

A Comparison Between The ORAC Value And The Novel PHOLASIN Test : Preliminary Results



Vincent DENYZET
Sales Manager
ARCHIMEX
sales@archimex.com

Pierre L. METRA
Ph.D, General Manager
LAREAL
pmetra@lareal.com

Christophe CHESNE
Ph.D, General Manager
BIOPREDIC International
christophe.chesne@biopredic.com



ORAC VALUE Procedure

(Oxygen Radical Absorbance Capacity)

The ORAC VALUE procedure was first published 10 years ago (1993) by CAO et al., and further improved by PRIOR (U.S.D.A). It has become a reliable reference method for the measurement of the overall antioxidant capacity of nutraceuticals and plant extracts.

Principle

Fluorescein is a naturally fluorescing molecule, which is very sensitive to free radicals. Free radicals generated by AAPH* in the reaction mixture react with the antioxidants from the plant extract. Once all radical scavengers are consumed, the free radicals destroy fluorescein, and the fluorescence vanishes.

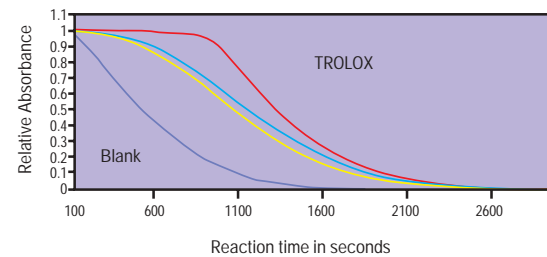
AAPH* = 2,2'-Azobis (2-AmidinoPropane) diHydrochloride

The quantification is made by measuring the area "under the curve", hence taking into account both the inhibition time and the inhibition percentage (XLS sheet used). Results are compared to the TROLOX figures, and expressed as micromole TEQ per kg or per litre. Analyses are run in duplicates.

Reaction

Directly in the spectrophotometer cuvette : sample extract + diluted fluorescein, temperature 37°C.

At time = zero : addition of the AAPH as the radical source
Measurement of the decrease of fluorescence every 100 seconds (Exc. 493 nm / Emi. 515 nm)
Subtract blank from sample figure.

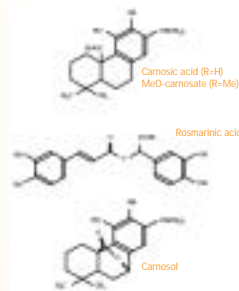


Values (in micromol TEQ per kg)

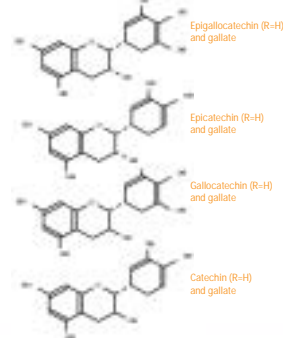
Values (in micromol TEQ per kg)

Orange juice	5 à 10 000	Blach tea leaves	1,5 à 2 000 000
Strawberry juice	10 à 20 000	Ascorbic acid	3 000 000
Fresh garlic	20 à 30 000	Tea polyphenol extracts	3 à 5 000 000
Human serum	20 à 30 000	Ethoxyquin	6 000 000
Red berries, Red wine	20 à 50 000	Rosemary extracts	5 à 7 000 000
Human blood	40 à 60 000	Butylhydroxyanisole (BHA)	15 000 000
Black berries (black current, ...)	30 à 90 000	Cocoa extracts	6 à 25 000 000
Cranberry powder	400 000		

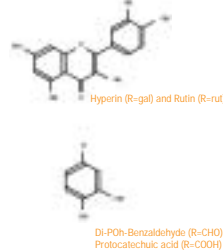
Rosemary leaves



Green tea leaves



Buckwheat kernel



Summary

As part of an ongoing study of the local plant materials, the efficacy and yield of extraction during the preparation of different extracts from rosemary leaves, green tea leaves, and buckwheat kernel (*Polygonum fagopyrum*) were studied using different analytical techniques.

The new Pholasin test, which has the ability to include different oxygen reactive species (ROS) like superoxide or peroxynitrite radicals, was compared to the more widely used ORAC value test taken as a reference. In a comparison between solvents, 50% refluxing ethanol was found to be the most effective according to the Folin polyphenol content, and both Pholasin and Orac tests.

On all extracts, the Pholasin Superoxide test was proved to be correlated to the ORAC value, giving higher results of a 5-fold magnitude. Both tests appear to give good information on the activity of the plant extracts against ROS.

PRO's and CON's - (ORAC and PHOLASIN tests)

	ORAC	PHOLASIN
Measurement of inhibition time	YES	YES
Measurement of inhibition degree	YES	YES
Detection of pro-oxidant species	NO	YES
End-point method	YES	NO
Sample colour interference	NONE	NONE
Need FOR several dilutions	YES	NO
High cost of equipment	YES	YES
High cost of reagents	NO	NO
High throughput v/s automation	NO*	YES
Several radical species	NO	YES
Easy-to-use	YES	YES
Hazardous reagents	NO	NO
Wide range of data in biochemistry	YES	YES
Wide range of data in food	YES	NOT YET

* Unless an expensive automatic instrument is used

RESULTS

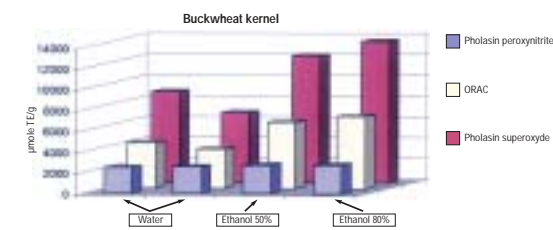
Experimental Procedure

For each plant, 4 different extracts were prepared using : Boiling water during 5 and 30 minutes, 50% and 80% aqueous ethanol at reflux for 30 minutes

Plant	Rosemary	Buckwheat	Green Tea
Dry matter yield (%)	22 - 23	2,5 - 4	20 - 31
Yield of polyphenolics (Folin) %	71 - 95	14 - 28	24 - 55

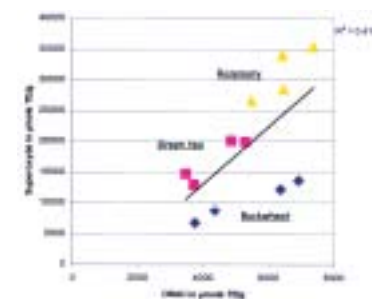
Conclusions

- Ethanol extracts show the best yields and the greatest activity,
- The superoxide test is the best indicator



Comparison ORAC v/s PHOLASIN

- Due to the differences between the molecules, the correlation is better plant by plant.
- The correlation between ORAC and Pholasin superoxide results is good, although figures are not of the same order of magnitude.
- Further studies involving the quantification of the molecules in more extracts are under way.



PHOLASIN Procedure

(ABEL® tests kits)

The PHOLASIN® ABEL® test kits were developed in U.K. by Knight Scientific Limited, PLYMOUTH. They are available in Europe since 2001 and are an interesting alternative to the other chemical tests.

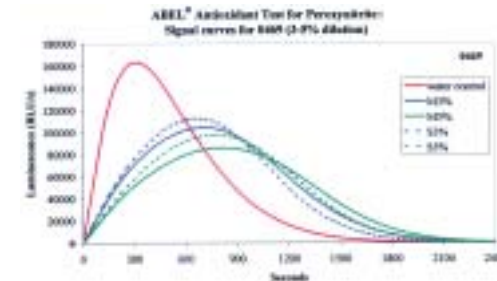
Principle

Pholasin is the photo-protein of the marine mollusc *Pholas dactylus*, it emits light in the presence of minute amounts of free radicals and other reactive oxygen species. Superoxide is generated in situ with or without the sample extract having an unknown antioxidant capacity. As Pholasin is present, it emits light. If there are antioxidants in the sample extract capable of scavenging superoxide, then these antioxidants will compete with Pholasin and less light will be emitted. On another hand, pro-oxidants will enhance the luminescence. Measurement is usually made over an hour. The same principle applies to the Peroxynitrite kit.

Results can be expressed as the percent reduction (or increase) of peak luminescence. If TROLOX is used for the calibration, results are given as micromole TEQ per kg or per litre. All standards and samples can be run simultaneously if an automated luminometer is used.

Reaction

Directly in the luminometer cuvette : buffer + sample extract + Pholasin + solution A, temp. 25°C.
At time = zero : addition of solution B as the free radical provider.
Measurement of the decrease (or increase) of light emission every 100 seconds in RLU/s (Relative Light Unit per second).



Typical values of a few beverages (in micromol TEQ per kg)

Champagne wine	2 to 5 000
Haut Médoc	25 000
Apple cider	10 to 30 000

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LAREAL
FOOD AND FEED RESEARCH
Postal Box 234
56006 VANNES - FRANCE