Riccarda Antiochia, Francesca Carbone,
Tania Gatta, Mauro Tomassetti, Luigi Campanella
Dipartimento di Chimica
Università di Roma "La Sapienza"
tania.gatta@uniroma1.it

ANTIOXIDANT PROPERTIES OF CURCUMIN, PAPAIN AND PAPAYA

The antioxidant properties were alleged to form the basis of the therapeutic properties attributed to curmunim, papain, papaya fruit and papaya integrators. Innovative electrochemical and more usual methods were used in order to verify these properties.

It is well known that the free radicals produced via an aerobic metabolism represent the main cause of ageing and cardiovascular or degenerative diseases. The antioxidant compounds contained in some foods are therefore important protective agents for human health [1, 2]. Therefore it is of great interest to develop new analytical methods and/or to apply the methods already developed for the evaluation of the antioxidant capacity of several chemical and natural species. Curcumin, usually used as drug and/or dietary spices, exhibits several pharmacological properties: it is reported to act as natural antioxidant [3-5] and it has been found to exhibit anti-mutagenic [6], anticarcinogenic [7], anti-inflammatory [8] properties. Papain, contained in the papaya fruit, is well known as anti-inflammatory agent, but the most recent studies on papaya stem from the growing interest aroused by Fermented Papaya Preparation (FPP) [9], the administration of which is believed, among other things, to reduce the symptoms of Parkinson's disease. FPP is obtained from the unripe fruit through a fermentation process, and has a wide range of different characteristics: it practically no longer contains any papain or vitamins but is rich in oligosaccharides [9]. So far no serious scientific study has confirmed the above-mentioned effects. Nevertheless, to the antioxidant and immunostimulating properties believed to be attributed its claimed therapeutic effect [10].

In order to exhaustively verify the antioxidant and thus "radical scavenging" properties, the curcumin, papain, papaya fruit and several papaya-based food integrators were studied. A biosensor method and a method based on cyclic voltammetry, recently trialled by the present authors for the determination of the antioxidant capacity of other compounds [11-15], were utilized. In addition a well known expensive spectrofluorimetric reference method [16] and a very cheap spectrophotometric method [17] were applied for determining and comparing the antioxidant capacity of considered samples. For a quantitative comparisons of antioxidant capacity obtained by different methods, homogeneous values were also achieved by finding the experimental correlation between the antioxidant capacity trends of the spectrofluorimetric reference method and the individual scales of the other first two used methods.

Finally, the antioxidant capacity of curcumin was compared to that of two well known antioxidant agents, ascorbic acid and phenol.

Methods

Superoxide dismutase (SOD) biosensor method and cyclic voltammetric method (CV) are widely described in previous papers [11-15, 18, 19], while spectrofluorimetric (ORAC) [16] and DMPD+Fe^{+3} [17] methods are well known and widely utilized in the literature devoted to this type of researches.

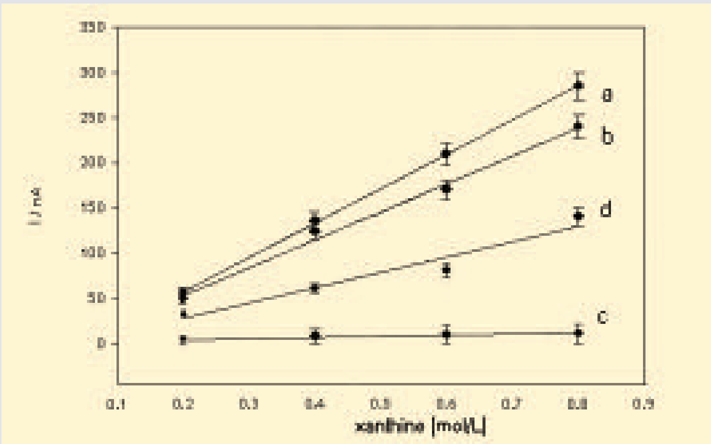

Fig. 1 - SOD biosensor method. Calibration curves obtained by plotting the current (i) *vs* the increasing xanthine concentration, relative to: a) blank, b) 10^{-2} mol/L curcumin, c) 10^{-2} mol/L ascorbic acid and d) 10^{-2} mol/L phenol. Error bars represent SD (n=5)

Results and discussion

In order to compare the efficiency of the antioxidant capacity of curcumin, ascorbic acid and phenol were also tested. These last two compounds have been chosen as they are two of the main antioxidant products naturally contained in several foods and wine.

All these samples were analysed using the SOD biosensor, the cyclic voltammetry (CV) method and the ORAC spectrofluorimetric method. Using the SOD biosensor method progressively lower values were obtained for the slope of calibration curves of curcumin (see curve b), in comparison by ascorbic acid (curve c) and phenol (curve d) in Fig. 1. The behaviour of these curves demonstrates that the antioxidant capacity can be arranged in the following order: ascorbic acid > phenol > curcumin. The results for the antioxidant capacity, obtained with the SOD biosensor, cyclic voltammetry and ORAC methods, are summarised in Tab. 1a. It is possible to note that curcumin shows quite good antioxidant activity but definitely lower than that observed for ascorbic acid and phenol.

Although each of the methods proposed for the determination of antioxidant capacity adopts an arbitrary specific scale to report the results, making impossible any direct comparison between the experimental values, it is in any case possible to compare the antioxidant capacity trends of found values (Fig. 2).

It is comforting to observe the good overall agreement found among the trends in antioxidant capacity, obtained using the biosensor method, the cyclic voltammetry (CV) method and the ORAC method. It can be easily noted that curcumin shows an antioxidant capacity which is quite limited if compared to that observed with ascorbic acid and phenol. In particular curcumin shows values of antioxidant activity which are about half of those obtained with phenol and almost one third of those obtained with ascorbic acid. This result is evident observing the data obtained using the SOD biosensor method and the ORAC method. The values found with the CV method show the same trend but the ratios among the values related to three compounds are differet in comparison with those obtained by the fluorimetric and SOD methods. This seems to be due to the fact that the sensitivity of the CV method is largely affected by the kind of the antioxidant compound analysed. It is still not fully clear what are the reasons for this fact. Indeed, the kinetics of the oxidative processes taking place at the glassy carbon electrode may be affected by particular substances present in the sample. Therefore the signals obtained for different products, when operating in the same conditions, could be dependent by this fact.

For as concerns the antioxidant capacity of papain, papaya fruit and papaya based integrators (see Tab. 1b), also in this case tests were checked for equal weight of each of the samples considered to allow a homogeneous comparison of their antioxidant capacity. By observing the trends showed in Fig. 3 it is interesting to underline that the so-called "fermented papaya" integrators, the one most publicised from the therapeutic point of view, are the products that usually displayed the lowest antioxidant capacity values among all the examined samples.

Lastly, pure papain shows a rather good antioxidant capacity, certainly higher than that of papaya based commercial integrators, but considerably lower than that of whole fruit, which shows the best antioxidant capacity value.

Tab. 1 - Values of antioxidant capacity

a) ***for curcumin, ascorbic acid and phenol, obtained using the SOD biosensor, the cyclic voltammetry and the ORAC fluorimetric methods***

Samples (concentration) ($1x10^{-2}$ mol/L)	SOD biosensor method (RAC units) (±SD) (n≥5)	Cyclic voltammetry method (cm^2) (±SD) (n≥5)	Fluorimetric method (ORAC units) (±SD) (n≥5)
curcumin	0.20±0.02	5.9±0.2	16±1
ascorbic acid	0.51±0.03	7.4±0.2	45±2
phenol	0.39±0.03	6.9±0.2	32±1

b) ***for papain, papaya, and papaya integrators, obtained using the SOD biosensor, the spectrophotometric DMPD+Fe+3 and the ORAC fluorimetric methods***

Samples	SOD biosensor method (RAC units) (±SD) (n≥5)	DMPD+Fe^{+3} method TEAC units) (±SD) (n≥5)	Fluorimetric method (ORAC units) (±SD) (n≥5)
a) papaya (pulp+skin)	0.62±0.02	28.4±0.3	42.8±0.8
b) papain (enzyme)	0.33±0.03	22.5±0.2	35.7±0.6
c) fermented papaya	0.39±0.01	15.3±0.5	36.2±0.9
d) papaya juice	0.30±0.03	16.2±0.6	33.0±0.4
e) fermented papaya	0.24±0.01	5.3±0.4	26.9±0.8
f) fermented papaya	0.13±0.02	2.2±0.2	23.2±0.8

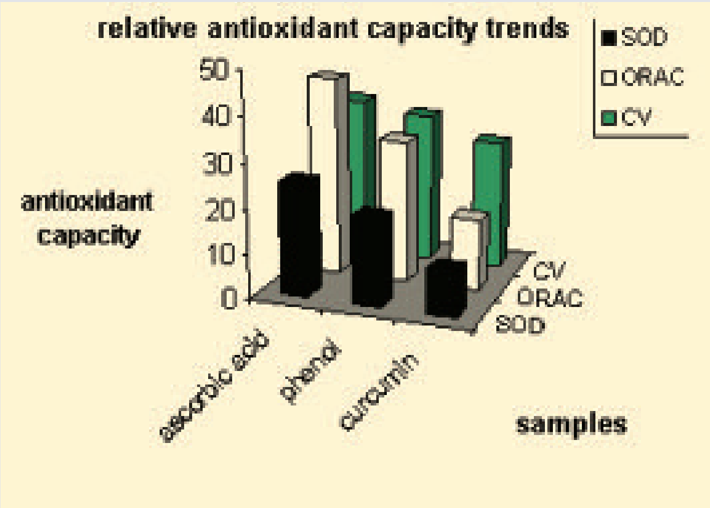

Fig. 2 - Antioxidant capacity trend obtained by SOD, ORAC and CV methods. To facilitate the comparison of the obtained values, showed in hystograms, the antioxidant capacity results, obtained by (SOD) biosensor method, were multiplied by a factor = 50, while the results, obtained by CV method, were multiplied by a factor = 5

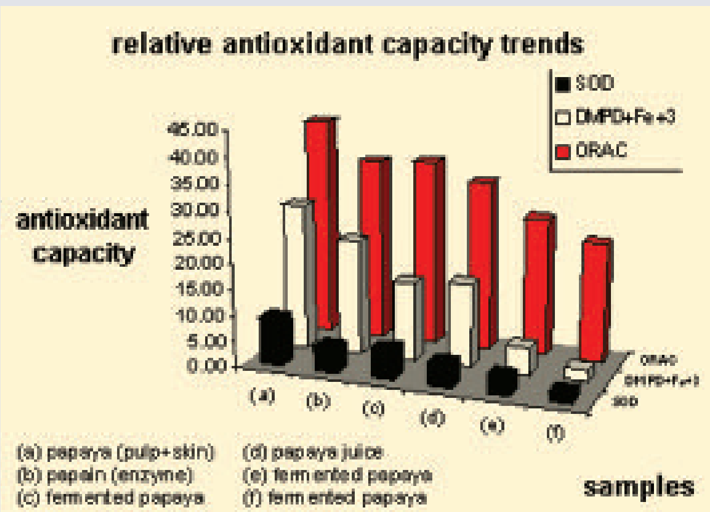

Fig. 3 - Antioxidant capacity trends obtained by SOD, ORAC and DMPD+Fe^{3+} methods. To facilitate the comparison of the obtained values, showed in hystograms, the antioxidant capacity results, obtained by (SOD) biosensor method were multiplied by a factor = 15

Clearly, also for these samples the comparison of the results obtained using different methods cannot be based on absolute values, as each method uses different units of measure, but rather on the trends emerging from the histograms obtained (Fig. 3). However, the qualitative trends displayed by three applied methods may be said to be very similar, with only small inversions. It is therefore interesting to stress the good agreement between the ORAC method (selected as reference method) and each of the other two methods. In fact the three analytical methods tested in the determination of antioxidant capacity, although based on different functioning principles, produced very similar results. This confirms the accuracy of the trends obtained and thus the reliability of the methods used. Furthermore, the accuracy, precision and robustness of the SOD biosensor method, recently proposed by the present author as had already emerged in previous studies [11-13] were again confirmed. The good agreement between the ORAC method (selected as reference method) and each of the other two methods it is also illustrated by the correlation straight lines equations reported in Tab. 2a obtained using the values of Tab. 1b. It is also significant that, by using these experimental equations of correlation straight lines, it was also possible to express all the values obtained using the three methods in the same units of measure, for instance ORAC units, which are the most commonly used.

By similar procedure, that is using the equations of correlation curves (see Tab. 2b) obtained by the values of Tab. 1a, also for curcumin, ascorbic acid and phenol it was possible to express all the antioxidant capacity values obtained by different methods in ORAC units.

The results shown in Tab. 3, obtained by the fluorimetric, the biosensor and CV methods, are all expressed in ORAC units. Now it is easier to observe the reasonably good agreement between the obtained values, in particular between the values obtained by the biosensor and the fluorimetric method. This result is in perfect agreement with those obtained with the same methods but relative to different compounds considered in previous papers [11, 12].

Tab. 2

a) *Correlation curve equations among values by ORAC method and values by SOD, or DMPD+ Fe^{+3} methods respectively, obtained for papain, papaya and papaya based integrators*

Correlation	Curve equations	Correlation coefficients
SOD vs ORAC	y = (40.3±2.2)x + (19.5±6.1)	R^2= 0.9149
(DMPD+ Fe^{+3}) vs ORAC	y = (0.68±0.09)x + (22.8±1.6)	R^2= 0.9282

b) *Correlation curve equations among values by ORAC method and values by SOD, or CV methods respectively, obtained for curcumin, ascorbic acid and phenol*

Correlation	Curve equations	Correlation coefficients
SOD vs ORAC	y = (0.0107±0.0006)x + (0.031±0.022)	R^2= 0.9962
CV vs ORAC	y = (0.0531±0.0066)x + (5.07±0.220)	R^2= 0.9848

Tab. 3 - Comparison of relative antioxidant capacity values for curcumin, ascorbic acid and phenol obtained by SOD biosensor, by spectrofluorimetric (ORAC) and by cyclic voltammetry methods (all expressed in ORAC units)

	Samples (concentration) ($1x10^{-2}$ mol/L)	(a) SOD biosensor method (ORAC units)	(b) Cyclic voltammetry method (ORAC units)	(c) Fluorimetric method (ORAC units)	Δ (%) (c-a)/c
curcumin	16.1	15.7	16.6	+3	+5
ascorbic acid	44.5	43.9	45.1	+1	+3
phenol	33.5	34.5	32.5	-3	-6

Conclusions

The present study indicates that curcumin exhibits quite good antioxidant properties which are responsible of the great importance of this substance in the prevention of human diseases. However, the antioxidant activity shown by curcumin resulted to be quite lower than that shown by other powerful natural antioxidants, like ascorbic acid and phenol. This was demonstrated using both the electrochemical methods based on the SOD biosensor and on cyclic voltammetry and the classic ORAC spectrofluorimetric method. The reproducibility of the two electrochemical methods resulted to be quite good, obtaining in the measurements on the three different products considered, respectively a RSD values ≤5% (n=5) in all RAC measures and a RSD values ≤8% (n=5) in all CV measures, thus proving that both methods are sufficiently precise. The antioxidant capacity of curcumin, ascorbic acid and phenol was also determined using the ORAC method, which was used as reference method. In the latter case, the RSD values was ≤5% (n=5), in all the spectrofluorimetric measures.

One interesting aspect of this research is the possibility of being able to express and then to compare, as already done for different substrates [11-12], the antioxidant capacity values obtained using the SOD biosensor, or using CV method, also in the same ORAC units, that is in the units of measure used by the fluorimetric method which, as already mentioned, is the one most frequently used. By expressing the results obtained with the same units of measure we were able to make not only a qualitative comparison of the trends in the antioxidant capacity values obtained using the different methods but also an homogeneous quantitative comparisons of all the values obtained. Lastly the SOD biosensor showed an excellent correlation with the ORAC method, while the results obtained with the cyclic voltammetry showed a less good correlation. This fact can be ascribed to the not uniform sensitivity of the method for all types of sample.

The same conclusions concerning the good repeatability of measurements and the excellent correlation among SOD biosensor, ORAC and DMPD+Fe^{+3} spectrophotometric methods may be formulated also as for as concerns the measurements of papain, papaya fruit and papaya based integrators carried out in this research.

Lastly the so-called "fermented papaya", the one most publicized from the therapeutic point of view, is the product that displayed the lowest antioxidant capacity among the products tested. Moreover, also in previous researches [14, 20] it was found that the antioxidant capacity of commercially food integrators resulted always appreciably lower than that found in the whole fresh fruits.

References

[1] L.W. Oberley, *Free Radical Biology and Medicine,* 1988, **5**, 113.

[2] P. Pietta *et al., J. Pharm. Biomed. Anal.*, 2000, **23**, 223.

[3] I. Nishigaki *et al., J. Clin. Biochem.Nutr.,* 1922, **13**, 23.

[4] K.K. Soudamini *et al., Indian J. Physiol. Pharmacol.*, 1922, **36**, 239.

[5] K. Elisabeth, M.N.A. Rao, *Int. J. Pharm.*, 1990, **58**, 240.

[6] M. Nagabushan *et al., Food Chem. Toxicol.*, 1981, 25, 545.

[8] K.K. Soudamini, R. Kuttan, *J. Ethnopharmacol.,* 1989, **27**, 227.

[9] A. Mukholadyay *et al., Anti inflammatory and irritant activities of curcumin analogues in rats. Agents Actions,* 1982, **12**, 508.

[10] Available from: www.saninforma.it.

[11] O. Aruoma, *Entr. de Bichat. Ther.*, 2006, 1.

[12] L. Campanella *et al., J. Pharm. Biomed. Anal.*, 2004, **35**, 303.

[13] L. Campanella *et al., J. Pharm. Biomed. Anal.*, 2004, **36**, 91.

[14] L. Campanella *et al., Biosens. Bioelectron.*, 2004, **19**, 641.

[15] L. Campanella *et al., J. Pharm. Biomed. Anal.*, 2001, **24**, 1055.

[16] L. Campanella *et al., Anal. Lett.,* 1999, **32(13)**, 2559.

[17] G. Cao *et al., Clin. Chem.*, 1995, **41**, 738.

[18] V. Fogliano *et al., J. Agric. Food Chem.*, 1999, **47**, 1035.

[19] L. Campanella *et al., J. Pharm. Biomed. Anal.*, 2000, **23**, 69.

[20] S. Chevion *et al., Free Rad. Biol. Med.*, 2000, **28**, 860.

[21] L. Campanella *et al., J. Pharm. Biomed. Anal.*, 2003, **32**, 725.

ABSTRACT

Proprietà antiossidanti della curcumina, della papaina e della di papaia determinate usando diversi metodi analitici

Le presunte proprietà antiossidanti ed antiradicaliche alla base delle proprietà terapeutiche attribuite alla curcumina, alla papaina ed alla papaia sono state studiate con due metodi elettrochimici innovativi. I risultati sono stati confrontati con quelli ottenuti con un metodo spettrofluorimetrico di riferimento ed uno spettrofotometrico; i valori della capacità antiossidante della curcumina sono stati confrontati con quelli di due composti di riferimento: acido ascorbico e fenolo.