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FRACTIONATION OF COCOA PROCYANIDINS ACCORDING TO THE DEGREE OF POLYMERIZATION BY CENTRIFUGAL PARTITION CHROMATOGRAPHY

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ABSTRACT Procyanidins, known also as condensed tannins (Fig. 1 and 2) , are very common constituents in plants and believed to be the second after lignans most common class of natural phenolic substances found in nature. Because they are well represented in western diet in foods such as cocoa, apples, and many berries, there is a growing interest in determining their pharmacological properties and significance as dietary antioxidants. Responding to these demands, we have designed a novel approach to the preparative fractionation of cocoa procyanidins according to their degree of polymerization (DP) by applying Fast Centrifugal Partition Chromatography.
In an experiment optimized for the best separation of DP families between 5 and 10, we employed a bi-phasic solvent system, consisting of ethyl acetate – ethanol – water (6:1:5) in ascending mode. The procyanidin oligomers (DP2 to DP10) eluted with the mobile phase in an order of increasing DP. The separation of bands between DP5 and DP10 was impressive in a context of the best preparative approaches reported so far (Fig. 3). The fractionation was monitored by a normal phase HPLC analysis on a polyvinyl alcohol (PVA) column (Fig. 4) as well as by a C18 RP column. Supplemental supporting information was obtained from a spectroscopic mass analysis of selected fractions by MALDI-TOF (Fig. 5). The RP HPLC analysis revealed that in each DP family, the dominant peaks represented the linear epicatechin (4β→8) oligomers. The FCPC fractionation appears to be an efficient approach to produce higher procyanidin oligomers in multi-milligram or gram quantities.

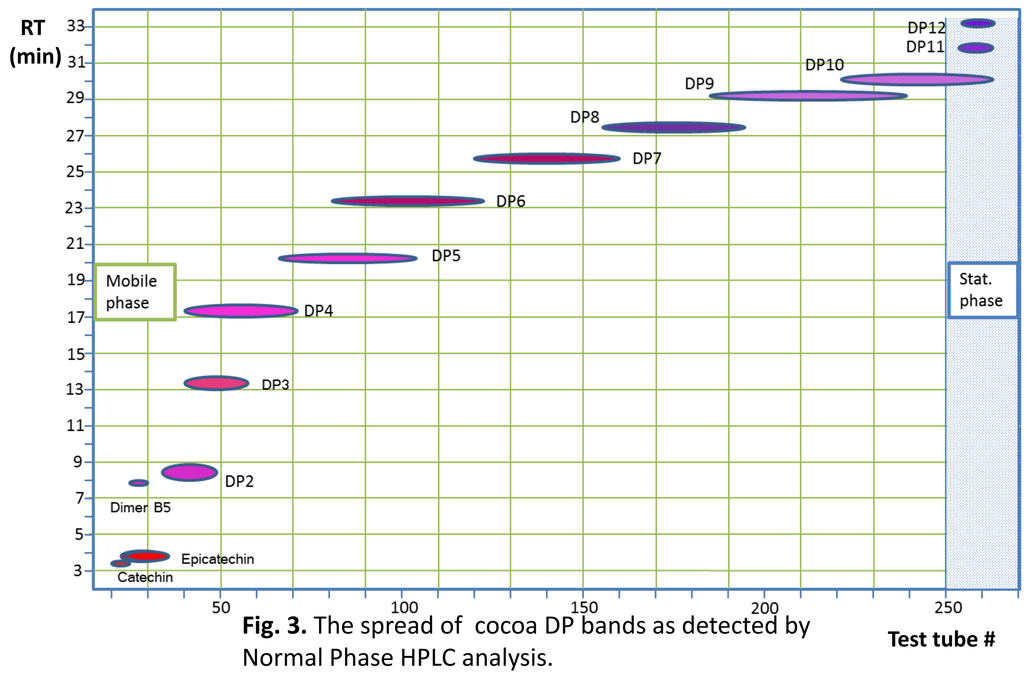

Fig. 3. The spread of cocoa DP bands as detected by Normal Phase HPLC analysis.

EXPERIMENTAL Centrifugal Partition Chromatography was carried out on a FCPC Kromaton (Rousellet, Robatel; France) equipped with a 1 L rotor. A Waters pump model 590 was used to deliver the stationary and mobile phases to the CPC. The stationary phase was pumped into the rotor at 15 ml/min, while keeping the rotor spinning at 200 RPM. A sample of 8.5 grams of cocoa procyanidins was dissolved in a mixture of the stationary and mobile phases and injected into the rotor using a Rainin pump model SDS-200. The rotational speed of the rotor was set to 800 and the mobile phase pumped at 10 ml/min. During the run the back pressure was between 38 and 42 atm. The eluent was collected in 26 ml increments into test tubes, using a Gilson fraction collector model FC-220. Test tube content was determined by HPLC using two approaches. The RP analysis, was carried out on a HP model 1050 HPLC consisting of a quaternary pump, an autosampler, diode array detector. A YMC C18 150 x 4.6 mm, 5 micron PackPro column was used in a gradient of 10 – 25% acetonitrile. The reverse phase analysis provided determination for the main components of each DP: epicatechin, catechin, procyanidin B5, and the sequence of epicatechin linear oligomers, which produced well-defined peaks starting with procyanidin B2 and ending with an already broad peak of epicatechin decamer. Higher oligomers did not produce useful signals. The NP HPLC analysis was carried out on a YMC 250 x 4.6 mm, 5 micron, PVA column using a gradient of MeOH:AcOH:H_2O (95:2:3) into MeCN:AcOH (98:2), Gu *et al. JAFC* **50**, pp 4852-60, 2002. This approach provided a separation of procyanidins into bands according to the degree of polymerization (Fig. 4).

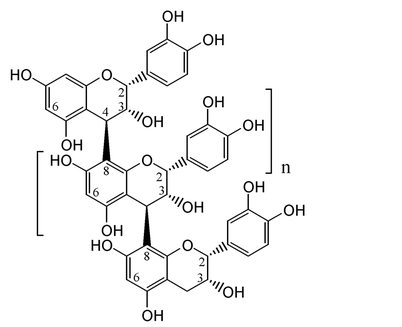

Fig. 1. General structure of polymeric linear epicatechin proanthocyanidins .

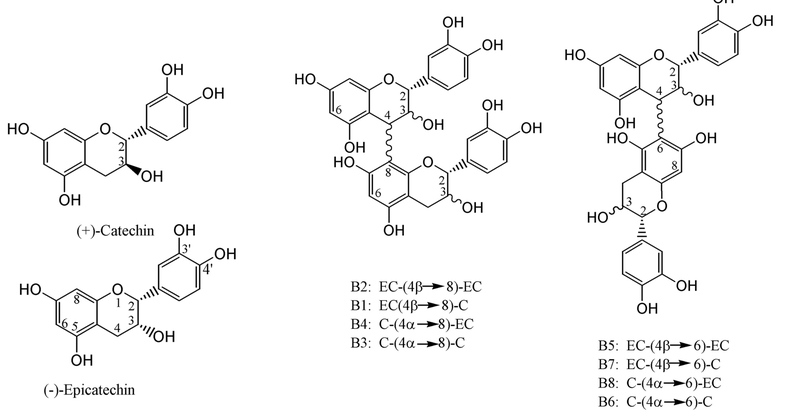

Fig. 2. Structures of monomers and dimeric procyanidins.

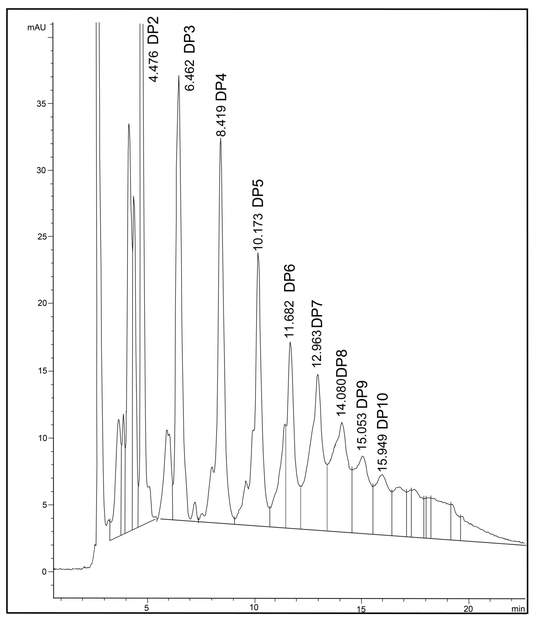

Fig. 4. Normal phase HPLC analytical pattern of oligomeric procyanidins eluting according to their degree of polymerization.

Degree of polymerization	Test tube range	Experimental Partition co-efficient	Primary Mass for middle test tube by MALDI-TOF
1 (catechin)	22-24	0.94	ND
1 (epicatechin)	25-36	1.19	ND
2 (procyanidin B5)	25-30	1.06	ND
2 (procyanidin B2)	34-48	1.59	601
3	34-57	1.92	889
4	40-68	2.44	1177
5	65-96	3.03	1465
6	81-124	3.85	1753
7	121-160	5.56	2041
8	156-195	7.14	2320
9	186-240	9.09	2617
10	221-262	12.50	2907
11, 12	254-262	not done	not done

Fig. 5. The spread of DP bands of oligomeric procyanidins in the CPC run, estimated partition coefficient and the dominant MW.