

Institution: University of Reading		
Unit of Assessment: 6 (Agriculture, Food and Veterinary Sciences)		
Title of case study: Optimising the health benefits of polyphenol-rich food products.		
Period when the underpinning research was undertaken: Between May 2004 and February 2013		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Jeremy PE Spencer	Professor of Molecular Nutrition, Reader, Lecturer	Between May 2004 and present
Gunter Kuhnle	Professor of Nutrition and Food Science, Associate Professor, Lecturer	Between April 2010 and present
Period when the claimed impact occurred: Between August 2013 and December 2020		
Is this case study continued from a case study submitted in 2014?		
<p>Yes: The 2014 REF case study presented clinical evidence linking the intake of flavanol-containing foods/beverages with improved cognition, digestive health and vascular function. This supported the launch of CocoaVia®, a dietary supplement developed by Mars, Inc. The findings from subsequent research determined the dose-dependent effects of flavanols on vascular function, which led Mars to reformulate CocoaVia® in 2019. The current case study also presents research on the benefits of consuming fruit juice with added pomace, which underpinned the development of novel, flavanone-rich food products by PepsiCo.</p>		
1. Summary of the impact		
<p>The beneficial effects of flavonoid compounds on cardiovascular function, validated through randomised control trials led by University of Reading researchers, has underpinned the development of functional food products by two major multi-national companies. PepsiCo has developed a new process for retaining flavanones and fruit fibre that are normally lost in the juicing process. This led to the launch of a new premium drink range, Tropicana Whole Fruit [text removed for publication]. Early findings on the benefits of flavanols supported the development to market of CocoaVia [text removed for publication]. Following the team's determination of the optimum daily intake of cocoa flavanols for promoting vascular health, in 2019 Mars, Inc. increased the concentration of cocoa flavanols in CocoaVia® to 450mg per serving. Collaborative research with Mars, as part of the EU FLAVIOLA project, also led to first AOAC (Association of Official Analytical Chemists) method for analysing cocoa flavanols in food products, which is steering consistent product labelling across the vitamin and supplement industry.</p>		
2. Underpinning research		
<p>Evidence from observational studies suggests that diets high in polyphenol-rich foods, such as apples, blueberries, cocoa, and black tea, are associated with a lower risk of cardiovascular disease. However, the ability to fully harness the health benefits from dietary polyphenols requires an understanding of the bioactivity of individual compounds, their optimum dose and recommended daily intake from food products.</p> <p>Since 2004, research by Professor Jeremy Spencer and colleagues at the University of Reading has helped to define the paradigm-changing concept of how flavonoids and other dietary polyphenols act, via non-antioxidant mechanisms of action, to mediate clinically significant health benefits. This body of research has included studies supported by Mars, Inc and BBSRC to understand the human absorption and metabolism of dietary flavanols, providing evidence linking the intake of flavanol-containing foods/beverages to beneficial changes in the gut microflora as well as to cognitive and vascular function [1].</p>		

Establishing the optimum intake of cocoa flavanols for promoting vascular health

Between September 2009 and February 2013, as part of the EU-funded FLAVIOLA consortium, Spencer and colleagues determined the dose-dependent effects of pure (-)-epicatechin (a cocoa-derived flavanol) on human vascular function. This randomised, double-blind, placebo-controlled clinical trial in 20 people established that **significant increases in vascular function and improved blood flow occurred at intake amounts as low as 0.5 mg/kg bodyweight (BW) (-)-epicatechin intake but were optimal at 1 mg/kg BW** (hence 70mg is the optimal intake for a 70kg individual). These data provided **further evidence that (-)-epicatechin is a causal vasoactive molecule within flavanol-containing foods/beverages** [2].

In further FLAVIOLA studies using a CocoaVia test product containing 73 mg (-)-epicatechin (450mg total cocoa flavanols) the team also demonstrated:

1. in a study of 42 healthy men, that (-)-epicatechin improves vascular function (improved endothelial function in large arteries and enhanced vasodilator function in resistance arteries) and **reduces systolic blood pressure in elderly individuals** (n = 20), in a similar manner to that observed in the younger individuals (n= 22) [3]. Spencer *et al.*'s findings provided clear evidence and novel insights that scientifically **underpin the potential of dietary cocoa flavanols to reverse, or at least to attenuate, features of circulatory dysfunction associated with vascular ageing in healthy men**, a major contributor to atherogenic risk independent of age.
2. that male and female vascular responses to cocoa flavanol intake were statistically similar (in a randomised, controlled trial (RCT) in 100 healthy men and women aged 35-60 years) [4].
3. that substantial interactions between cocoa flavanols and methylxanthines such as caffeine and theobromine (which were also present in the test product) occur during absorption; the methylxanthines mediate an increased plasma concentration of (-)-epicatechin metabolites (up to 37% higher than from cocoa flavanols alone). This coincides with enhanced vascular effects commonly ascribed to cocoa flavanol intake (evidence from four RCTs in 47 healthy volunteers which measured flow-mediated vasodilation, brachial pulse wave velocity, circulating angiogenic cells and blood pressure) [5].

Spencer and Kuhnle also addressed a key challenge of attaining an accurate measurement of flavanol concentration in blood after eating. They established a new protocol which minimised the loss or degradation of flavanols and metabolites during sample preparation [6]. This work was validated across the FLAVIOLA consortium through inter-laboratory ring trials and was successfully employed to analyse the large number of samples that emanated from FLAVIOLA activities.

Demonstrating the health benefits of flavanone-rich orange juice

Research at Reading showed **for the first time that dietary flavanones (found in citrus fruits) are capable of counteracting transient impairments in vascular function induced postprandially** by a double meal challenge [7]. In a randomised, controlled, double-masked, cross-over intervention study in 28 healthy middle-aged men, improvements in vascular function coincided with the peak of naringenin/hesperetin metabolites in the circulation (7h) following the consumption of flavanone-containing beverages (flavanone-rich orange juice and homogenised whole orange). Sustained levels of plasma nitrite indicated that citrus flavanones are effective at counteracting the negative impact of a sequential double meal on human vascular function, potentially through the actions of flavanone metabolites on nitric oxide.

The flavanone content of standard orange juice is lower than in whole fruit: flavanones are removed during the juicing process along with pomace – the (edible) fibrous component of orange segment walls. In additional RCTs, Spencer and colleagues found that the addition of pomace to orange juice elicited a significantly beneficial effect on blood glucose and insulin responses after sequential meals in men with moderate cardiometabolic risk factors [8]. They postulated that the high levels of polyphenols in pomace may have contributed to the observed postprandial responses, along with higher levels of soluble fibre in pomace (compared to whole

fruit), which they suggested may have slowed stomach emptying and thereby contributed to enhanced satiety.

3. References to the research

The flavanol research outlined in this case study has been supported by competitive awards from BBSRC and Mars (BB/F008953/1, BB/G005702/1) and through the EUR 4,000,000 FLAVIOLA project ([Grant ID 226588](#)) in which Spencer led work package 1. Research on flavanone-rich orange juice was supported by a grant from PepsiCo (PEP1122), Human trials were registered via clinicaltrials.gov (e.g. NCT01639781; NCT02149238). Presented below is a small selection of peer-reviewed papers, which were published in internationally recognised journals, including the *American Journal of Clinical Nutrition*. This includes work that underpinned the first AOAC accredited method for cocoa flavanol analysis in food products.

1. **Williams C.M., Abd El Mohsen M., Vauzour D., Butler L.T., Ellis J.A., Whiteman M., Spencer J.P.E.** (2008). 'Blueberry-induced changes in spatial working memory are associated with changes in hippocampal CREB phosphorylation and BDNF levels'. *Free Radical Biology and Medicines*, **45** (3), 295-305. DOI: <https://doi.org/10.1016/j.freeradbiomed.2008.04.008>
2. **Alañón M.E., Castle S.M., Serra G., Lévèques A., Poquet L., Actis-Goretta L., Spencer J.P.E.** (2020). 'Acute study of dose-dependent effects of (-)-epicatechin on vascular function in healthy male volunteers: A randomized controlled trial'. *Clinical Nutrition*, **39**(3), 746-754. DOI: <https://doi.org/10.1016/j.clnu.2019.03.041>
3. Heiss C., Sansone R., Karimi H., Krabbe M., Schuler D., Rodriguez-Mateos A., Kraemer T., Cortese-Krott M.M., **Kuhnle G.G., Spencer J.P.**, Schroeter H., Merx M.W., Kelm M.; FLAVIOLA Consortium, European Union 7th Framework Program (2015). 'Impact of cocoa flavanol intake on age-dependent vascular stiffness in healthy men: a randomized, controlled, double-masked trial'. *AGE*, **37**(3), 9794. DOI: <https://doi.org/10.1007/s11357-015-9794-9>.
4. Sansone R., Rodriguez-Mateos A., Heuel J., Falk D., Schuler D., Wagstaff R., **Kuhnle G.G., Spencer J.P.**, Schroeter H., Merx M.W., Kelm M., Heiss C.; Flaviola Consortium, European Union 7th Framework Program. (2015). 'Cocoa flavanol intake improves endothelial function and Framingham Risk Score in healthy men and women: a randomised, controlled, double-masked trial: the Flaviola Health Study'. *British Journal of Nutrition*, **28**, 114(8), 1246-55. DOI: <https://doi.org/10.1017/S0007114515002822>.
5. Sansone R., Ottaviani J.I., Rodriguez-Mateos A., Heinen Y., Noske D., **Spencer J.P.**, Crozier A., Merx M.W., Kelm M., Schroeter H., Heiss C. (2017). 'Methylxanthines enhance the effects of cocoa flavanols on cardiovascular function: randomized, double-masked controlled studies'. *American Journal of Clinical Nutrition*, **105**(2), 352-360. DOI: <https://doi.org/10.3945/ajcn.116.140046>.
6. Ottaviani Javier I., Momma Tony Y., **Kuhnle Gunter G.**, Keen Carl L., Schroeter Hagen (2012). 'Structurally related (-)-epicatechin metabolites in humans: Assessment using de novo chemically synthesized authentic standards'. *Free Radical Biology and Medicine*, **52** (8), 1403-1412. DOI: <https://doi.org/10.1016/j.freeradbiomed.2011.12.010> .
7. **Rendeiro C., Dong H.**, Saunders C., Harkness L., Blaze M., Hou Y., Belanger R.L., **Corona G., Lovegrove J.A., Spencer J.P.E.** (2017). 'Flavanone-rich citrus beverages counteract the transient decline in postprandial endothelial function in humans: a randomised, controlled, double-masked, cross-over intervention study'. *British Journal of Nutrition*, **118**(8), 638. DOI: <https://doi.org/10.1017/S0007114516004219>.
8. **Dong H., Rendeiro C., Kristek A., Sargent L.J.**, Saunders C., Harkness L., **Rowland I., Jackson K.G., Spencer J.P., Lovegrove J.A.** (2016). 'Addition of Orange Pomace to Orange Juice Attenuates the Increases in Peak Glucose and Insulin Concentrations after Sequential Meal Ingestion in Men with Elevated Cardiometabolic Risk'. *The Journal of Nutrition*, **146**(6), 1197-203. DOI: <https://doi.org/10.3945/jn.115.226001>.

4. Details of the impact

Research led by Spencer *et al.* to better understand how dietary flavonoids can mediate clinically significant health benefits has underpinned the development of two functional food products by two leading multinational food and drink companies.

PepsiCo develops and commercialises Tropicana Whole Fruit

Collaborative research between PepsiCo and the University of Reading, to understand the physiological impact of flavanone-rich, high-fibre pomace and juice consumption, was used as justification by PepsiCo for three additional clinical studies [text removed for publication]. *“Ultimately, University of Reading research contributed to a body of research demonstrating that orange pomace is a dietary fiber with functionality in glycemic control, satiety, and digestive health. This nutrition science research was instrumental in driving a commitment to R&D investment and business innovation using fruit pomace.”* Principal Scientist, PepsiCo USA [E1].

As a result, PepsiCo installed new equipment [text removed for publication] to develop and commercialize a proprietary enzyme-treated pomace ingredient, and numerous patent applications have been filed to protect PepsiCo’s method for producing pomace with an improved sensory and nutritional profile (e.g. US20200236976A1, and [E2]). *“This was a significant and first-of-its-kind innovation for Tropicana to be able to add more nutritional value to the daily diet of juice drinks with a functional dietary fibre from the fruit itself.”* Principal Scientist, PepsiCo USA.

Tropicana Whole Fruit, consisting of both apple and orange drinks, was launched in May 2019. The new range was viewed by PepsiCo as a premium addition to the brand, and not merely a line extension, as evidenced by the 83% higher price (GBP 0.44 per 100ml) compared to standard Tropicana orange and apple juice products (GBP 0.24 per 100ml, November 2019 prices). [text removed for publication] [E3].

Tropicana Whole Fruit products contained 150% more fibre than standard Tropicana orange and apple juices, when compared in November 2019 [E4]. A single portion of Whole Fruit juice (both orange and apple) contained 2.3g fibre, equating to 7.6% of UK recommended average intake. Only 30% of UK adults and approximately 10% of 11–18-year-olds meet the ‘5-a-day’ recommendation for fruit and vegetables (Public Health England, 2019). This is a public health concern: diets low in the essential nutrients (vitamins, minerals, dietary fibre) as well as polyphenols provided by fruit and vegetables are at higher risk of developing chronic diseases such as CVD and diabetes. The Tropicana Whole Fruit range was developed with this concern in mind, appealing to health conscious but time-poor consumers. *“Some of the reasons people give for not eating enough fruit and veg include lack of convenience and messiness,” ... “This is especially true of people living an on-the-go lifestyle. The Whole Fruit range seeks to break that down – by packaging up more of the whole fruit in an easy to consume 150ml format”.* Marketing Manager for juices at PepsiCo UK [E5].

Mars, Inc. reformulates CocoaVia® with higher flavanol content

Initial flavanol research conducted at the University of Reading, which demonstrated the beneficial changes to the gut microflora from consumption of flavanols as well as improvements to cognitive and vascular function, supported the market launch of the CocoaVia® dietary supplement in 2010. The original product formulation contained 250mg total cocoa flavanols/30mg of (-)-epicatechin per serving. Further collaborative research with Mars, Inc., led by Spencer as part of the EU FLAVIOLA project, showed that this flavanol concentration was below the active amount required for a physiologically beneficial effect. As a result, Mars, Inc. reformulated CocoaVia® to contain 450mg of total flavanols and 73mg of (-)-epicatechin per serving, which was in line with the intake range demonstrated by Spencer and colleagues to increase vascular function. Furthermore, scientific evidence on the benefits of cocoa flavanols is highlighted on the ‘See the Science’ page of the CocoaVia® website with Reading research providing eight of the 30 papers cited. On the benefits of heart health specifically, two of the three scientific papers cited are from Reading research [3],[4], [E8]. Since 2016, CocoaVia’s product packaging has also highlighted the role of cocoa flavanols in promoting healthy blood flow [E6].

The reformulated 450mg product has been on sale to US consumers since August 2019. Sales data for the 450mg product was not available [text removed for publication] [E7].

Enabling accurate flavanol analysis across the food industry

Spencer and Kuhnle's analytical expertise and validated protocol for measuring flavanol intake in biological samples underpinned a collaborative study, with Mars, Inc scientists, that resulted in the first AOAC-accredited method (2012.24) for the analysis of cocoa flavanols and procyanidins in food products [E9, Robbins 2013]. In August 2020, the AOAC adopted a new method of analysis (2020.05) which 'revised and expanded' this previous methodology, on which Mars commented: *"This opens the door for consistent product labelling, ultimately empowering consumers by providing them reliable information to compare products, which is particularly important given the wide variability in flavanol content that exists in the marketplace today,"* [E10]. Independent laboratory analysis confirmed that CocoaVia® contained the highest level of flavanols of any supplement on the market in 2020 [E8].

Summary: Evidence from clinical trials conducted by Spencer and colleagues has underpinned the development and launch of the Whole Fruit drink range by PepsiCo, which contained higher amounts of a functional dietary fibre compared to standard juice products, and the reformulation of a Mars, Inc dietary supplement to include higher levels of flavanols to boost vascular function. The team's expertise and protocol has also underpinned the first AOAC-accredited method for the analysis of cocoa flavanols and procyanidins in food products, enabling consistent food labelling across products to aid consumer choice.

5. Sources to corroborate the impact

- [E1] Testimonial from Principal Scientist, PepsiCo USA.
- [E2] News article: [Patent watch: PepsiCo explores how to make juice as nutritious as whole fruit](#), September 2014.
- [E3] Tropicana Whole Fruit Sales Data, May 2019 to June 2020.
- [E4] Publicly available nutritional data for Tropicana Whole Fruit and original fruit juices.
- [E5] Grocery Trader Article: [Tropicana launches a 'whole' new way to drink fruit](#), May 2019.
- [E6] News article: [CocoaVia available in Walgreens stores](#), November 2016.
- [E7] US sales data for CocoaVia 250mg product, 2015-2018.
- [E8] CocoaVia ['See the Science'](#) webpage.
- [E9] R. J. Robbins, J. Leonczak, J. Li, J. C. Johnson, T. Collins, C. Kwik-Urbe, H. H Schmitz, Collaborators: including Kuhnle G., and Spencer J. (2013). 'Flavanol and Procyanidin Content (by Degree of Polymerization 1–10) of Chocolate, Cocoa Liquors, Cocoa Powders, and Cocoa Extracts: First Action 2012.24'. *Journal of AOAC INTERNATIONAL*, **96** (4), 705–711. DOI: <https://doi.org/10.5740/jaoacint.13-109>
- [E10] AOAC press release: [Revised and expanded analytical test adopted as new Official Method of Analysis by AOAC INTERNATIONAL food science experts](#), August 2020.