

TRANSLATION IN ENGLISH FROM THE ORIGINAL ARTICLE

QUALITOLOGY IN PHYTOTHERAPY

The quantitation of cranberry proanthocyanidins (PAC) in food supplements: challenges and latest developments¹

G.Haesaerts

Pharmatoka, 118 avenue Paul Doumer, F-92563 Rueil-Malmaison cedex, France
Correspondance : g.haesaerts@pharmatoka.fr

Sommaire : En avril 2004, l'Agence française de sécurité sanitaire des aliments (AFSSA maintenant « ANSES ») a publié un avis positif concernant l'anti-adhésion bactérienne des proanthocyanidines (PAC) de cranberry (*Vaccinium macrocarpon* exclusivement). La dose efficace validée est de 36 mg de PAC/j selon la méthode de dosage renseignée par le pétitionnaire (méthode DMAC/PAC003). D'autres méthodes existent, mais toutes donnent des résultats non comparables, variant de un à cinq dans la quantification des PAC. La méthode BL-DMAC a été spécifiquement développée, ensuite validée par cinq laboratoires internationaux et récemment publiée afin de proposer pour le dosage des PAC une méthode fiable, peu coûteuse et donnant des résultats statistiquement identiques à la méthode DMAC/PAC003. Cette standardisation est nécessaire pour garantir des produits bien dosés, comparables et efficaces pour le consommateur.

Mots clés : Proanthocyanidines – Cranberry *Vaccinium macrocarpon* – Anti-adhésion bactérienne – Infections urinaires – Méthode de mesure BL-DMAC – AFSSA

Abstract : In April 2004, AFSSA (Agence française de la sécurité des aliments, now « ANSES ») published a positive opinion concerning the bacterial anti-adherence of cranberry (*Vaccinium macrocarpon*) PAC. The validated efficacious dosage is 36 mg PAC/day using the petitioner's method (DMAC/PAC003). Other methods exist, however they all produce non comparable results, varying from 1 to 5 in PAC quantitation. The BL-DMAC method has been specifically developed and then validated by 5 international laboratories before its recent publication, in order to propose a reliable and cheap quantitation method for PAC, yielding statistically identical results to the DMAC/PAC003 method. This standardisation is indispensable in order to guarantee well dosed, comparable and efficacious products to the consumer.

Key words : Proanthocyanidins – Cranberry *Vaccinium macrocarpon* – Bacterial anti-adherence – Urinary infections - BL-DMAC quantitation method – AFSSA

The traditional use of cranberry juice to protect against urinary tract infections (UTI) has been established in North America for centuries but its scientific validation is of a recent date.

In April 2004, the French Agency for food health safety (AFSSA) has published a first positive opinion (2) (table 1) regarding the bacterial anti-adherence of cranberry (*Vaccinium macrocarpon*) proanthocyanidins (PAC). It was the first time worldwide that a national Authority issued a health claim for a food product based on fruits. This opinion was followed by two other positive opinions in 2005 and 2007 (3), attributing the same beneficial effects to other cranberry ingredients.

Table 1. Conclusion – opinion of AFSSA of 6 April 2004

Afssa concludes expressing their opinion that the data presented suggest that the consumption of *Vaccinium Macrocarpon* juice (containing 36 mg measured proanthocyanidins (PAC) leads to a decrease in frequency of urinary tract infections caused by certain uropathogenic P-fimbriated *E.coli* bacteria in adult women. The same effect has been reported with an encapsulated powder of *Vaccinium macrocarpon*.

The claim " contributes to decrease the fixation of certain *E.coli* bacteria to the walls of the urinary tract " is acceptable only for the fruit juice and the powder of the fruit juice of *Vaccinium macrocarpon*.

(1)Article extracted from a conference presented by the author at the 22nd Meeting of AMPP galenics and qualitology, June 2009.

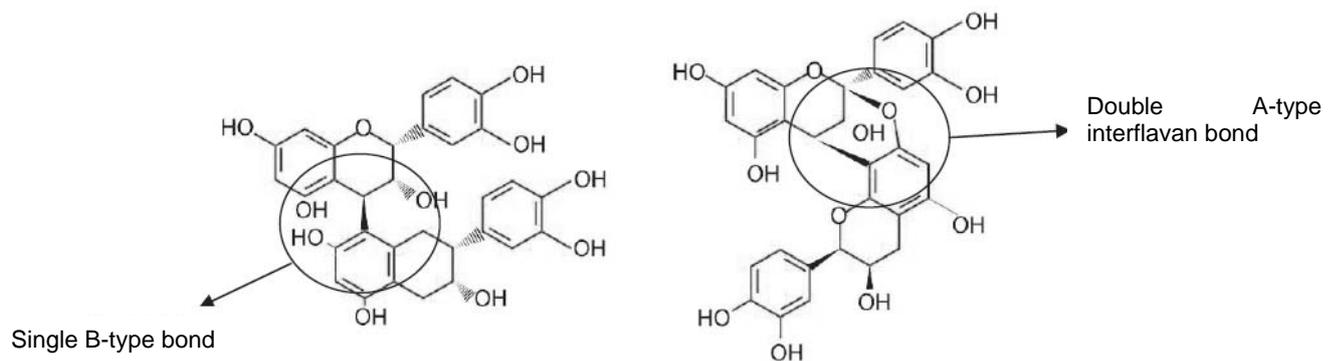


Fig. 1. Type B and type A PAC molecules

In June 2008, AFSSAPS (French Agency for health security of drugs) has published its good practise recommendations “ Diagnostic and anti-biotherapy of common bacterial urinary tract infections with adults” . Regarding non antibiotic prophylaxis for urinary tract infections, AFSSAPS reiterated the principles of the conclusions that AFSSA had validated before.

Bacterial anti-adherence results from the presence of double interflavan bonds between (epi)catechines, the building blocks of A-type PAC (Fig. 1). PAC are condensed tannins and part of its oligomeric fraction is absorbed so that the anti-adherence effect becomes measurable in urines four hours after uptake. B-type PAC (Fig. 1) do not show anti-adherence. The latter results from the strong interaction between the tannins and the protein structures of the E.coli bacteria's P-fimbriae.

36 mg minimum *Vaccinium macrocarpon* PAC per day in one uptake

Where does this quantity of 36 mg PAC come from?

Based on the clinical studies provided by the petitioner (Pharmatoka) , AFSSA endorsed, among others, the significant results of a consumption of 300 ml/day of cranberry juice cocktail : the clinical study of J.Avorn published in 1994(4). In this study, the participants had consumed 300 ml of cranberry juice cocktail per day and a significant decrease of bacteriuria and pyuria was observed.

This quantity of 300 ml contains 36 mg PAC (fig.2) measured by the method the petitioner had joined to his dossier, the colorimetric dimethylaminocinnamaldehyde or DMAC/PAC003.

This quantitation method was selected and developed by the US cranberry cooperative Ocean Spray (65 pct of the world production of cranberries) and preferred to other methods because very specific for cranberry PAC.

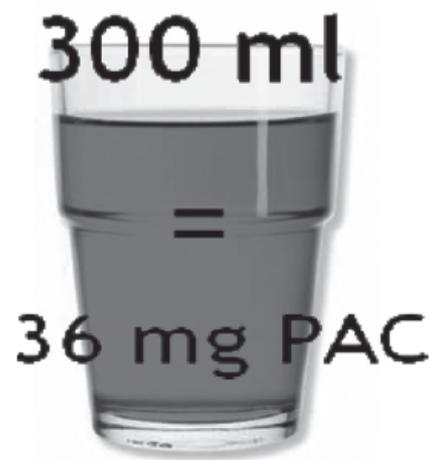


Fig. 2 Quantity of PAC in 300 ml cranberry juice cocktail

Optimum dosage and Escherichia coli virulence

After these first clinical observations, Pharmatoka has between 2006 and 2010 undertaken various studies (published in peer reviewed Journals) that have confirmed the dose dependent effect. These were the questions asked :

- which is the optimum dosage ? 18 mg (half dosage) , 36 mg , 72 or perhaps 108 mg ?
- is it recommendable to fraction the daily dosage in two 12 hour cycles ?
- what is the effect of PAC or their metabolites on the virulence of uropathogenic E.coli ?

Cranberry(Vaccinium macrocarpon) and urinary tract infections : study and review of literature (10) (study 1)

The efficacy of cranberry PAC was evaluated in eight healthy participants, in a double blinded randomised cross over protocol, comparing the uptake of product A (0 – 36 or 108 mg PAC measured by the DMAC method) against placebo. The ex vivo effect (inhibition of E.coli growth in the participants' urines) was highly significant for 36 mg PAC (one capsule of product A).

There was further demonstration that the decrease of bacterial adherence depends on the dosage of PAC: the adhesion index (average amount of adhering bacteria per T24 epithelial cell for 100 cells) was as follows :

- 5,61 after consumption of three capsules of 36 mg, i.e. 108 mg PAC ;
- 14,40 after consumption of one capsule of 36 mg PAC ;
- 22,30 after consumption of the placebo.

Conclusion : dose dependence is very significant between 36 and 108 mg PAC/day (all $p < 0,001$)

twice a day would be more efficacious than the uptake of a single 36 mg dose per day (9)

- The analysis of Prof. A.Sotto (SIFUD June 2010) : “ for urines taken after six hours, the dosages of 36/72 mg produce a significantly higher anti-adherence effect than 18 mg “ (13)
- That the daily uptake of 36 mg PAC brings a optimum anti-adherence and a better protection during 24 hours ;
- That the daily uptake of 72 mg PAC shows a maximum efficacy, which could perhaps justify a splitting of the 2 x 36 mg dose over 24 hours. This observation requires clinical confirmation.

In vitro and in vivo evidence of dose dependent decrease of uropathogenic Escherichia coli virulence after consumption of commercial Vaccinium macrocarpon capsules (study 2)

This study is an extension of the previous study. The in vivo model (with Caenorhabditis elegans worms) shows that E.coli when soaked in urines of participants who had consumed 108 mg PAC/day showed less virulence and a clearly reduced capacity to kill the worms that had absorbed them ($p < 0,0001$) (11)

Conclusion : the virulence of uropathogenic E.coli decreases significantly in the presence of urines of volunteers through the action of PAC and their metabolites.

Dosage effect on uropathogenic Escherichia coli anti-adhesion activity in urine following consumption of cranberry powder standardized for PAC content : a randomized double blind study. (study 3)

Thirty two healthy participants in four countries consumed 18-36 and 36-72 mg PAC/day in one uptake. The study was placebo controlled, double blind and randomized (8).

Two different anti-adherence tests, the HRBC (human red blood cells) test of Howell and Foo and the Lavigne test using T24 epithelial cells were performed. For the second time, the team of researchers could observe a significant decrease of the virulence of E.coli in the presence of urines of the participants.

One daily uptake

In study 3, the ex vivo adherence test on T24 epithelial cells showed :

- that the dosage of 18 mg PAC/day reaches, after 6 hours, a significantly lower level of anti-adhesion than that obtained by the dosage of 36 mg and 72 mg. This suggests that two partial doses of 18 mg taken with a 12 hour interval do not reach an efficacious level of anti-adhesion (Fig. 3)
- The comment of Dr.Howell on the splitting of 36 mg PAC in two times 18 mg : “There are no published studies which demonstrate that the consumption of products that bring 18 mg PAC

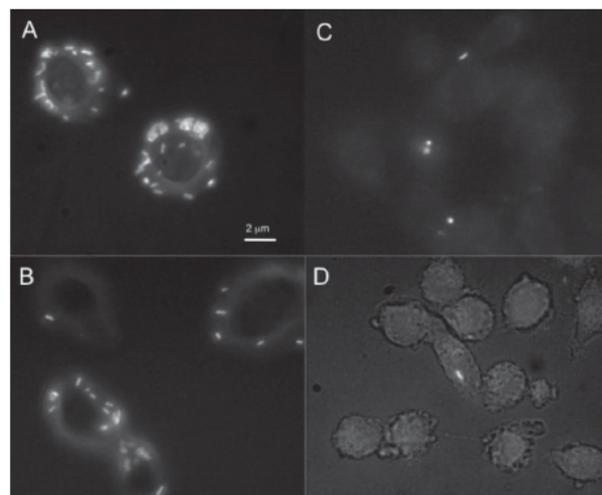


Fig. 3. Fluorescent microscopy presented in the study. A. Escherichia coli cultivated in urines collected after placebo consumption. B. Escherichia coli cultivated in urines collected six hours after consumption of a capsule containing 18 mg PAC. C. Escherichia coli cultivated in urines collected six hours after consumption of one capsule containing 36 mg PAC. D. Escherichia coli cultivated in urines six hours after consumption of two capsules containing 36 mg PAC, in total 72 mg PAC.

36 mg PAC of Vaccinium macrocarpon

AFSSAPS published their recommendations in 2008 with strong reservations : “ There are arguments in favour of the efficacy against E.coli of certain preparations (Vaccinium macrocarpon) that bring 36 mg PAC/day. However, the present evidence is insufficient for a general recommendation taking into account that the composition of the available preparations is very variable”.

One may conclude that the experts of AFSSAPS have observed enormous difference among available products (very few contain 36 mg PAC measured by the petitioner’s method, DMAC)

Furthermore, in the addendum (8.3.4.1 : “ non antibiotic prophylactic treatment of AFSSAPS’ recommendations) the following is mentioned :

- grape juice contains only B-type PAC and has no anti-adherence effect shown in vitro. Products composed of 36 mg PAC of which a majority is from grapes do not qualify and bring confusion to the consumer.
- Certain commercial arguments, such as the importance of consuming all the ingredients of the cranberry (the whole berry) are not validated by AFSSA.

The consumption of the other ingredients of the cranberry (fibers, anthocyanins, flavonols, phenolic acids) does not influence bacterial anti-adhesion, this having been shown only for PAC.

The absence of anti-adhesion in B-type PAC (like raisin, apples, tea, cocoa, etc.) has been demonstrated by Howell et al in a study published in 2005 (6)

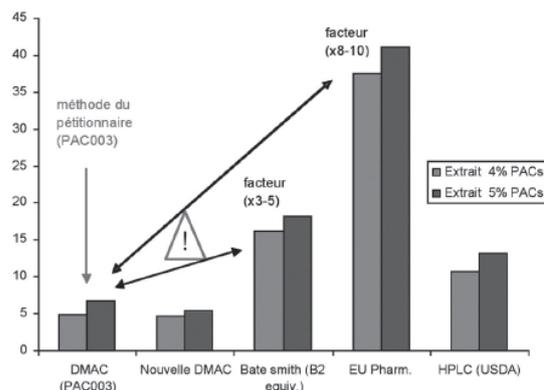


Fig. 4 . Comparison of quantitation methods of PAC contained in two extracts standardised at 4 and 5 pct using DMAC in catechin equivalent

The method has been automated to make it more reliable, more economical and quicker. It now carries the codename “BL-DMAC” and yields the same results as the previous “petitioner’s” method DMAC/PAC003.

The method has been validated by five international specialized laboratories and published in the *Journal of the science of food and agriculture* after acceptance by its peers (14)

The scientists who cooperated on this issue have created an Association for the promotion of the use of the analytical method BL-DMAC for the quantification of *Vaccinium macrocarpon* PAC. An institutional website is available to the public at large and delivers detailed explanations on this issue (16)

$$36 \text{ mg DMAC/PAC003} = 36 \text{ mg PAC BL-DMAC}$$

36 mg PAC measured by the petitioner’s method, DMAC

In its opinions published between 2004 and 2007, AFSSA had omitted to mention the quantitation method corresponding to the 36 mg PAC their experts had validated. This omission has been heavy with consequences and has contributed to cause anarchy in the “cranberry” food supplement category sold through pharmacies. At the request of a consumer’s Association in France, AFSSA finally confirmed in 2009 that the efficacious dose (36 mg PAC/day) had been measured by the petitioner’s method, DMAC (5)

Unfortunately the harm had been done! Taking advantage of this “scientific void” , new French extractors started to propose their powders using different quantitation methods yielding results that vary from one to five compared with the DMAC method.

Aubert has published a very clear summary of the present situation (1) (Fig. 4): “We can easily observe that, according to the method of analysis used for PAC quantitation, we do not obtain the same results. The PAC concentration can even vary with a factor up to 8 and 10 when the DMAC method (’s results) are compared with those of the “European pharmacopoeia”. The author was referring to the “Euracran method” derived from the “European Pharmacopoeia Hawthorn method” which, according to its authors, was “adapted to the cranberry “ but in reality it is totally inadapted to quantitate cranberry PAC (7). This method (Euracran) is these extractors’ favourite method and one easily understands the reason why.

BL-DMAC METHOD

In order to make the DMAC method available to all scientists and interested laboratories, an ad hoc working group simplified the procedures and selected a freely available A 2 procyanidin standard.

Limitation of the health claim to specific ingredients mentioned by AFSSA, excluding all others

In their opinion of 6 April 2004 , AFSSA have clearly mentioned that “ the claim is acceptable only for the juice of the *Vaccinium macrocarpon* and the powder of the juice of this plant’s berry.

The extension of the claim to other cranberry ingredients in 2007 (fresh cranberries, frozen cranberries, puree) had no impact on this issue because these ingredients are not used in food supplement preparations.

However, there are numerous extracts (powders) and food supplements that contain dried cranberry pomace as their main ingredient. This pomace, usually sold as cow-fodder, can be purchased at a very low price in comparison with the cost of the ingredients mentioned by AFSSA: 10 to 20 times cheaper. The one explains the other ...

Another inconvenience of these pomace powders is that the PAC have a very low bioavailability. In fact, PAC stick to the cellulose of the plant material, having not undergone

an acid hydrolysis and they will not be absorbed in the intestine so that their bioactivity remains very limited. To that purpose, a recent comparative study with product A containing 36 mg bioactive cranberry PAC and product B containing 500 mg of a powder infused with cranberry concentrate , against placebo has been completed ; Howell, Lavigne et al. the study remains unpublished because of a conflict of interest pointed out by the French Journal where it was submitted (12).

However, a recent study points out that enzymatic fermentation in the colon in animals could set free proanthocyanidins in form of phenolic acids which might play a certain part in anti-adhesion. This study's conclusions, are very hypothetical (15).

The inconvenience of pomace based cranberry powders is the reduced bioactivity (during HRBC and T24 cell tests) and the fact that the PAC are not measurable with the BL-DMAC method.

Advice for users of “cranberry” food supplements - traps to avoid

Hereafter as a conclusion our advice for all health professionals and users concerned :

- ask for a product that mentions 36 mg PAC in one daily uptake
- verify on the ingredients list that PAC are exclusively from cranberry *Vaccinium macrocarpon*
- verify that the PAC have been measured by the BL-DMAC method ; in case of doubt, proceed with verification using one of the laboratories that validated the BL-DMAC method
- ask for information about and proof of the bioactivity of the proposed products. Their 36 mg PAC can be “dead” , meaning that they produce no anti-adherent effect because of an inadequate production process or because of the ingredients they contain.
- Consult the web site of the Association for the promotion of the BL-DMAC method (16)

Using these tips could avoid that many patients who suffer from recurrent cystitis engage in useless costs for products without guaranteed efficacy.

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