

# Multi-laboratory validation of a standard method for quantifying proanthocyanidins in cranberry powders

Ronald L Prior,<sup>a\*</sup> Ellen Fan,<sup>b</sup> Hongping Ji,<sup>c</sup> Amy Howell,<sup>d</sup> Christian Nio,<sup>e</sup> Mark J Payne<sup>f</sup> and Jess Reed<sup>g</sup>

## Abstract

**BACKGROUND:** The objective of this study was to validate an improved 4-dimethylaminocinnamaldehyde (DMAC) colorimetric method using a commercially available standard (procyanidin A2), for the standard method for quantification of proanthocyanidins (PACs) in cranberry powders, in order to establish dosage guidelines for the uropathogenic bacterial anti-adhesion effect of cranberry.

**RESULTS:** Commercially available cranberry samples were obtained (five from U.S. sources and six from European sources) for PAC quantification in five different analytical laboratories. Each laboratory extracted and analyzed the samples using the improved DMAC method. Within-laboratory variation (mean  $\pm$  SD) was  $4.1 \pm 1.7\%$  RSD (range, 2.3–6.1% RSD) and the between laboratory variability was  $16.9 \pm 8.5\%$  RSD (range, 8–32% RSD). For comparative purposes, the cranberry samples were alternatively quantified using weights of extracted PACs (gravimetric). The correlation coefficient between the two methods was 0.989.

**CONCLUSION:** This improved DMAC method provides a simple, robust and relatively specific spectrophotometric assay for total PACs in cranberry samples using commercially available procyanidin A2 dimer as a standard. DMAC is most useful within a given type of food such as cranberries, but may not be appropriate for comparing concentrations across different food types, particularly in those cases where large differences exist among the relative amounts of each oligomer and polymer.

© 2010 Society of Chemical Industry

**Keywords:** proanthocyanidins; cranberry; DMAC; BL-DMAC; urinary tract infection; bacterial adhesion

## INTRODUCTION

Cranberry (*Vaccinium macrocarpon* Ait.) has been utilized traditionally for the prevention of urinary tract infections (UTIs), and clinical research supports this claim.<sup>1,2</sup> An important mechanism of action may be the bacterial anti-adhesion activity attributed to ingesting cranberry products.<sup>3</sup> Proanthocyanidins (PACs) in cranberry have been shown to inhibit primarily P-fimbriated *Escherichia coli* adhesion to uroepithelial cells *in vitro*<sup>4–7</sup> and *ex vivo*,<sup>8</sup> interfering with this primary step in the infection process.<sup>9</sup> PACs, also referred to as condensed tannins, are oligomers and polymers of monomeric flavan-3-ols, such as catechin and epicatechin (Fig. 1). The PACs in cranberry contain a high proportion of the unusual A-type double linkages which may be important structural features in the anti-adhesion process.<sup>5</sup> In one study, other food sources of PACs that contain only B-type linkages (chocolate, grape, apple and green tea) were consumed. However, they did not elicit *ex vivo* bacterial anti-adhesion activity in urine following ingestion. Only cranberry juice with A-type PACs prevented bacterial adhesion.<sup>10</sup>

The current recommended daily dosage of cranberry for UTI prevention is based on the efficacious levels that have been administered in human intervention trials. Often, the daily consumption of 300 mL of Cranberry Juice Cocktail (a 27% cranberry

juice preparation made by Ocean Spray Cranberries, Inc., containing 36 mg PACs, measured by the DMAC colorimetric method

\* Correspondence to: Ronald L Prior, USDA, Arkansas Children's Nutrition Center, 15 Children's Way, Little Rock, AR 72202, USA.  
E-mail: priorronaldl@uams.edu

a USDA-ARS, Arkansas Children's Nutrition Center, 15 Children's Way, Little Rock, AR 72202, USA

b Brunswick Laboratories, 50 Commerce Way, Norton, MA 02766, USA

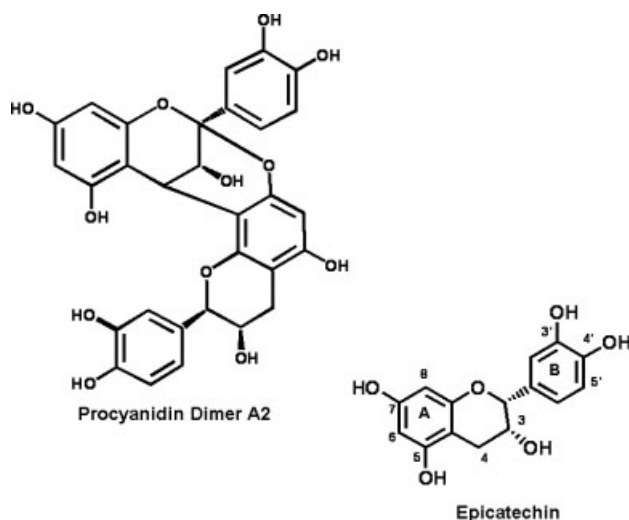
c Brunswick Laboratories (China), 320, A3 Building, 218 Xing Hu Rd, Suzhou Industrial Park, Suzhou, Jiangsu, China

d Rutgers University, Marucci Center for Blueberry Cranberry Research, 125A Lake Oswego Rd., Chatsworth, NJ 08019, USA

e Agrobio, ZAC des Trois Marches, 3, impasse de la jonchée, F-35132 Vezin Le Coquet, France

f Hershey Center for Health and Nutrition, The Hershey Company, 1025 Reese Avenue, Hershey, PA 17033, USA

g Department of Animal Sciences, Environmental Toxicology Center, International Agriculture, 256 Animal Science Bldg., 1675 Observatory Drive, Madison, WI 53706, USA



**Figure 1.** Numbering system for flavanoid structure (epicatechin) and structure of procyanidins dimer A2.

(an aldehyde condensation of 4-dimethylaminocinnamaldehyde), has been recommended. This dose has been shown to reduce bacteriuria and pyuria in a large clinical trial.<sup>11</sup> Other types of cranberry products, including encapsulated powders, have also demonstrated activity *in vitro*,<sup>6</sup> *ex vivo*<sup>12,13</sup> and *in vivo*.<sup>14</sup>

A number of analytical procedures including colorimetric, gravimetric, chromatographic and mass spectrometric methods are currently being employed for the measurement of cranberry PACs;<sup>15–17</sup> however, due to the complexities of the PAC structures and A-type linkages, the results can often be erroneous and may not be reproducible.<sup>18</sup> Gravimetric methods involve fractionating PACs and weighing the total content. This technique can be expensive and labor intensive. Mass spectrometry may be useful for authentication, but it is not suitable for quantification unless standards are available and response factors for the individual oligomers are known. Normal-phase high-performance liquid chromatography methods are effective at quantifying PACs with B-type linkages, such as those in chocolate, grapes and other foods,<sup>19,20</sup> but may be less effective at estimating total PACs for cranberry due to the heterogeneous A-linked PACs and lack of appropriate standards.

Colorimetric assays are advantageous because they are normally inexpensive, rapid, and simple to perform. Certain colorimetric assays are specific for flavan-3-ols and PACs and depend on the reaction of chemical reagents with sites on the PAC molecule. However, a lack of appropriate standards and interferences from other sample components, such as anthocyanins and extraction solvents, can lead to over- or underestimation of PACs. One such method that is subject to overestimation of PACs is the European Association for the Valorization of Cranberry (EuraCran) method, a modification of the acid catalyzed autoxidation reaction that leads to the formation of anthocyanidins from PACs. The resulting anthocyanidins are red in color and are then quantified by measuring their absorbance at 545 nm. Since the native anthocyanins present in the whole cranberry sample are not removed prior to the reaction, their presence may confound the results, leading to an overestimation of PACs. Even though this acid–butanol assay is a classical method, it is not well suited to quantification.<sup>21,22</sup> Several of the shortcomings of this method have been outlined in a review.<sup>22</sup> An additional review

of colorimetric quantification methods for cranberry PACs can be found in the paper by Howell.<sup>23</sup>

DMAC is a colorimetric method that appears to be more accurate than other methods and has been successfully used to quantify cranberry PACs.<sup>15</sup> It is less likely to have interferences from sample components, such as anthocyanins, since the reaction is read at 640 nm. The DMAC method was used to substantiate French health claims for the bacterial anti-adhesion activity of cranberry juice concentrate and juice concentrate extract powder granted in 2004, cranberry juice cocktail in 2005, and fresh frozen and puréed cranberry in 2007 by the French food safety authority, AFSSA (Agence française de sécurité sanitaire des aliments), which concluded that the daily intake of 36 mg of PAC in cranberry contributes to decreasing adhesion of certain uropathogenic P-fimbriated *E. coli* to the walls of the urinary tract. However, the quantification method used in the 2002 petition, the ‘old DMAC (ref. PAC 003)’ involves using a proprietary standard which is not commercially available. This limits the widespread use of this method for quantification of PACs in cranberry products and justifies the need to develop an improved method.

Accurate standardization of PAC content is vital to establishing dosage guidelines for consumers, monitoring efficacy and shelf-life of dietary supplements and other products, and to formulate standardized test materials for use in research studies. Currently, there is no universally accepted method for quantification of cranberry PACs. The objective of this study was to validate an improved ‘DMAC’ method (originally modified and improved by Brunswick Labs, Norton, MA, USA) which uses a commercially available A2 dimer standard (Fig. 1). This method could be utilized worldwide as an accurate, reproducible, inexpensive and rapid standard method for quantification of PACs in cranberry powders with an outcome similar to that obtained using the previous DMAC method for the reference quantity of 300 mL of Cranberry Juice Cocktail (36 mg PAC). Under these circumstances, the existing health claims in France can be upheld with the current value of 36 mg PAC and also be validated by competent authorities.

## EXPERIMENTAL

### Chemicals

1. HPLC grade water was from a Millipore MilliQ R/O water purification system (Millipore, Millipore, Bedford, MA)
2. HPLC grade methanol (99.9%) was from Fisher Scientific, Houston, TX; cat. #A452-4
3. HPLC grade reagent alcohol (91%) was from Fisher Scientific; cat. #A995-4
4. HPLC grade acetone was from Burdick & Jackson, Morris Township, NJ; cat. #AH010-4
5. 4-Dimethylaminocinnamaldehyde (DMAC; F.W. 175.23) was from Sigma, St Louis, MO, USA; cat. #EC-228-267-0
6. Hydrochloric acid (37%; 12.5 mol L<sup>-1</sup>) was from Sigma; cat. #320331-500
7. Acetic acid (glacial) was from Fisher Scientific; cat. #A35-500
8. Procyanidin A2 (HPLC; purity >99%) was from Extrasynthèse, Genay Cedex, France; cat. #0985 S, lot #0808041

### Preparation of working reagents for the BL-DMAC method

1. *PAC extraction solvent.* Acetone (75 mL) was transferred to a glass bottle containing 24.5 mL of deionized water and 0.5 mL of acetic acid was added. This solution is stable for 1 year at 18–25 °C.

2. **Acidified ethanol.** Concentrated (36%) hydrochloric acid (12.5 mL) was added to 12.5 mL distilled water and 75 mL of ethanol (91%) in a glass bottle and mixed. This solution is stable for 1 year at 18–25 °C.
3. **Dilution solution.** Ethanol (91%) (80 mL) was added to 20 mL of deionized water and mixed.
4. **Elution solution.** Ethanol (91%) (80 mL) was added to 19.5 mL of deionized water, 0.5 mL acetic acid and mixed.
5. **DMAC reagent (0.1%).** DMAC (0.05 g) was weighed out and added to 50 mL of acidified ethanol (reagent 2, above). This reagent was made fresh daily.

#### Procyanidin A2 calibration standard

Each laboratory was responsible for purchasing the standard (100 µg mL<sup>-1</sup>). Procyanidin A2 (5 mg) was weighed out and quantitatively transferred to a 50 mL volumetric flask. Ethanol (91%) was added to give a final concentration of 100 µg mL<sup>-1</sup>. Aliquots were placed in 1.5 mL HPLC sample vials and stored at -80 °C. These standards were stable for at least 6 months. A procyanidin A2 control was prepared by transferring 1 mL of the 100 µg mL<sup>-1</sup> working procyanidin A2 solution into a 1.5 mL conical tube, and 250 µL of ethanol (91%) was added and vortexed to give a 80 µg mL<sup>-1</sup> procyanidin A2 as a quality control sample.

#### Extraction of PACs from cranberry powders

Dried powders of either whole cranberry or non-selective food grade concentrates of cranberry were weighed (20–1000 mg) into a 50 mL conical tube. The PAC extraction solution (20 mL) was added to the samples. The samples were vortexed for 30 s followed by sonication at room temperature for 30 min. Samples were then placed on an orbital shaker for 1 h and subsequently centrifuged at 2000 × *g* at 20 °C for 10 min. The supernatant was collected for analysis.

#### Extraction of PACs from cranberry juice sample

A C18 column (1cc, LCR 100 mg; Varian, Palo Alto, CA; part# 121130001, lot# 0710105MFG) was washed with water with care taken to keep the column wet with the water meniscus just touching the surface of the C18 packing. One milliliter of cranberry juice was added to each column and allowed to pass through the column by gravity. The column was washed twice with 1.0 mL water and eluted twice with 1.0 mL of elution solution (reagent 4, above). Two milliliters of the eluted extract was collected in a 5 mL tube and vortexed to mix well prior to analysis.

#### 96-Well plate layout

The plate reader protocol was set to read the absorbance (640 nm) of each well in the plate every minute for 30 min. The frequency of reading may vary with the particular microplate reader used. The plate included blanks, standards, controls, and unknowns at serial dilutions of 1-, 2-, 4-, 8-, 16-, and 32-fold as appropriate.

#### Analysis

The incubation chamber was pre-heated to 25 °C, and the system was allowed to equilibrate. A digital pipettor was used to dispense into wells of a 96-well plate one of the following: (1) 70 µL of 80% ethanol for blanks; or (2) 70 µL of control, standard, and samples. The DMAC solution (210 µL) was added using a multi-channel pipettor into all 96 wells (containing blanks, standards,

controls, and samples). Depending upon the particular brand of plate reader, some used an automatic pipettor in the plate reader to add the DMAC solution. The final volume was 280 µL well<sup>-1</sup>. The microplate was read for 25 min.

#### Calculations and statistical analyses

The maximum absorbance readings were used for calculation, which generally occurred before 20 min, depending on the dilution of the sample. Corrected absorbencies were calculated by subtracting the average blank absorbance and a calibration curve was generated from the standards. PAC concentrations were calculated by using a regression equation ( $Y = a + bX$ ) between procyanidin A2 concentration ( $Y$ ) (µg) and the maximum absorbance minus the blank ( $X$ ).

Concentrations of sample extracts were calculated as total PACs =  $(C \times D \times V)/(1000 \times S)$ , where the total PACs are in mg g<sup>-1</sup>;  $C$  is the concentration of PACs in a sample extract, in g L<sup>-1</sup>;  $D$  is the dilution factor;  $V$  is the extraction volume, in milliliters; and  $S$  is the sample size, in grams.

Data were expressed as milligrams of procyanidin A2 equivalents per gram or per 300 mL (juice) of sample.

The means and relative standard deviation (%RSD) for replicate analyses were calculated for each sample within each laboratory. The overall mean and %RSD between laboratories was calculated from the individual laboratory means. Two-way analysis of variance was performed on all powdered samples with laboratory and sample as independent factors. Interaction of laboratory and sample was also evaluated.

#### Gravimetric isolation of PACs

Cranberry powder was extracted to isolate total PACs using a gravimetric method<sup>10</sup> by only one laboratory (Rutgers University). Reverse phase (C18) followed by adsorption chromatography (Sephadex LH-20) were used to fractionate and isolate the total PACs effective at preventing P-type *E. coli* bacterial adhesion. An aqueous sample extract was loaded onto a C18 column, washed with water, and then a 15% methanol elution of sugars and acids, followed by acidified methanol (1% acetic acid) to elute the total polyphenolics. The total polyphenolics sample was dried under reduced pressure, reconstituted in 50% ethanol and loaded onto a Sephadex LH-20 column. The flavonol glycosides, anthocyanins and other low molecular weight flavonoid compounds were washed off with 50% ethanol, followed by elution with 70% acetone to recover the PAC fraction. The PAC elution was lyophilized and weighed to quantify the total PACs.

#### Cranberry sample materials

A total of 11 commercially available samples were obtained (five from US sources and six from European sources) for PAC quantification analysis. Powdered samples were coded and sent blinded to five different analytical laboratories (three in the US, one in China and one in Europe) for analysis of PACs by the BL-DMAC method. In order to obtain a reference quantification value for PAC levels in cranberry powder samples and cranberry juice, each laboratory (except one) extracted a juice sample, and all laboratories analyzed all cranberry powder samples. Laboratories were instructed to perform triplicate extractions and to make serial dilutions of the extracts (1:2, 1:4, 1:8, 1:16, and 1:32) and analyze each dilution in triplicate.

**Table 1.** PAC concentrations<sup>†</sup> of cranberry samples analyzed in five different laboratories

ID#	Lab #1		Lab #2		Lab #3		Lab #4		Lab #5		Overall		P <sup>‡</sup>
	Mean	RSD %	Mean	RSD %	Mean	RSD %	Mean	RSD %	Mean	RSD %	Mean	RSD %	
09-0150	0.77	1.30	0.63	2.26	0.58	15.58	0.65	4.94	0.53	6.98	0.63	14.3	NS
08-3733	2.46	2.09	2.41	1.17	2.86	2.21	2.05	8.00	2.03	0.00	2.36	14.4	NS
08-3731	5.61	0.57	6.10	1.74	4.81	7.43	2.37	6.77	4.73	3.35	4.72	30.3	NS
09-0145	11.86	3.10	10.24	7.32	10.62	1.87	4.22	6.06	8.75	5.19	9.14	32.4	NS
09-0148	11.14	0.99	11.08	2.23	11.27	9.64	10.05	3.02	8.52	1.43	10.41	11.2	NS
09-0149	24.27	0.96	23.24	5.36	22.04	8.35	20.27	0.74	18.61	1.30	21.68	10.5	NS
09-0147	33.08 <sup>a</sup>	5.10	33.52 <sup>ab</sup>	2.97	37.80 <sup>c</sup>	2.48	27.22 <sup>a</sup>	9.14	27.96 <sup>a</sup>	3.35	31.92	13.7	<0.001
08-3732	33.89 <sup>a</sup>	1.80	36.42 <sup>ab</sup>	2.12	40.34 <sup>c</sup>	11.44	25.34 <sup>a</sup>	8.75	29.33 <sup>a</sup>	1.20	33.06	17.8	<0.001
08-3730	37.26 <sup>b</sup>	4.37	40.76 <sup>c</sup>	0.78	46.15 <sup>d</sup>	1.12	–	–	32.73 <sup>a</sup>	2.65	38.62	13.3	<0.05
09-0146	144.4 <sup>b</sup>	4.58	155.6 <sup>c</sup>	0.04	152.8 <sup>c</sup>	5.18	139.5 <sup>b</sup>	1.85	128.5 <sup>a</sup>	3.13	144.2	7.5	<0.001
09-0144	185.9 <sup>b</sup>	10.16	185.3 <sup>b</sup>	0.21	195.2 <sup>c</sup>	2.50	165.3 <sup>a</sup>	7.99	160.0 <sup>a</sup>	3.80	179.0	9.4	<0.001

<sup>†</sup> Concentrations of PACs expressed as g kg<sup>-1</sup> dried powder except for sample 08–3730 which was a cranberry juice cocktail in which the concentration is expressed as mg of PACs in 300 mL of juice.

<sup>‡</sup> Significance of interaction term of laboratory by sample in two-way analysis of variance for all samples except cranberry juice (08–3730) which was analyzed by one-way analysis of variance.

<sup>a–d</sup> Results without a common superscript are significantly different ( $p < 0.05$  or  $p < 0.001$ ). RSD, relative standard deviation (%).

## RESULTS AND DISCUSSION

The DMAC reagent has been used previously for the detection of catechins and PACs for their selective detection following HPLC separation of crude extracts;<sup>24</sup> as a sensitive, selective derivatization method for densitometric determination of catechins<sup>25</sup> and as a reliable and sensitive method for PAC screening in plants.<sup>26</sup> More recently the DMAC method has been used for the determination of total PACs in chocolate.<sup>27</sup> In this manuscript we report on an extension of the use of this method for determination of PACs in cranberry which inherently contain both B-type and A-type linkages.

The reaction mechanism of the DMAC reagent with a molecule is not clearly defined although it seems to react with compounds having free *meta*-oriented hydroxyl groups in the flavonoid molecule and with a single bond at the 2,3-position of the C-ring (Fig. 1).<sup>28</sup> Previous work with the DMAC reagent has shown that there is a high degree of selectivity for flavanols.<sup>27,28</sup> Color development appears to depend on the structural conformation of the flavanol with the color yield with procyanidin B<sub>3</sub> being less than 50% of that with catechin or epicatechin.<sup>28</sup> Procyanidin B<sub>3</sub> has a C–C intermonomer linkage involving the C-4 position of one monomer and the C-8 position of the other monomer. Although it has been suggested that the DMAC reagent reacts with only one monomeric unit in each PAC molecule,<sup>28,29</sup> this has not been clearly demonstrated in the literature. Depending upon their structural configuration, large polymeric compounds may not be detected with as much sensitivity with the DMAC reagent as a monomer.

Concentrations of PACs in the powdered cranberry samples obtained commercially ranged from 0.63 to 177 mg g<sup>-1</sup> based upon the mean concentrations determined from the analyses by five different laboratories (Table 1). Samples were extracted in triplicate by these laboratories and thus the overall variability within a laboratory represents errors associated with extraction as well as analytical variability of the BL-DMAC method. The within-laboratory variation (mean  $\pm$  SD) across all samples was 4.1  $\pm$  1.7% RSD (range, 2.3–6.1% RSD) which is slightly better

than observed previously with chocolate samples (6.6% RSD). The between-laboratory variability was 16.9  $\pm$  8.5% RSD (range, 8–32% RSD).

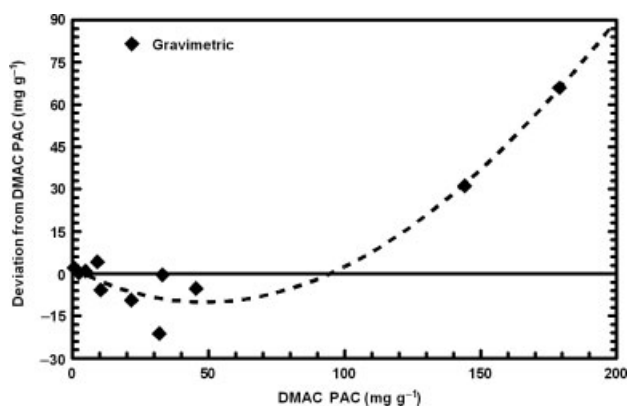
Data from cranberry powders were analyzed by two-way analysis of variance with laboratory and sample as independent variables. Interactions of laboratory by sample were also analyzed. The interaction term was not significant ( $P > 0.05$ ) for samples with lower PAC concentrations (Table 1), but was at higher concentrations. Repeatability of the PAC concentrations was best within an optical density reading on the photometer between 0.2 and 0.8 OD. The responses for the calibration curve were linear in the concentration range of 3.125–100  $\mu$ g mL<sup>-1</sup>. The laboratories were not given specific instructions relative to dilutions to use for quantification which may account for the greater variability among labs at the higher concentrations. Thus, for best repeatability, concentrated samples should be diluted so that they fall within the range of linearity below 100  $\mu$ g mL<sup>-1</sup>.

Cranberry samples were also analyzed quantitatively by a gravimetric method<sup>10</sup> in which the PACs were isolated, dried and weighed (Table 2). This technique was performed by the Rutgers University laboratory, where it is used routinely, and in this study served as a comparative check for the accuracy of the BL-DMAC method. The within-laboratory variation of the gravimetric method was 15.2  $\pm$  15.7% RSD. Because of the complexity of the PACs, there is no 'gold standard' to use in validating a chemical method and the gravimetric method seemed to be a reasonable approach for comparison, realizing that it is not without deficiencies. The amount of PACs determined to be present in the samples using gravimetric weighing was greater than that determined with the BL-DMAC method in two samples with high concentrations of PACs (Fig. 2 and Table 2). The linear relationship between the two methods (Fig. 2) had a correlation coefficient of 0.9892, but there was deviation below the BL-DMAC mean with the gravimetric method at concentrations in the range of 20–30 mg g<sup>-1</sup> and a large deviation above in samples with higher PACs concentrations. These two samples also had larger relative quantities of polymers than the other samples. In these later sam-

**Table 2.** Comparison of analytical methods for the analysis of PACs in cranberry products

Sample	DMAC (D)		Gravimetric (G)		Deviation (G – D) (mg g <sup>-1</sup> )
	Mean	RSD (%)	Mean	RSD (%)	
09-0150	0.63	14.3	2.67	43.0	2.04
08-3733	2.36	14.4	2.67	43.0	0.31
08-3731	4.72	30.3	5.67	10.0	0.95
09-0145	9.14	32.4	13.33	4.0	4.19
09-0148	10.41	11.2	4.67	25.0	-5.74
09-0149	21.68	10.5	12.33	20.0	-9.35
09-0147	31.92	13.7	10.67	11.0	-21.25
08-3732	33.06	17.8	32.67	4.0	-0.39
08-3730	45.40	24.7	40.14	5.0	-5.26
09-0146	144.15	7.5	175.33	1.0	31.18
09-0144	178.98	9.4	244.00	1.0	66.00

Data expressed as means (mg g<sup>-1</sup>) with coefficient of variation (%) of triplicate analyses from five laboratories for BL-DMAC and one laboratory for the gravimetric method. RSD, relative standard deviation (%).



**Figure 2.** Deviation in proanthocyanidin (PAC) concentrations using gravimetric method (Y) of quantitation versus the BL-DMAC method (X). Correlations coefficient was 0.972. Polynomial regression coefficients ( $Y = a_0 + a_1X + a_2X^2 + a_3X^3$ ) were as follows: (2.2572, -0.4980, 0.00497).

ples, the BL-DMAC method would be expected to underestimate the quantity relative to the gravimetric method (Fig. 2), if indeed the DMAC reagent reacts with only one monomeric unit per PAC molecule. There were also differences in extraction of PACs for the gravimetric determination compared to the extract used for DMAC determination. Because of these differences, we do not expect a strong direct correlation between the two methods.

## CONCLUSION

Quantification of the total PACs in cranberry powder samples using the improved BL-DMAC method is important in the evaluation and standardization process. Universal adoption of this DMAC standard reference method worldwide by the cranberry industry will allow producers to use one standard method to ensure accurate labeling of PAC levels in products, and aid consumers in selecting products with sufficient PAC levels to obtain bacterial anti-adhesion activity. Currently, there is no 'perfect' method for the quantification of PACs in cranberry powders and other botanical materials. The

biggest limitation to most methods is lack of a commercially available standard. The new BL-DMAC method provides a simple and relatively specific spectrophotometric assay for total PACs in cranberry powders, utilizing a commercially available procyanidin A2 standard. BL-DMAC is most useful within a given type of food such as cranberries, but may not be appropriate for comparing concentrations across different food types, particularly in those cases where large differences exist among the relative amounts of each oligomer and polymer.

Correlation of the PAC level with anti-adhesion bioactivity may be important to ensure that a particular cranberry product contains PACs that are efficacious and have not been degraded during processing, irreversibly bound to excipients, etc. This will help to ensure that an adequate anti-adhesion benefit is delivered to the consumer.

Furthermore, techniques such as mass spectrometry could be considered to authenticate cranberry powders by guaranteeing the presence of A-type linkages in the PACs. This would guard against adulteration of cranberry products with B-linked PACs or flavan-3-ols (epicatechin or catechin) from other less expensive food sources.

## ACKNOWLEDGEMENTS

Analytical laboratories which participated in the BL-DMAC validation tests were: USDA-ARS, Arkansas Children's Nutrition Center, 15 Children's Way, Little Rock, Arkansas, USA; Agrobio, ZAC des Trois Marches, 3, impasse de la jonchée, Vezin Le Coquet, France; Brunswick Laboratories, 50 Commerce Way, Norton, Massachusetts, USA; Brunswick Laboratories (China), 320, A3 Building, 218 Xing Hu Rd, Suzhou Industrial Park, Suzhou, Jiangsu, China; and Hershey Center for Health and Nutrition, The Hershey Company, 1025 Reese Avenue, Hershey, Philadelphia, USA. The research presented in this manuscript was supported in part by: USDA, ARS; Cranberry Institute, 3203-B Cranberry Highway East Wareham, Massachusetts, USA; Brunswick Laboratories, Norton, Massachusetts, USA; and Cranberry Marketing Committee, 219A Main Street, Wareham, Massachusetts, USA.

## REFERENCES

- Jepson RG and Craig JC, A systematic review of the evidence for cranberries and blueberries in UTI prevention. *Mol Nutr Food Res* **51**:738–745 (2007).
- Jepson RG and Craig JC, Cranberries for preventing urinary tract infections. *Cochrane Database of Systematic Reviews* **1**:(2008).
- Sobota AE, Inhibition of bacterial adherence by cranberry juice: potential use for the treatment of urinary tract infections. *J Urol* **131**:1013 (1984).
- Howell AB, Inhibition of the adherence of P-fimbriated *Escherichia coli* to uroepithelial-cell surfaces by proanthocyanidin extracts from cranberries. *N Engl J Med* **339**:1085–1086 (1998). [Erratum appears in *N Engl J Med* **339**:1408 (1998).]
- Foo LY, Lu Y, Howell AB and Vorsa N, A-type proanthocyanidin trimers from cranberry that inhibit adherence of uropathogenic P-fimbriated *Escherichia coli*. *J Nat Prod* **63**:1225–1228 (2000).
- Gupta K, Chou MY, Howell AB, Wobbe C, Grady R and Stapleton AE, Cranberry products inhibit adherence of uropathogenic *Escherichia coli* to primary cultured bladder and vaginal epithelial cells. *J Urol* **177**:2357–2360 (2007).
- Liu Y, Black MA, Caron L and Camesano TA, Role of cranberry juice on molecular-scale surface characteristics and adhesion behavior of *Escherichia coli*. *Biotechnol Bioeng* **93**:297 (2006).
- Howell AB, Leahy MM, Kurowska E and Guthrie N, *In vivo* evidence that cranberry proanthocyanidins inhibit adherence of P-fimbriated *E. coli* bacteria to uroepithelial cells. *FASEB J* **15**:A284 (2001).

- 9 Beachey EH, Bacterial adherence: Adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. *J Infect Dis* **143**:325 (1981).
- 10 Howell AB, Reed JD, Krueger CD, Winterbottom R, Cunningham DG and Leahy M, A-type cranberry proanthocyanidins and uropathogenic bacterial anti-adhesion activity. *Phytochemistry* **66**:2281–2291 (2005).
- 11 Avorn J, Monane M, Gurwitz JH, Glynn RJ, Choodnovskiy I and Lipsitz LA, Reduction of bacteriuria and pyuria after ingestion of cranberry juice. *J Am Med Assoc* **271**:751 (1994).
- 12 Howell AB, Cranberry capsule ingestion and bacterial anti-adhesion activity of urine. *FASEB J* **20**:LB454 (2006).
- 13 Lavigne JP, Bourg G, Combescure C, Botto H and Sotto A, *In-vitro* and *in-vivo* evidence of dose-dependent decrease of uropathogenic *Escherichia coli* virulence after consumption of commercial *Vaccinium macrocarpon* (cranberry) capsules. *Clin Microbiol Infect* **14**:350 (2008).
- 14 Walker EB, Barney DP, Mickelsen JN, Walton RJ and Mickelsen RA Jr, Cranberry concentrate: UTI prophylaxis. *J Fam Pract* **45**:167–168 (1997).
- 15 Cunningham DG, Vannozzi S, O'Shea E and Turk R, Analysis and standardization of cranberry products, in *Quality Management of Nutraceuticals*, ed. by Ho CT, Zheng QY, American Chemical Society, Washington, DC, pp. 151–166 (2002).
- 16 Gu L, Kelm MA, Hammerstone JF, Zhang Z, Beecher G, Holden J, *et al.*, Liquid chromatographic/electrospray ionization mass spectrometric studies of proanthocyanidins in foods. *J Mass Spectrom* **38**:1272–1280 (2003).
- 17 Gu L, Kelm MA, Hammerstone JF, Beecher G, Holden J, Haytowitz D, *et al.*, Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *J Agric Food Chem* **51**:7513–7521 (2003).
- 18 Mole S and Waterman PG, A critical analysis of techniques for measuring tannins in ecological studies. *Oecologia* **72**:148–156 (1987).
- 19 Hammerstone JF, Lazarus SA, Mitchell AE, Rucker R and Schmitz HH, Identification of procyanidins in cocoa (*Theobroma cacao*) and chocolate using high-performance liquid chromatography mass spectrometry. *J Agric Food Chem* **47**:490–496 (1999).
- 20 Gu L, Kelm MA, Hammerstone JF, Beecher G, Holden J, Haytowitz D, *et al.*, Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *J Nutr* **134**:613–617 (2004).
- 21 Schofield P, Mbugua DM and Pell AN, Analysis of condensed tannins: a review. *Anim Feed Sci Technol* **91**:21–40 (2001).
- 22 Waterhouse AL, Ignelzi S and Shirley JR, A comparison of methods for quantifying oligomeric proanthocyanidins from grape seed extracts. *Am J Enol Vitic* **51**:383–389 (2000).
- 23 Howell AB, Bioactive compounds in cranberries and their role in prevention of urinary tract infections. *Mol Nutr Food Res* **51**:732–737 (2007).
- 24 Treutter D, Chemical reaction detection of catechins and proanthocyanidins with 4-dimethylaminocinnamaldehyde. *J Chromatogr* **467**:185–193 (1989).
- 25 Glavnik V, Simonovska B and Vovk I, Densitometric determination of (+)-catechin and (–)-epicatechin by 4-dimethylaminocinnamaldehyde reagent. *J Chromatogr A* **15**:4485–4491 (2009).
- 26 Li Y-G, Tanner G and Larkin P, The DMACA-HCl protocol and the threshold proanthocyanidin content for bloat safety in forage legumes. *J Sci Food Agric* **70**:89–101 (1996).
- 27 Payne MJ, Hurst WJ, Stuart DA, Ou B, Fan E, Ji H, *et al.*, The use of the DMAC assay for the determination of total procyanidins in selected chocolate and confectionery products. *JAOAC Int* (In press) (2010).
- 28 McMurrough I and McDowell J, Chromatographic separation and automated analysis of flavanols. *Anal Biochem* **91**:92–100 (1978).
- 29 Nagel CW and Glories Y, Use of a modified dimethylaminocinnamaldehyde reagent for analysis of flavanols. *Am J Enol Vitic* **42**:364–366 (1991).