

This article was downloaded by:[University of Pittsburgh]  
 On: 10 May 2008  
 Access Details: [subscription number 773394239]  
 Publisher: Informa Healthcare  
 Informa Ltd Registered in England and Wales Registered Number: 1072954  
 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Free Radical Research

Publication details, including instructions for authors and subscription information:  
<http://www.informaworld.com/smpp/title~content=t713642632>

### Betanin inhibits the myeloperoxidase/nitrite-induced oxidation of human low-density lipoproteins

Mario Allegra<sup>a</sup>; Luisa Tesoriere<sup>a</sup>; Maria A. Livrea<sup>a</sup>

<sup>a</sup> Department of Pharmaceutical, Toxicological and Biological Chemistry, Università di Palermo, Palermo, Italy

Online Publication Date: 01 January 2007

To cite this Article: Allegra, Mario, Tesoriere, Luisa and Livrea, Maria A. (2007) 'Betanin inhibits the myeloperoxidase/nitrite-induced oxidation of human low-density lipoproteins', *Free Radical Research*, 41:3, 335 — 341

To link to this article: DOI: 10.1080/10715760601038783

URL: <http://dx.doi.org/10.1080/10715760601038783>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article maybe used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

## Betanin inhibits the myeloperoxidase/nitrite-induced oxidation of human low-density lipoproteins

MARIO ALLEGRA, LUISA TESORIERE, & MARIA A. LIVREA

Department of Pharmaceutical, Toxicological and Biological Chemistry, Università di Palermo, Palermo, Italy

Accepted by Professor H. Sies

(Received 23 June 2006; in revised form 5 September 2006)

### Abstract

Production of nitrogen dioxide by the activity of myeloperoxidase (MPO) in the presence of nitrite is now considered a key step in the pathophysiology of low-density lipoprotein (LDL) oxidation. This study shows that betanin, a phytochemical of the betalain class, inhibits the production of lipid hydroperoxides in human LDL submitted to a MPO/nitrite-induced oxidation. Kinetic measurements including time-course of particle oxidation and betanin consumption, either in the presence or in the absence of nitrite, suggest that the antioxidant effect is possibly the result of various actions. Betanin scavenges the initiator radical nitrogen dioxide and can also act as a lipoperoxyl radical-scavenger. In addition, unidentified oxidation product(s) of betanin by MPO/nitrite inhibit(s) the MPO/nitrite-induced LDL oxidation as effectively as the parent compound. In the light of betanin bioavailability and post-absorption distribution in humans, present findings may suggest favourable *in vivo* activity of this phytochemical.

**Keywords:** Betanin, myeloperoxidase, nitrite, low-density lipoproteins, atherosclerosis

### Introduction

Oxidation of low-density lipoproteins (LDL), considered a pivotal process in the progression and eventual development of atherosclerosis [1–3], has been studied for years. It has recently been suggested that myeloperoxidase (MPO) has a prominent role in promoting oxidative reactions in LDL *in vivo* [4,5]. MPO, a heme-enzyme which occurs in neutrophils and monocytes, utilizes hydrogen peroxide and a variety of co-substrates to generate reactive oxidants as intermediates [2,4,6]. The enzyme follows the classical peroxidase cycle [7], in accordance with equations (1)–(3):



A single two-electrons oxidation of the native enzyme to compound I (MPO-I) by  $\text{H}_2\text{O}_2$  is followed by two successive one-electron reductions by reducing substrates ( $\text{AH}_2$ ) to native enzyme, via compound II (MPO-II). MPO-I, which is two oxidative equivalents above the ground state, is an extremely reactive species but cannot easily oxidize large molecular complexes, including LDL, because its active site is buried in a hydrophobic cleft. Indeed, it relies on low molecular weight compounds to convey oxidizing equivalents from the heme group to its target [8–10]. The hypochlorous acid formed via a single two-electrons oxidation of chloride ions by MPO-I [11], has been considered implicated in the pro-oxidant activity of MPO on the LDL protein (LDL prot) [12–14]. On the other hand, it has recently been shown that the MPO activity also depends on the metabolism of nitric oxide (NO) being nitrite, the final oxidation product of NO metabolism, a substrate for the enzyme [15–18]. Nitrogen dioxide radical ( $\text{NO}_2^\cdot$ ), the one-electron oxidation product of nitrite by MPO-I, has

Correspondence: M. A. Livrea, Department of Pharmaceutical, Toxicological and Biological Chemistry, Università di Palermo, Via Carlo Forlanini 1, 90123 Palermo, Italy. Tel: 39 091 214855. Fax: 39 091 214855. E-mail: mal96@unipa.it

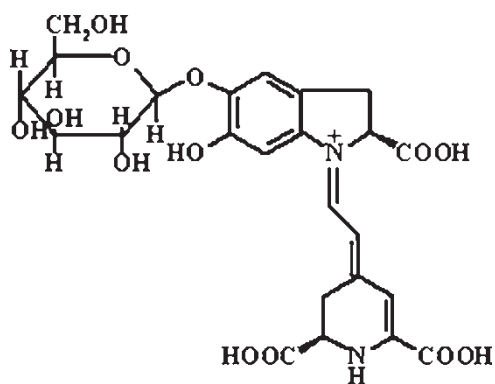


Figure 1. Chemical structure of betanin.

been proposed as the reactive species to start massive oxidation of the LDL lipids [19–20].

Bioactive components of fruits and vegetables are considered of great importance in the prevention of various degenerative pathologies, including cardiovascular diseases [21]. Betalains are phytochemicals characteristic of flowers and fruits of ten families of *Caryophyllales* plants, including red beet and cactus pear, and of few superior fungi of the genus *Amanita* of the *Basidiomycetes* [22]. These pigments are betalamic acid derivatives and depending on the components bonded to the main structure, betacyanins or betaxanthins arise, the former when the group is 3,4-dihydroxyphenylalanine which may or may not be glycosylated and the latter if the conjugation partners are amino acids or the derived amines. Various *in vitro* studies showed that the betacyanin betanin (5-*O*-glucose betanidine) (Figure 1), a molecule with three redox potentials at 404, 616, and 998 mV [23], reduces numerous radical species in both chemical and biological environments. This molecule can partition in lipid compartments [24,25] and increases the resistance of human LDL to oxidation induced by copper [25] as well as by H<sub>2</sub>O<sub>2</sub>-activated metmyoglobin [24]. This study assessed the antioxidant activity of betanin the MPO/nitrite-dependent oxidation of human LDL.

## Materials and methods

Betanin was extracted from cactus pear fruits (red cultivar) as reported by Butera et al. [23] and then purified according to Stintzing et al. [26].

Unless stated otherwise, all materials and solvents were from Sigma (Milan, Italy) and of the highest purity or high-performance liquid chromatography (HPLC) grade.

### LDL preparation

LDL (density: 1.019–1.063 g/ml) was isolated from EDTA plasma by ultracentrifugation at 110,000*g* for 4 h at 4°C in a L8-70 ultracentrifuge (Beckman, CA,

USA) fitted with a 50 Ti rotor and using potassium bromide for density adjustments according to Kleinveld et al. [27]. The LDL fraction was shown to be free of other lipoproteins by electrophoresis on agarose gel. EDTA, salts and plasma components were removed from LDL by gel filtration on a Sephadex G-25 column (Pharmacia Biotech, Milan, Italy). Proteins were determined by using the Bio-Rad colorimetric method [28].

### Lipoprotein oxidation

Reactions were carried out at 37°C in 0.1 M potassium phosphate buffer, pH 7.4, supplemented with 0.1 mM diethylenetriaminepentacetic acid. The reaction mixture contained 50 µg LDL prot, 50 µM nitrite, 53 nM MPO ( $\epsilon_{430} = 170 \text{ mM}^{-1} \text{ cm}^{-1}$ , Planta Natural Products, Vienna, Austria), 310 ng/ml (1.29 nkat/ml) glucose oxidase (GOD) and 0.56 mM D-glucose (final concentrations). In some experiments nitrite and/or LDL were omitted. Betanin was added to the reaction mixture dissolved in PBS. The oxidation of LDL was followed spectrophotometrically by measuring the formation of conjugated diene (CD) lipid hydroperoxides at 234 nm. The absorbance of the sample was recorded every 2 min for a total of 100 min on a Beckman DU 640 spectrophotometer, equipped with a temperature control device.

### Betanin analysis

Betanin was extracted from the incubation mixtures by mixing one volume of the incubation medium with three volumes of chloroform/methanol (2:1, v/v). The methanol phase was then dried under nitrogen and re-suspended in 1% acetic acid in water and analysed on a Varian Microsorb C-18 column (250 × 4.6 mm, Varian, Palo Alto, CA, USA), eluted with a 20 min linear gradient elution from solvent A (1% acetic acid in water) to 20% solvent B (1% acetic acid in acetonitrile) with a flow of 1.5 ml/min and revelation at 536 nm [22]. Quantitation was by reference to a standard curve constructed with 5–100 ng of betanin. A computer-assisted analysis (TableCurve 2D, Jandel, CA, USA) of the linear portion of the kinetic traces of the molecule decay was used to determine the initial rate of betanin consumption ( $v_i$ ).

Spectral changes of betanin during oxidation were evaluated by analysing the pigment methanol extracts in the range 250–750 nm, before and at time-intervals from the reagent mixing.

### LDL-vitamin E consumption

α-Tocopherol was extracted from LDL by mixing aliquots of the incubation mixture (50 µg LDL prot) with two volumes of absolute ethanol and eight volumes of petroleum ether. The organic extracts

containing vitamin E were then dried under nitrogen, re-suspended with several microliters of methanol and analyzed on a Supelco Supelcosil (Bellefonte, PA) LC-18 column ( $0.46 \times 25$  cm), with methanol as eluent at a flow rate of 1.0 ml/min. Detection was at 290 nm. Quantitation was by reference to a standard curve constructed with 5–100 ng of  $\alpha$ -tocopherol.

#### Statistical analysis

Conventional methods were used for calculation of means and standard errors.

### Results

When nitric dioxide, produced by the catalytic activity of MPO on nitrite, attacks LDL lipids, formation of CDs is first counteracted by the endogenous LDL antioxidants, which is spectrophotometrically monitored as a lag phase, followed by a net rise of the absorbance at 234 nm. The time-course of the MPO/nitrite-induced LDL oxidation is shown in Figure 2. A lag phase of 25 min is followed by a linear increase of the sample absorbance as a result of the chain propagation process. Betanin in a  $0.5$ – $5.0$   $\mu\text{M}$  range inhibited the MPO/nitrite-induced LDL oxidation, decreasing the propagation rate in a dose-dependent manner, without affecting the lag period. At the highest concentration tested ( $10$   $\mu\text{M}$ ), betanin prevented the CD formation over a period of 100 min (Figure 2).

Spectral changes of the pigment were assessed during LDL oxidation in the presence of  $5$   $\mu\text{M}$  betanin. Scanning of the absorbance spectrum at time-intervals showed a net decrease of the  $536$  nm peak (Figure 3), while did not provide evidence of absorbance peaks revealing formation of betanin product(s). The spectrophotometric traces showed that depletion of the molecule occurred within 10 min.

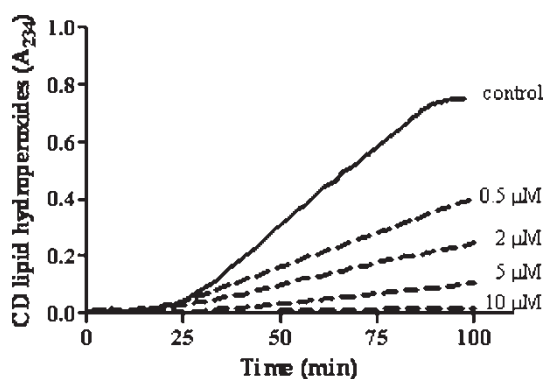


Figure 2. Time-course of LDL lipid oxidation by MPO/GOD/glucose/nitrite either in the absence (control) or in the presence of various betanin concentrations ( $0.5$ – $10$   $\mu\text{M}$ ). That reported is representative of six experiments with different LDL preparations. Oxidation of LDL was as reported in Methods.

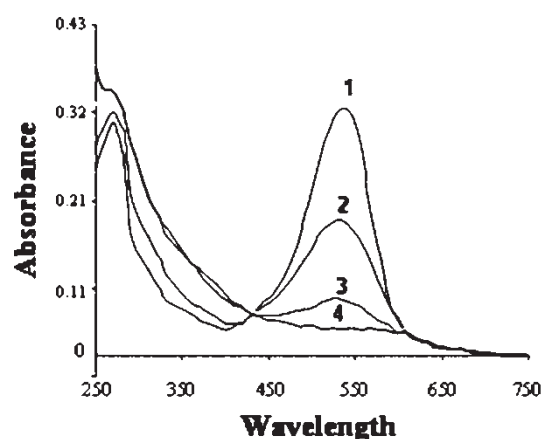


Figure 3. Spectral evidence of the consumption of betanin ( $5$   $\mu\text{M}$ ) during LDL oxidation by MPO/nitrite. The spectral traces before (1) and after 2 min (2); 5 min (3); and 10 min (4) represent one of a large series of experiments. Oxidation of LDL was as reported in Methods.

Consumption of the endogenous vitamin E, the main LDL antioxidant, was monitored during LDL oxidation, either in the absence or in the presence of  $5$   $\mu\text{M}$  betanin. Vitamin E was consumed in 20 min and betanin did not modify its temporal disappearance (not shown).

The MPO/nitrite-dependent LDL oxidation model entails the formation and interaction of various reactive species in succession. For a fuller understanding of the protective action of betanin in counteracting the LDL oxidation, a number of experiments were performed. Betanin has recently been shown as a substrate for the highly reactive intermediates of the MPO cycle [29]. To distinguish consumption of betanin by peroxidase activity from oxidation by nitrogen dioxide, the consumption of the molecule in a MPO-dependent nitrogen dioxide generating system was monitored in the absence of LDL and compared to its consumption in the presence of MPO and hydrogen peroxide alone. The time-course of betanin disappearance (Figure 4) provides evidence that the consumption rate is much higher in the presence of nitrite ( $v_i = 1.01 \times 10^{-8} \text{ M s}^{-1}$ ) than in its absence ( $v_i = 0.75 \times 10^{-8} \text{ M s}^{-1}$ ), thus suggesting reaction with nitrogen dioxide. On the other hand, betanin was not consumed by hydrogen peroxide alone (not shown). It should be noted that consumption of betanin during the MPO/nitrite-dependent LDL oxidation was even faster ( $v_i = 1.8 \times 10^{-8} \text{ M s}^{-1}$ , Figure 4).

Oxidation of LDL lipids by MPO can occur even in the absence of nitrite [30,31], though at much a lower rate [20]. Then assays were performed to check about the activity of betanin against the MPO-induced LDL oxidation in the absence of nitrite. The time-course profile of LDL oxidation in this system is shown in Figure 5. Betanin ( $0.5$ – $10$   $\mu\text{M}$ ) inhibited the CD lipid hydroperoxide formation in a dose-dependent

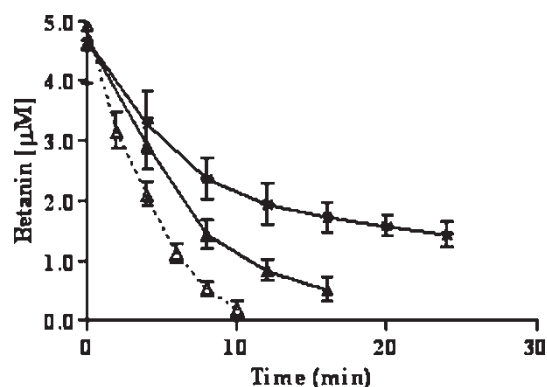


Figure 4. Time-course of the consumption of betanin ( $5 \mu\text{M}$ ) during the catalytic cycle of MPO, either in the absence ( $*$ ) or in the presence ( $\blacktriangle$ ) of nitrite and during the MPO/nitrite-dependent LDL oxidation ( $\triangle$ , dotted line). Values are means  $\pm$  SE of three separate experiments. The experimental conditions are reported in Methods.

manner, with a lag phase the length of which linearly increased at the increase of the betanin concentration. On the other hand, betanin did not have any effect on the propagation rate and was consumed within the lag phase (not shown).

Activity of betanin oxidation product(s) on the MPO/nitrite-stimulated LDL oxidation was assessed by incubating  $5 \mu\text{M}$  betanin with a MPO-dependent  $\text{NO}_2$ -generating system for 15 min, to obtain oxidation of the molecule and then adding the LDL. The oxidation kinetics compared with those in the presence of the native compound, provided evidence that the oxidised betanin was as effective as betanin in counteracting LDL oxidation (Figure 6).

## Discussion

Current knowledge on the nutrition's role in disease prevention, highlights the importance of phytochemicals in health promotion. Characterization of the activity of these substances in various either chemical

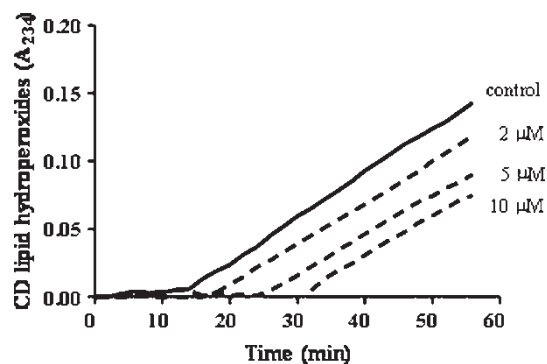


Figure 5. Time-course of LDL lipid oxidation by MPO/GOD/glucose, either in the absence (control) or in the presence of various betanin (BET) concentrations ( $2.0$ – $10 \mu\text{M}$ ). That reported is representative of six experiments with different LDL preparations. Oxidation of LDL was as reported in Methods.

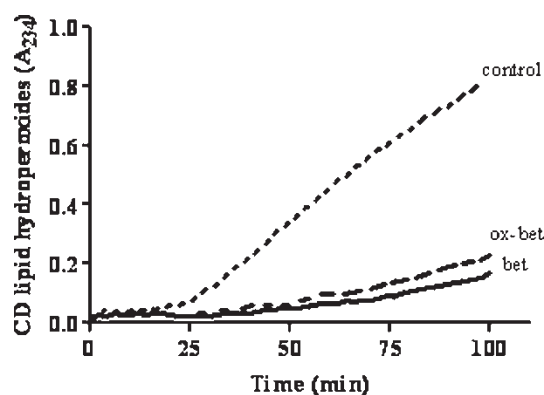


Figure 6. Time-course of the MPO/nitrite-dependent LDL lipid oxidation in the absence (control) or in the presence of either  $5 \mu\text{M}$  betanin (BET), or the betanin oxidation product(s) (ox-bet). LDL and ox-betanin were as reported in Methods. That reported is representative of six experiments with different LDL preparations.

or bio-mimetic models may suggest eventual actions of these molecules in the body. This study shows that betanin, a betalain with recently acknowledged radical-scavenging properties [23–25,29,32,33], protects human LDLs from oxidative damage in a MPO/nitrite-dependent oxidation model, which is considered relevant to the pathophysiological conditions supporting the atherogenic process [34–36].

Because of its physicochemical properties, betanin intercepts radicals in the water phase [23], or can interact with lipid moieties, and affords antioxidant protection [24,25,32]. We have previously shown that *ex vivo* spiking of human plasma with varied betanin concentrations resulted in a concentration-dependent binding to LDLs, while the incorporated compound prevented a copper-induced oxidation of the isolated particles, acting as a lipoperoxyl radical-scavenging and chain-breaking antioxidant [25]. The inhibition kinetics and the time-course of betanin consumption during the MPO/nitrite-induced LDL oxidation suggested more complex antioxidative mechanisms.

Nitrogen dioxide, the oxidation product of nitrite with MPO-I, has been proposed as the main reactive intermediate able to promote LDL lipid oxidation [19,20]. This amphiphilic molecule can attack lipid moieties and start oxidation by abstracting hydrogen atoms from bis-allylic methylene groups of polyenoic fatty acids [37]. Since betanin can partition between LDL and water, it is supposed to be able to protect lipids by reacting with either lipoperoxyl radicals [25] and/or possibly with hydrophilic species, including  $\text{NO}_2$  radicals. Our data show that betanin is consumed by nitrogen dioxide in a MPO-dependent  $\text{NO}_2^-$  generating system, supporting the idea that it can indeed inhibit the MPO/nitrite-stimulated LDL oxidation by intercepting  $\text{NO}_2$  radicals, though additional radical scavenging activity in the lipid phase cannot be ruled out. The faster rate of consumption of the molecule in the MPO/nitrite-LDL system, as

compared to its disappearance in the presence of MPO/nitrite alone, suggests that betanin reacts with other radical species in the presence of LDL.

Betanin has been shown to act as a reducing substrate for the intermediates of the peroxidative MPO cycle, namely compound I and II, with a rate constant of the order of  $10^6$  and  $10^5 \text{ M}^{-1} \text{ s}^{-1}$ , respectively [29], which could have affected the activity of the betanin in the MPO/nitrite-dependent-LDL oxidation. In particular, reduction of MPO compound I/II could result in formation of diffusible oxidants that may actually enhance the efficacy of the enzyme to oxidise the particles [38]. On the contrary, when assayed in the absence of nitrite, betanin was not pro-oxidant, providing evidence that, though capable of activating the catalytic cycle of MPO [29], it does not export the oxidizing power of the enzyme from the active protein site to the medium. Rather, betanin clearly counteracted the activity of the peroxidase to oxidize LDL. Thus, these data ruled out that the effects of betanin in the MPO/nitrite-induced LDL oxidation, could result from overlapping of antioxidant actions and the eventual pro-oxidative activity from the MPO-mediated conversion of the betanin to a reactive species.

In contrast with the kinetics observed in the presence of nitrite, the inhibition kinetics of LDL oxidation by MPO in the absence of nitrite revealed a classical chain-breaking antioxidant behaviour of betanin, with dose-dependent prolongation of the lag phase and consumption of the molecule within the lag phase. In accordance with previous findings [25], amounts of betanin partitioned in the LDL particle can account for this evidence. Apparently, such a lipoperoxyl radical-scavenging activity is masked in the presence of nitrite by the effects from scavenging nitrogen dioxide.

Finally, the early consumption of betanin, as compared with the extent of the protective effects during the MPO/nitrite-induced LDL oxidation, revealed peculiar aspects of the molecular activity of the phytochemical. Interactions and recycling are common features in the action of antioxidant molecules and cooperative interactions with the endogenous vitamin E, the main antioxidant in biological membranes and LDL [39,40], can amplify the effects of various compounds [41,42]. As already observed in other metal ion-dependent LDL oxidation model [25], the time-course of vitamin E consumption, either in the presence or in the absence of betanin, ruled out that interactions with the LDL-vitamin E were involved. On the other hand, oxidation product(s) from the reaction of betanin with MPO have appeared to prevent LDL oxidation as effectively as the parent compound, which allows to extend the antioxidative protection beyond the time in which betanin is consumed. Oxidation products of betanin by MPO are not known. Products from the reaction

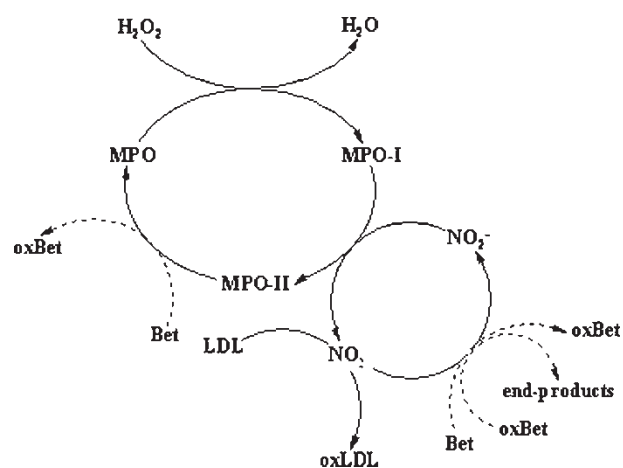


Figure 7. Catalytic cycle of MPO and putative sites of action of betanin (Bet). Oxidised betanin(OxBet).

between betanin and horseradish peroxidase have been described as stable phenoxyl radicals and betalamic acid [43], however, we failed to have any spectral evidence of such compounds in our system. It is tempting to speculate that a rapid reaction of betanin and/or MPO-oxidised betanin with NO<sub>2</sub> led to the accumulation of end-products that do not exhibit a characteristic absorbance spectrum.

On the whole, our work shows that betanin may protect human LDLs from a MPO/nitrite-induced oxidation and suggests that it may affect the oxidative process at various levels. Based on the present findings and previous observations [25,29], a proposal as to how betanin may act in this system is shown in Figure 7. It is hypothesized that, after partitioning between water phase and LDL lipid, betanin could act as an electron donor towards what are currently believed to be the focal oxidants generated in this system, i.e. the MPO catalytic intermediates and their main downstream oxidation product nitrogen dioxide. The rate constant for the reaction between MPO-I and nitrite [15], of the same magnitude order than that of MPO-I and betanin [29] and the actual concentrations of the reductants in our assays, prevent betanin from competing with nitrite for the enzyme compound I, which results in production of nitrogen dioxide radical. By contrast, being the rate constant for the reduction of nitrite by MPO-II of the order of  $10^2 \text{ M}^{-1} \text{ s}^{-1}$  [15], betanin can effectively reduce the MPO-II intermediate, being oxidised and promoting the enzyme cycle. This potentially pro-oxidant behaviour appears to be counteracted by the antioxidant activity of betanin and/or possibly a betanin oxidised product, through scavenging of NO<sub>2</sub>. Finally, it is not possible to rule out that betanin partitioned in the LDL may be involved in additional lipoperoxyl radical-scavenging activity within the particle.

By blunting undesirable MPO-mediated actions of nitrite, betanin could modulate NO metabolism in

a favourable direction, thus counteracting LDL oxidation. In this regard, it is remarkable that recent nutritional studies have shown that betanin is bioavailable in humans [24,44], accumulates time-dependently in circulating LDL and attains a 0.2  $\mu\text{M}$  plasma concentration after a single ingestion of a fruit meal providing 16 mg of the molecule.

### Acknowledgements

This work has been carried out by a grant from Assessorato Agricoltura e Foreste Regione Sicilia.

### References

- [1] Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 1989;320:915–924.
- [2] Heinecke JW. Oxidants and antioxidants in the pathogenesis of atherosclerosis: Implications for the oxidized low density lipoprotein hypothesis. *Atherosclerosis* 1998;141:1–15.
- [3] Lusis AJ. Atherosclerosis. *Nature* 2000;407:233–241.
- [4] Daugherty A, Dunn JL, Rateri DL, Heinecke JW. Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. *J Clin Invest* 1994;94:437–444.
- [5] Heller JI, Crowley JR, Hazen SL, Salvay DM, Wagner P, Pennathur S, Heinecke JW. *p*-Hydroxyphenylacetaldehyde, an aldehyde generated by myeloperoxidase, modifies phospholipid amino groups of low density lipoprotein in human atherosclerotic intima. *J Biol Chem* 2000;275:9957–9962.
- [6] Klebanoff SJ. Oxygen metabolism and the toxic properties of phagocytes. *Ann Intern Med* 1980;93:480–489.
- [7] Marquez LA, Dunford HB. Mechanism of the oxidation of 3,5,3',5'-tetramethylbenzidine by myeloperoxidase determined by transient- and steady-state kinetics. *Biochemistry* 1997;36:9349–9355.
- [8] Leeuwenburgh C, Hardy MM, Hazen SL, Wagner P, Ohishi S, Steinbrecher UP, Heinecke JW. Reactive nitrogen intermediates promote low density lipoprotein oxidation in human atherosclerotic intima. *J Biol Chem* 1997;272:1433–1436.
- [9] Savenkova ML, Mueller DM, Heinecke JW. Tyrosyl radical generated by myeloperoxidase is a physiological catalyst for the initiation of lipid peroxidation in low density lipoprotein. *J Biol Chem* 1994;269:20394–20400.
- [10] Leeuwenburgh C, Rasmussen JE, Hsu FF, Mueller DM, Pennathur S, Heinecke JW. Mass spectrometric quantification of markers for protein oxidation by tyrosyl radical, copper, and hydroxyl radical in low density lipoprotein isolated from human atherosclerotic plaques. *J Biol Chem* 1997;272:3520–3526.
- [11] Furtmuller PG, Burner U, Obinger C. Reaction of myeloperoxidase compound I with chloride, bromide, iodide, and thiocyanate. *Biochemistry* 1998;37:17923–17930.
- [12] Hazell LJ, Stocker R. Oxidation of low-density lipoprotein with hypochlorite causes transformation of the lipoprotein into a high-uptake form for macrophages. *Biochem J* 1993;290 (Pt 1):165–172.
- [13] Hazell LJ, Arnold L, Flowers D, Waeg G, Malle E, Stocker R. Presence of hypochlorite-modified proteins in human atherosclerotic lesions. *J Clin Invest* 1996;97:1535–1544.
- [14] Hazen SL, Heinecke JW. 3-Chlorotyrosine, a specific marker of myeloperoxidase-catalyzed oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima. *J Clin Invest* 1997;99:2075–2081.
- [15] Burner U, Furtmuller PG, Kettle AJ, Koppenol WH, Obinger C. Mechanism of reaction of myeloperoxidase with nitrite. *J Biol Chem* 2000;275:20597–20601.
- [16] Eiserich JP, Hristova M, Cross CE, Jones AD, Freeman BA, Halliwell B, van der Vliet A. Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. *Nature* 1998;391:393–397.
- [17] van der Vliet A, Eiserich JP, Halliwell B, Cross CE. Formation of reactive nitrogen species during peroxidase-catalyzed oxidation of nitrite. A potential additional mechanism of nitric oxide-dependent toxicity. *J Biol Chem* 1997;272:7617–7625.
- [18] Sampson JB, Ye Y, Rosen H, Beckman JS. Myeloperoxidase and horseradish peroxidase catalyze tyrosine nitration in proteins from nitrite and hydrogen peroxide. *Arch Biochem Biophys* 1998;356:207–213.
- [19] Byun J, Mueller DM, Fabjan JS, Heinecke JW. Nitrogen dioxide radical generated by the myeloperoxidase–hydrogen peroxide–nitrite system promotes lipid peroxidation of low density lipoprotein. *FEBS Lett* 1999;455:243–246.
- [20] Kostyuk VA, Kraemer T, Sies H, Schewe T. Myeloperoxidase/nitrite-mediated lipid peroxidation of low-density lipoprotein as modulated by flavonoids. *FEBS Lett* 2003;537:146–150.
- [21] Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *Am J Med* 2002;113(Suppl 9B):71S–88S.
- [22] Piattelli M. The betalains: Structure, biosynthesis and chemical taxonomy. In: Conn EE, editor. *The biochemistry of plants: A comprehensive treatise*. New York: Academic Press; 1981. p 557–575.
- [23] Butera D, Tesoriere L, Di Gaudio F, Bongiorno A, Allegra M, Pintaudi AM, Kohen R, Livrea MA. Antioxidant activities of sicilian prickly pear (*Opuntia ficus indica*) fruit extracts and reducing properties of its betalains: Betanin and indicaxanthin. *J Agric Food Chem* 2002;50:6895–6899.
- [24] Kanner J, Harel S, Granit R. Betalains—a new class of dietary cationized antioxidants. *J Agric Food Chem* 2001;49:5178–5185.
- [25] Tesoriere L, Butera D, D'Arpa D, Di Gaudio F, Allegra M, Gentile C, Livrea MA. Increased resistance to oxidation of betanin-enriched human low density lipoproteins. *Free Radic Res* 2003;37:689–696.
- [26] Stintzing FC, Schieber A, Carle R. Identification of betalains from yellow beet (*Beta vulgaris* L.) and cactus pear [*Opuntia ficus-indica* (L.) Mill.] by HPLC–electrospray ionization mass spectrometry. *J Agric Food Chem* 2002;50:2302–2307.
- [27] Kleinveid HA, Hak-Lemmers HL, Stalenhof AF, Demacker PN. Improved measurement of low-density-lipoprotein susceptibility to copper-induced oxidation: Application of a short procedure for isolating low-density lipoprotein. *Clin Chem* 1992;38:2066–2072.
- [28] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal Biochem* 1976;72:248–254.
- [29] Allegra M, Furtmuller PG, Jantschko W, Zederbauer M, Tesoriere L, Livrea MA, Obinger C. Mechanism of interaction of betanin and indicaxanthin with human myeloperoxidase and hypochlorous acid. *Biochem Biophys Res Commun* 2005;332:837–844.
- [30] Heinecke JW, Li W, Daehnke HL 3rd, Goldstein JA. Dityrosine, a specific marker of oxidation, is synthesized by the myeloperoxidase–hydrogen peroxide system of human neutrophils and macrophages. *J Biol Chem* 1993;268:4069–4077.
- [31] Wieland E, Parthasarathy S, Steinberg D. Peroxidase-dependent metal-independent oxidation of low density lipoprotein of

- low density lipoprotein *in vitro*: A model for *in vivo* oxidation? Proc Natl Acad Sci USA 1993;90:5929–5933.
- [32] Tesoriere L, Butera D, Allegra M, Fazzari M, Livrea MA. Distribution of betalain pigments in red blood cells after consumption of cactus pear fruits and increased resistance of the cells to *ex vivo* induced oxidative hemolysis in humans. J Agric Food Chem 2005;53:1266–1270.
- [33] Escribano J, Pedreno MA, Garcia-Carmona F, Munoz R. Characterization of the antiradical activity of betalains from *Beta vulgaris* L. roots. Phytochem Anal 1998;9:124–127.
- [34] Brennan ML, Wu W, Fu X, Shen Z, Song W, Frost H, Vadseth C, Narine L, Lenkiewicz E, Borchers MT, Lusi AJ, Lee JJ, Lee NA, Abu-Soud HM, Ischiropoulos H, Hazen SL. A tale of two controversies: Defining both the role of peroxidases in nitrotyrosine formation *in vivo* using eosinophil peroxidase and myeloperoxidase-deficient mice, and the nature of peroxidase-generated reactive nitrogen species. J Biol Chem 2002;277:17415–17427.
- [35] Brennan ML, Penn MS, Van Lente F, Nambi V, Shishehbor MH, Aviles RJ, Goormastic M, Pepoy ML, McErlean ES, Topol EJ, Nissen SE, Hazen SL. Prognostic value of myeloperoxidase in patients with chest pain. N Engl J Med 2003;349:1595–1604.
- [36] Zhang R, Brennan ML, Shen Z, MacPherson JC, Schmitt D, Molenda CE, Hazen SL. Myeloperoxidase functions as a major enzymatic catalyst for initiation of lipid peroxidation at sites of inflammation. J Biol Chem 2002;277:46116–46122.
- [37] Huie RE. The reaction kinetics of NO<sub>2</sub><sup>•</sup>. Toxicology 1994;89:193–216.
- [38] Santanam N, Parthasarathy S. Paradoxical actions of antioxidants in the oxidation of low density lipoprotein by peroxidases. J Clin Invest 1995;95:2594–2600.
- [39] Sies H, Murphy ME. Role of tocopherols in the protection of biological systems against oxidative damage. J Photochem Photobiol B 1991;8:211–218.
- [40] Burton GW, Traber MG. Vitamin E: Antioxidant activity, biokinetics, and bioavailability. Annu Rev Nutr 1990; 10:357–382.
- [41] Palozza P, Krinsky NI. The inhibition of radical-initiated peroxidation of microsomal lipids by both alpha-tocopherol and beta-carotene. Free Radic Biol Med 1991;11:407–414.
- [42] Tesoriere L, Bongiorno A, Pintaudi AM, D’Anna R, D’Arpa D, Livrea MA. Synergistic interactions between vitamin A and vitamin E against lipid peroxidation in phosphatidylcholine liposomes. Arch Biochem Biophys 1996;326:57–63.
- [43] Martinez-Parra J, Munoz R. An approach to the characterization of betanine oxidation catalyzed by horseradish peroxidase. J Agric Food Chem 1997;45:2984–2988.
- [44] Tesoriere L, Allegra M, Butera D, Livrea MA. Absorption, excretion, and distribution of dietary antioxidant betalains in LDLs: Potential health effects of betalains in humans. Am J Clin Nutr 2004;80:941–945.