Solid/liquid extraction as key step for quality assessment of commercial cranberry products using HPTLC-densitometry

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A novel tag-free probe for targeting molecules interacted with flavonoid catabolites

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Quercetin is one of the most widely distributed flavonoids in vegetables and fruits and has attracted much attention because of its beneficial biological effect. Quercetin-4'- glycoside (Q4'G) is one of the major quercetin conjugates in onion. Q4'G reaching the colon is subjected to hydrolysis by intestinal bacteria into 3,4-dihydroxyphenylacetic acid (DOPAC), and then DOPAC is converted into 3-hydroxyphenylacetic acid (OPAC) or protocatechuic acid (PCA) in the large intestine. We have reported that DOPAC has the strongest ability to induce expression of phase 2 enzyme genes compared to other metabolites. However, the underlying mechanism in which DOPAC exerts the antioxidant effect via inducing phase 2 enzymes is not clarified. Therefore, developing a new probe of DOPAC give an important clue to reveal the mechanism of its biological activity.

In the present study, to detect directly binding target of DOPAC, we designed a novel DOPAC probe using the copper (I)-catalyzed alken azide 1,3-dipolar cycloaddition (CuAAC) reaction. First, we introduced an alkyne group into DOPAC by esterification with propalgyl alcohol, thereby yielded DOPAC propargyl ester (DPE). To examine its efficacy, DPE was incubated with a model protein, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and followed by CuAAC click reaction with azide-labelled biotin. Finally we detected the DOPAC-modified protein by a labelled streptavidin biotinylated antibody (LSAB) method. The formation of 1,2,3-triazole by the Huisgen cycloaddition was also confirmed by both mass spectrometry and nuclear magnetic resonance spectroscopy. These results provide an alternative approach to understand how polyphenol catabolites modify cellular proteins.

Financial support: MEXT KAKENHI Grant Number 25292073.

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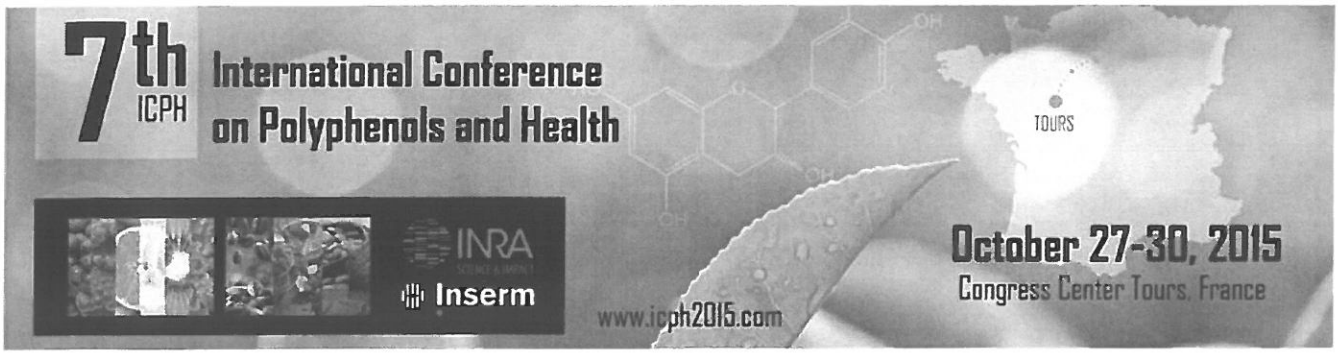
Increasing number of dietary supplement containing cranberry polyphenols are commercialized every year. The composition of cranberry ingredients varies from whole fruit powder to cranberry extract or mixture with blueberry. In order to analyze the quality of cranberry based commercial products, researchers proceed to quality control based on BL-DMAC analysis after one step extraction. In our previous work, we demonstrated that BL-DMAC analysis is not sufficient alone to assess the quality of cranberry ingredients. BL-DMAC as quality control couple to High Performance Thin Layer Chromatography -densitometry bring complementary information, especially rate of epicatechin, PAC-$A_2$and PAC-B_2.

In this work, different extraction solvents were screened for extraction of PACs from a range of commercial dietary supplements. Thus, solid / liquid extraction step was repeated until total extraction of polyphenols was achieved, i.e no coloration of the residue in contact with DMAC reagent. If the solvent nature was found to have a minor impact, the number of required extraction steps varied significantly between commercial products. This step was then demonstrated to be a key factor to control for meaningful comparison between various food supplements.

Quality assessment of cranberry extracts was then conducted using both BL-DMAC and HPTLC-densitometry protocols, in order to obtain global PACs content and metabolic profile. A large diversity of PACs global content and profile was observed

This work highlighted the need of new standardization protocols to control and asses the quality of cranberry ingredients, to guarantee biological effect.



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