Review Article

# Cocoa flavanols: measurement, bioavailability and bioactivity

Catherine Kwik-Uribe PhD[1] and Roger M Bektash PhD[2]

[1]*Mars Inc., Hackettstown, NJ, USA*
[2]*Mars Inc, Petcare Place, Wodonga, Victoria, Australia*

There has been growing interest in the potential cardiovascular benefits associated with cocoa consumption. As a result of accurate analytical methodologies, there is evidence to support that the flavanols in cocoa can be absorbed, are bioactive, and may be responsible for the cardiovascular benefits associated with regular cocoa consumption. The flavanols in cocoa exist in a multitude of different stereochemical configurations, thus giving rise to a unique and complex mixture of compounds. Given this complexity, the quantitative analysis of cocoa flavanols in foods can be challenging. While there are published methods suitable for the analysis of these compounds, these methods require sophisticated instrumentation and can be challenging to set up. As such, simpler techniques that measure such things as total phenolic content or antioxidant potential have been used as indicators of flavanol content. However, as these simpler assays are prone to interferences and are not specific for flavanols, these methods are not appropriate for use in studies that aim to examine the physiological effects of cocoa flavanols. It is only through the use of methods that can accurately quantify these flavanols that it will be possible to make meaningful dietary recommendations regarding the consumption of cocoa flavanol containing foods.

**Key Words: cocoa, flavonoids, flavanols, analysis, bioavailability**

## INTRODUCTION

Over the past decade, there has been increasing scientific interest in the potential cardiovascular health benefits associated with regular cocoa consumption. This interest stems from data gathered from both epidemiological investigations and human dietary intervention trials.[1] Despite the complex matrix of cocoa, there is evidence to support that flavanols, a group of naturally occurring plant compounds, are bioactive and may be responsible for the reported cardiovascular health benefits associated with cocoa consumption.

Flavanols are a sub-class of a larger group of plant compounds known as flavonoids. Flavanols (also known as flavan-3-ols, or catechins) are present in a number of commonly consumed foods and beverages including grapes and grape products, apples, teas, cocoa and cocoa products. While flavanols can be particularly rich in the original plant material, a number factors including plant genetics, agricultural and post-harvesting practices, food manufacturing, as well as food preparation in the home can all dramatically alter the flavanol content of the food item that is eventually consumed. In the context of cocoa and cocoa containing products including chocolate, this is particularly relevant as the practices commonly used in cocoa processing (e.g., fermentation, roasting, alkalization, etc.) can dramatically reduce the cocoa flavanol content of the finished food product. These issues lead to immense variability in the flavanol content of foods, and prevent the reliable use of published data for the selection of "flavanol-rich" foods.

At this time, published data can only be used to identify a food as a potential source of flavanols, with the accurate determination of actual flavanol content only possible through the use of appropriate analytical methodologies.

While grapes, teas, apples, and cocoa products can all be identified as containing flavanols, the number and type of flavanols varies considerable between these food items. When discussing flavanols, it is important to keep in mind that the flavanols can exist in both simple monomeric forms (Figure 1, A), as well as more complex linked forms, known as proanthocyanidins (Figure 1, B). (Within this paper, the term flavanols will be used to collectively describe the monomeric and oligomeric forms of flavanols). Further adding to this complexity is the fact that the number and stereochemical position of the hydroxyl groups (-OH group) on the molecule, as well as the number and type of linkages between monomers can vary, potentially leading to the natural presence of thousands of different molecules within one flavanol-containing food. Separated using high performance liquid chromatography (HPLC), examples of the different flavanols in a cocoa, a green tea, and an apple sample are shown in Figure 2.

In these traces, it is easy to see that while all of these foods may be classified as containing flavanols, the number and type of flavanols within each of these foods are not

**Corresponding Author:** Dr. Catherine Kwik-Uribe, Mars Inc. 800 High Street Hackettstown NJ 07840 USA.
Email: catherine.kwik-uribe@effem.com
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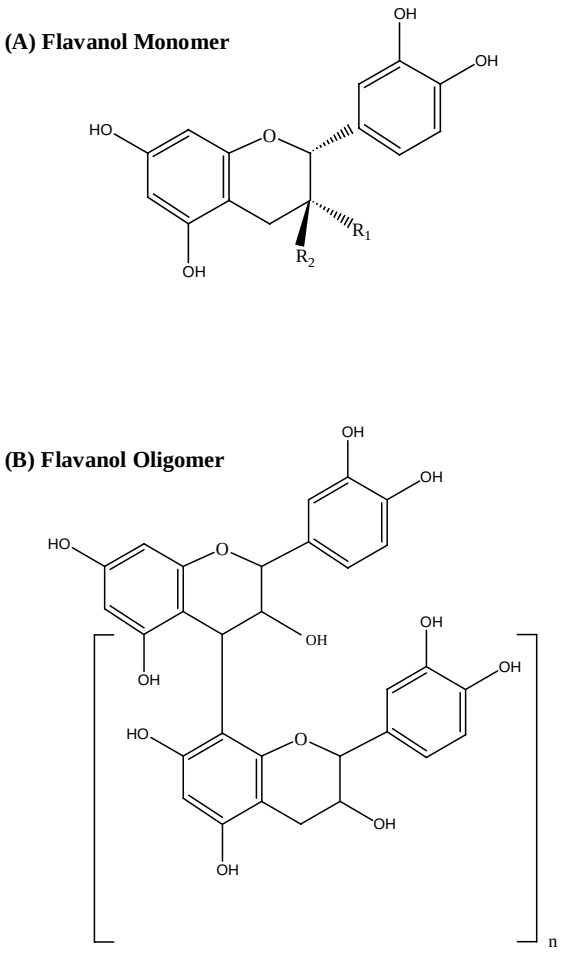


**Figure 1.** Examples of a (A) flavanol monomer and a (B) flavanol oligomer.

identical. This is interesting from an analytical perspective, as well as a biological perspective as these differences in flavanol composition could be expected to lead to differences in both the bioavailability and physiological effects of the flavanols.

## MEASUREMENT OF COCOA FLAVANOLS

The structural diversity in the flavanols in cocoa makes their analysis quite complex. Analytical separations are typically accomplished utilizing HPLC coupled with ultraviolet/visible spectrophotometry, fluorescence detection, or mass spectrometry. For the flavanol monomers (which in the cocoa seed are predominately (-)-epicatechin and to a much lesser extent, (+)-catechin), separations are most typically done using C-18 as the stationary phase (reversed phase HPLC). Reversed phase HPLC has also been utilized for the separation of the flavanol oligomers, but separations for oligomers larger than trimer are typically ineffective. For cocoa, which contains a distinct mixture of flavanol monomers and oligomers, normal phase HPLC has been used most successfully for the separation and quantification of these compounds in cocoa and cocoa containing products. First published in 1993, Rigaud *et al.*[2] demonstrated the use of normal-phase (silica) HPLC for the separation of monomers through heptamers in cocoa seeds (beans) and grape seeds. In 1999, Hammerstone *et al.*[3] further modified this method to achieve the separation and resolution of oligomers with a degree of polymeration of 10 (ie, decamers). Most recently, the monomers and oligomers in cocoa have been separated using normal phase HPLC, but this time employing a diol stationary phase in place of silica. [4] This novel modification allows not only for the enhanced resolution of oligomers, but also enables the isolation of oligomeric fractions on a preparative scale.

Despite the existence of published methods for the analysis of the flavanols in cocoa, these methods are complex and challenging to set up. This analytical complexity has made the use of alternative methods that measure such things as phenolic chemistry (ie, Folin-Ciocalteu) or antioxidant potential (ie, ORAC) appealing. While these assays can provide some details about the chemistry of the molecules within a food product, they are all non-specific for flavanols.

Folin-Ciocalteu is a method with a long history of use. First developed in the early 1900's for the analysis of proteins, this assay has since been developed as a method for determination of total phenolic content. This assay takes advantage of the reducing capacity of phenols. Despite the ease of use and high sensitivity, the fact that any compound within a sample which contains a phenolic group will be detected (ie, reducing sugars, ascorbic acid, amino acids, etc) limits the utility of this method for the specific determination of the flavanols (or flavonoids) in food samples. Although this method has been modified over the years to deal with potential interferences and has even been adapted for use with biological samples, the non-specific nature of this method will always prohibit its use in situations where (flavanol) specific information is required.

ORAC, which stands for Oxygen-Radical Absorbance Capacity, is another assay that has gained increasing popularity over the years. As its name implies, this assay measures the ability of a compound or group of compounds to "absorb" or quench oxygen radicals, and is among a larger group of assays that has been developed to provide information on the antioxidant potential of foods. Like the Folin assay, this assay does provide information regarding the chemical nature of a sample, and like the Folin assay, it is non-specific for flavanols (flavonoids) and is prone to interferences. In addition to the simplicity and convenience offered by antioxidant assays, the appeal of these antioxidant measures is also the fact that there are many published papers in the scientific literature which suggest that the potential health benefits of flavanol (flavonoid) containing foods relates to their ability to act as antioxidants *in vivo*. Thus, a measurement of the *in vitro* antioxidant potential has often been used to support arguments that compounds with strong antioxidant capacity *in vitro* are likely to be strong antioxidants *in vivo*, and thus "good for your health". In recent years, the function of flavonoids as antioxidants has been brought into question.[5] Furthermore, it is well established that the flavanols in foods, upon consumption, are subjected to extensive biotransformation *in vivo*, resulting in a variety of *O*-methylated, *O*-glucuronidated, and *O*-sulfated metabolites with markedly reduced "antioxidant" capacity. Given this, it is perhaps not too surprising then that the vascular effects observed following the consumption of cocoa flavanols have been shown to occur without concomitant

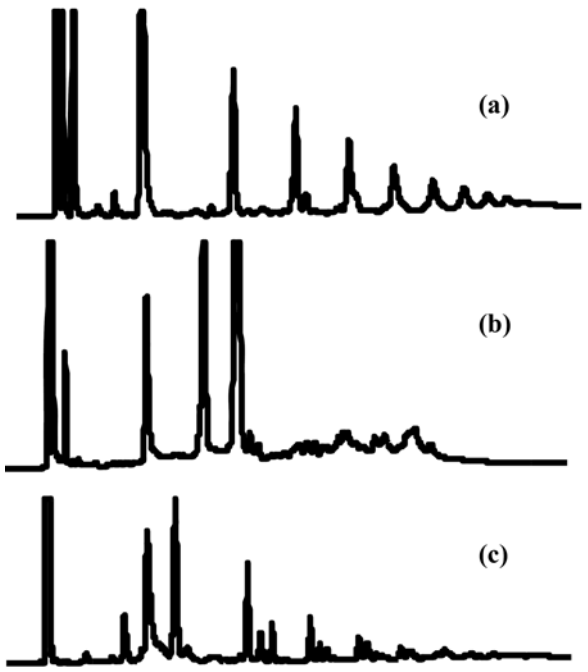


**Figure 2.** Sample HPLC traces of (a) cocoa, (b) green tea, and (c) apple.

changes in established markers of oxidative stress and damage.[6,7]

***Bioavailability of Cocoa Flavanols***

In order to establish a causal relationship between the consumption of cocoa flavanols and specific physiological effects, it is critical not only to establish that the food product which is consumed contains cocoa flavanols, but it is also critical to establish that these compounds are absorbed into the circulation. Since the early 1990's, several groups have examined the bioavailability and metabolism of cocoa flavanols.[8-10] From these studies, it is clear that the flavanols in cocoa products are absorbed and do enter the circulation. While the acidic environment of the stomach was initially thought to degrade the flavanol oligomers in cocoa, it has been shown that little to no degradation of flavanol oligomers occurs in the stomach and that the flavanol monomers and flavanol oligomers from cocoa reach the upper intestine intact.[11] Once in the upper intestine, the flavanol monomers and dimers can be absorbed, and have been shown to begin to appear in plasma within 30 minutes to one hour post-consumption.[8,10] The flavanol monomers have been shown to undergo extensive metabolism, with biotransformation initiated within the enterocyte and carried on by enzymes located predominantly in the liver and kidney. This extensive metabolism gives rise to a range of *O*-methylated, *O*-glucuronidated, and *O*-sulfated flavanol derivatives in plasma. While the bioavailability and kinetics of absorption can be affected by product matrix, the total concentration of flavanol metabolites has been shown to achieve a concentration of 1-2 μM level in plasma following the consumption of a flavanol-containing cocoa beverage.[11] In contrast, the dimers in cocoa have only been detected in plasma in the 40 nM range.[12] This is likely an underestimation of their concentration in plasma as the gaps in our knowledge regarding the biotransformation of these oligomeric compounds and the lack of available analytical standards have prevented the development of suitable methods for their analysis. Despite their presence in cocoa, oligomers larger than dimers have not been detected in human plasma following the consumption of flavanol (oligomer) containing cocoa products, suggesting that the monomers and dimers are the predominate flavanols in cocoa that are absorbed intact from the upper intestine.

The flavanol monomers and flavanol oligomers that are not absorbed in the small intestine travel to and are metabolized by colonic microflora within the large intestine. Once within the large intestine, the flavanols are metabolized by the microflora to produce a wide range of simple phenolic acids. A feeding study in humans found that the majority of the phenolic acid metabolites could be detected in human urine within 9 hours post-consumption of a flavanol-containing dark chocolate, with some of the highest concentrations of these phenolic acids detected in urine 24-48 hrs after the consumption of the chocolate.[13] Thus, it is possible to speculate that the flavanols and flavanol metabolites absorbed from both the upper and lower intestinal tracts may work in an additive or synergistic manner within the body to support cardiovascular health.

***Bioactivity of Cocoa Flavanols***

Data from epidemiological studies have found an inverse correlation between the consumption of flavanol containing foods, including cocoa, and the risk of cardiovascular diseases.[1] The findings of these epidemiological investigations have been substantiated in part by human dietary intervention trials with flavanol-containing cocoa products. A comprehensive review of the findings from all of the published studies on cocoa is beyond the scope of this article; however, in the context of cardiovascular health, it is notable that among these intervention studies, several have shown that the consumption of cocoa flavanols can improve insulin sensitivity, vascular function, and blood pressure, as well as attenuate platelet reactivity.[1] While the mechanisms underlying these effects are still under investigation, there are multiple lines of evidence which suggest that the modulation of nitric oxide concentrations by flavanols is central to their physiological effects, and this modulation may underlie the cardiovascular health benefits associated with the consumption of cocoa.[14]

**SUMMARY**

Data from epidemiological studies, and data from dietary intervention trials provide support for the concept that the flavanols in cocoa may offer cardiovascular health benefits, and may even be cardioprotective. Additional clinical investigations are needed to clearly identify the efficacy (and safety) of these flavanols. While the accurate analysis of cocoa flavanols in foods is inherently complex, these analytical characterisations are essential in order to establish a causal relationship between the consumption of cocoa flavanol containing foods and cardiovascular health benefits. Furthermore, it is only through this level of analytical investment that it will be possible to execute well-designed studies that will enable the development of

meaningful dietary recommendations for the public regarding the consumption of flavanol containing foods.

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**AUTHOR DISCLOSURES**

Catherine Kwik-Uribe and Roger M Bektash, no conflicts of interest, except that this paper was authored in Mars Inc.

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