applications. The effective utilization such as nutrition for ruminants was discussed.

Chapter 3: Structural Features of Predominant Polyphenolic Compounds in Cacao (*Theobroma cacao* L.) Hulls

Extracts with 70% acetone before (E-1) and after (E-2) ball-milling of cacao (*Theobroma cacao* L.) hulls were investigated for their chemical compositions as well as comprehensive structural characterization of predominant polyphenolic compounds. The extract after ball-milling E-2) contained much higher amounts of rhamnose, arabinose, and galactose than E-1. Total yields of those sugars were 20.9% and 47.2% of the total determined neutral sugar in E-1 and E-2, respectively. In addition, small amounts of uronic acid (0.2%) and calcium (8 μg g^{-1}) in E-1 were determined and relatively high amounts of uronic acid (4.3%) and calcium (46 μg g^{-1}) in E-2. These results suggest that E-2 is composed higher amounts of pectic substances than E-1. Klason residue (KR) and acid soluble phenolics (ASP) in E-1 were 22.8% and 23.7% respectively, while E-2 contained 50.4% KR and 9.3% ASP. These results suggest that E-1 is highly composed of low molecular weights and acid soluble phenolics, which have usually contained carboxyl groups. The suggestion was confirmed by FTIR. The pyrogram of E-2 showed high intensities of catechol and 4-methylcatechol, while E-1 pyrogram showed high intensities of alkaloid compounds such as theobromine, theophylline and caffeine, phenol, and *p*-cresol. The existence of catechol units in E-2 was confirmed by ^{13}C-NMR spectrometry. According to all results, lignin is not dominant constituent in cacao hull and unknown polyphenolic compounds which gave catechol by analytical pyrolysis would be predominant polyphenolic compounds.

Chapter 4: Assumption of Lignin Content Under Co-Existence with Non-Lignin PolyphenolicCcompounds in Cacao (*Theobroma cacao* L.) Hulls and Shells

Structural characteristics of polyphenolic components of cacao (*Theobroma cacao* L.) bean hulls and shells were investigated by the use of four indications, Klason

residue, methoxyl group, alkaline nitrobenzene oxidation, and ozonation products. Trials were made to assume the lignin content on the basis of these analyses and to compare the assumed lignin contents with the amount of Klason residue. Although Klason residues were obtained in rang of 272-320 g kg^{-1} of those samples, the methoxyl content and the yields of nitrobenzene oxidation and ozonation products were much smaller than the value expected from the level of Klason lignin content. Among those three indications, the methoxyl group content was relatively high for every sample but, even in this case, the lignin contents assumed from the methoxyl group content were in the range of 17-30, 67-115 and 73-126 g kg^{-1} of oven dry samples of cacao bean hulls as a residue from food industry, intact cacao bean hulls and shells of cacao fruits, respectively. Assumed lignin contents obtained from other indications were much smaller. All results of those approaches are suggesting that the lignin is not predominant polyphenolic component among cacao sub-products, especially cacao bean hulls.

Chapter 5: Morphological Characteristics of the Tracheary Elements in Cacao (*Theobroma cacao* L.) Hulls

The tracheary elements (TEs), which are the distinctive function of the xylem, are characterized during the formation of a secondary wall with annular, spiral, reticulate, or pitted wall thickenings. The spiral TEs are likely of functional significance due to their ability to expand and transport water to plant organs. The morphological structure of TEs of cacao hull was characterized by scanning electron microscopy as well as physical properties. The spiral structures of TEs covered with web-like thin layer of primary wall on the outside surfaces were observed. The TEs had various sizes of diameter of spiral circularity ranging from about 5-10 μm and the thickness of secondary wall about 1.3-2.7 μm. Polarized microscopy and X-ray diffraction analysis revealed that the orientation of cellulose microfibrils was aligned in parallel, following the spiral thickening and the crystallite size, similar to cellulose-I of the cotton. The data for this study provide three-dimensional understanding of such extensive TE structures including some valuable information for plant physiology.

Chapter 6: Differences in Chemical Constituents between Vascular Bundles and Non-Vascular Bundles of Cacao (*Theobroma cacao* L.) Hulls

Cacao hulls were physically separated into vascular bundles (VB) and non-vascular bundles (NVB) to investigate their chemical compositions as well as abundant and structural features of polyphenolic compounds. Xylose content of VB was significantly higher (13.1%) than that of NVB (2.8%). Non-vascular bundles were composed of relatively high proportions of rhamnose, arabinose, and galactose and significantly rich in uronic acid (12.9%), suggesting less secondary walls in NVB.

These data suggest that the high amount of glucose (21.4%) together with remarkable amount of xylose (13.1%) in VB is correlated with development of secondary wall formation. Total (poly)phenolic compounds (35.9-39.1%) quantified as Klason residues (KR) and acid soluble phenolic compounds (ASP) were similar in both cell types, however there were great differences in structural characteristics of polyphenolic compounds. The pyrogram of VB clearly showed high intensities of guaiacol and 4-vinylguaiacol, together with low intensities of catechol and 4-methylcatechol. On the other hand, that of NVB showed opposite trends. These results were also confirmed by total yields of vanillin and syringaldehyde of the products of alkaline nitrobenzene oxidation Therefore, the accumulation of different polyphenolic compounds in cacao hull is strongly relying on cell types, which have correlated with development of secondary wall.