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Modified-atmosphere packaging (MAP) does not affect the bioavailability of tocopherols and carotenoids from broccoli in humans: A cross-over study

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Abstract

Aim of the study: Ready-to-eat and pre-packed vegetables are increasingly accepted by consumers but little is known about the effect of these technological approaches on the bioavailability of the nutrients. To assess the effect of modified-atmosphere packaging (MAP) on the bioavailability in humans of carotenoids and tocopherols from broccoli.
Results: Serum lutein increased significantly upon broccoli intake but those of β-carotene, α- and γ-tocopherol did not reach statistical significance. Serum changes were observed regardless of the type of broccoli consumed.
Conclusions: Modified-atmosphere packaging does not affect significantly the *in vivo* bioavailability of carotenoids and tocopherols from broccoli, supporting its convenience for use by the food industry and consumers.

Keywords: Bioavailability; Food processing; Carotenoids; Vitamin E; Public health

1. Introduction

Fruits and vegetables constitute the major sources of biologically active compounds, many of which (i.e. ascorbic acid, carotenoids, tocopherols) may have beneficial effects against chronic diseases (i.e. cancer, cardiovascular disease) (WCRF & AICR, 1997; WHO, 2003). Although the underlying mechanisms are not completely understood, different biological activities have been suggested including the antioxidant capacity, modulation of inmune function, modification of inflammatory processes and signal transduction within and between cells (Biesalski, 2001).

Growing evidence, including the failure of supplementation trials with single nutrients to prevent certain chronic diseases (Hennekens, 1998) and the apparent advantage of providing mixtures of phytochemicals at dietary achievable levels, supports a holistic view of the diet-health relationship. This would involve consider it in terms of including the variety of foods, processing methods and food habits (Scali, Richard, & Gerber, 2001).

Thus, due to insufficient evidence and the lack of nutrient specificity, especially in relation with the prevention of cancer and CVD, a food-based rather than a compound-based approach is recommended (WHO, 2003). Translation of these guidelines for the general population means

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encouraging a balanced diet rich in a variety of fruits and vegetables (WCRF & AICR, 1997; WHO, 2003). To achieve the benefits associated with the consumption of fruits and vegetables, different approaches are considered to increase the content and/or the bioavailability of these components including agricultural practices, biotechnology and food technology (Olmedilla, Granado, & Herrero, 2001). However, several factors, both associated with the food matrix and the subjects, may alter the bioavailability of active compounds present in foods (West & Castenmiller, 1998). In addition, the majority of plant foods are stored and processed before consumption, a fact that lengthens their preservation and, possibly, affects the bioavailability of different micronutrients (Solomons & Bulux, 1997).

Nowadays, the food industry is increasingly using emerging technologies, including new approaches to preservation and packaging (i.e. minimal processing, modified-atmospheres), to safeguard and stabilize food products. This is having a relevant impact on the food quality and stability of micronutrients in foods (Lindley, 1998), as well as on the food supply and the dietary patterns of the population. Healthy eating campaigns and sociological factors have increased the demand for "ready-to-eat" vegetables, leading to a flourishing market of pre-packed fresh foods, packaged under conditions to extend shelf-life (i.e. modified-atmospheres packaging, MAP). While being increasingly used and accepted by consumers, little is known about the effect of these technological approaches on the retention, stability and bioavailability in humans of the nutrients and phytochemicals these foods provide. In addition, evidences suggest a differential effect of MAP on different nutrients in a given food as well as for a given nutrient in different foods (Erturk & Picha, 2002; Hansen, Moller, & Sorensen, 1995; Wright & Kader, 1997) and, in vitro bioaccesibility (i.e. changes in carotenoid content) and its potential predictive value regarding human absorption of phytochemicals should be validated in different *in vivo* situations. Within this context, our aim was to assess the effect of regular consumption of modified-atmospheres packaging (MAP) on the bioavailability in humans of carotenoids and tocopherols from broccoli.

2. Subjects and methods

2.1. Subjects

Fourteen apparently healthy volunteers (20–35 years) were enrolled in a cross-over dietary intervention study. Number of subjects were calculated based on an expected mean of differences (final-start) of 0.10 μmol/L (SD 0.05 μmol/L) (for α and β values of 0.05 and 0.10, respectively) and the length of the intervention was established according to the average shelf-life of MPA-packaged foods (ca. 1 week), as recommended to the consumer. Inclusion criteria were to display a normal biochemical and haematological profile and serum levels of vitamin A, E and carotenoids within the reference ranges (Table 1) (Olmedilla, Granado, Gil-Martìnez, & Rojas-Hidalgo, 1997). Exclusion criteria included the use of vitamin and/or herbs supplements, dieting, chronic medication and intercurrent disease or infection that could alter the bioavailability or status of the compounds of interest.

Dietary intervention consisted of the consumption of 200 g of cooked broccoli once a day, at lunch or dinner, for seven days. Subjects were randomized to consume firstly untreated ($n = 7$) or minimally-processed ($n = 7$) broccoli and, after 4-weeks wash-out period, subjects consumed the other type of broccoli. Cooking conditions of the broccoli were common for both periods and for all subjects, and it was performed with microwave ovens (800 W, 5 min plus 5 min to rest). Subjects were asked to consume the standard portion of broccoli (200 g) along with a fixed amount of olive oil (10 ml). No other changes were included in the diet and /or the lifestyle of the participants

Table 1
Characteristics of the subjects at the start of each intervention period (mean, $CI_{95\%}$)[a]

	Broccoli (control)	Broccoli (MAP)	*p* value
No. of subjects (male/female)	14 (7/ 7)		–
Age (years)	24 (21, 27)		–
BMI (kg/m^2)	23.1 (22, 25)		–
Total cholesterol (mmol/L)	4.16 (3.72, 4.63)	4.18 (3.66, 4.70)	NS[b]
HDL (mmol/L)	1.55 (1.44, 1.67)	1.57 (1.42, 1.72)	NS[b]
LDL (mmol/L)	2.28 (1.89, 267)	2.22 (1.76, 2.67)	NS[b]
Triglycerides (mmol/L)	0.80 (0.54, 1.05)	0.79 (0.60, 0.99)	NS[b]
Retinol (μmol/L)	1.79 (1.43, 2.16)	1.69 (1.41, 1.97)	NS[b]
Lutein (μmol/L)	0.20 (0.17, 0,24)	0.20 (0.17, 0.26)	NS[c]
β-Carotene (μmol/L)	0.40 (0.17, 0.63)	0.42 (0.21, 0.63)	NS[c]
α-Tocopherol (μmol/L)	24.71 (22.02, 27.39)	24.35 (21.22, 27.47)	NS[c]
α-Toc./Chol. (μmol/mmol)	6.75 (6.11, 7.40)	6.52 (6.07, 6.98)	NS[c]
γ-Tocopherol (μmol/L)	0.53 (0.41, 0.66)	0.51 (0.31, 0.72)	NS[c]

NS: not significant.
[a] CI: Confidence interval.
[b] ANOVA test.
[c] Kruskal–Wallis test.

except to avoid the consumption of other green-vegetables (i.e. spinach, swiss chard) or fortified foods (i.e. juices) and keep a record of their diets to check compliance with these dietary recommendations. Fasting blood samples for tocopherols and carotenoid analysis were obtained before and after the intervention study. The protocol study was approved by the Comité Ético de Investigación Clínica of the Hospital Universitario Puerta de Hierro and all subjects were informed and gave their signed consent.

2.2. Standards and reagents

Unless otherwise stated, all reagents and materials used in the blood sample analysis for vitamin E and carotenoids were purchased from Sigma Aldrich Química, VWR International Eurolab and Carlo Erba (Spain).

2.3. Food preparation

The foods were bought and prepared by the expert staff at the Instituto del Frío (CSIC, Madrid, Spain). In brief, broccoli was purchased at local stores on two occasions (each period of intervention) and at each time the purchase was divided into two homogenized batches to be prepared with and without MAP and distributed to the volunteers ($n = 7$ for each group, in each period). For non-treated broccoli, raw material was only cut, washed, dried, placed into bags (edible portions of 200 g), and stored at 4 °C throughout the study (up to 7 days). For foods processed with MAP, edible portions were dealt with as mentioned except that, afterwards, inflorescences were placed inside polypropylene bags (200 g of broccoli per bag), which were sealed with a 90 μm microperforated film (AMCOR 35PA90). Modified-atmospheres packaging was achieved by flushing the gases into the bags before sealing and concentrations of CO_2 and O_2 were analyzed by PBI-Dansensor AS, CheckMate 9900 (Denmark). Atmosphere determined at day 2 were 3–5% CO_2 and oxygen 16–18% and, under these conditions, the foods were stored at 4 °C during the study (up to 9 days).

2.4. Analytical methods

Analysis of carotenoids and tocopherols were performed in broccoli ($n = 5$, in triplicate) as ready-to-eat (after microwave processing) according to the methodology used in our lab (Granado, Olmedilla, Blanco, & RojasHidalgo, 1992; Granado, Olmedilla, Gil-Martinez, & Blanco, 2001). Analyses were carried out in broccoli samples stored with MAP (at day 2, 7 and 9) and without MAP (at day 1 and 7). For carotenoids and vitamin A and E analysis in serum, samples were processed as described elsewhere (Olmedilla et al., 1997). Briefly, 0.5 ml of serum were mixed with 0.5 ml of ethanol containing internal standard (retinyl acetate), vortexed and extracted twice with 2 ml of methylene chloride/hexane (1:5). Organic phases were pooled, evaporated to dryness and reconstituted to be injected onto the HPLC. The chromatographic system consisted of a Spheri-5-ODS column (Applied Biosystems, San José, CA) with gradient elution of acetonitrile/methanol (85/15) for 5 min to acetonitrile/methylene chloride/methanol (70/20/10) for 20 min. Ammonium acetate (0.025 M) was added to the methanol. Detection was carried out by a photodiode array (Model 996, Waters Associates, Milford, USA) set at 294 nm for tocopherols, 326 nm for retinol and 450 nm for carotenoids. Using this method, retinol, α-, γ-(+β) and δ-tocopherol, trans-lutein, zeaxanthin, 13/15-cis-lutein, α-carotene, all-trans-β-carotene, 9-cis-β-carotene and 13/15-cis-β-carotene, among other carotenoids, can be simultaneously determined. Identification of compounds was carried out by comparing retention times with those of authentic standards and on-line UV–VIS spectrum.

For carotenoid and vitamin A and E analysis, samples from the same individual (before and after the intervention of both periods) were analyzed the same day to reduce analytical variability. The short- and long-term precision and accuracy of the analytical method was within accepted values as contrasted periodically through our participation in the Fat-Soluble Quality Assurance Programme conducted by the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA). At the time of performing the study, the comparability score (CS) (average distance, in standard deviation units, from the consensus medians) was 1 for all the analytes of interest except for γ-(+β)-tocopherol (CS = 2).

2.5. Statistical analysis

For carotenoid and tocopherol content in broccoli, mean and standard deviation were calculated at each time point of the analysis. Since no significant differences were observed according to storage time, intake of the compounds of interest were estimated by pooling the results from all the analysis and descriptive statistics were calculated (mean, median, $CI_{95\%}$). Serum responses upon consumption of broccoli and the effect of the minimal processing were evaluated using both parametric (ANOVA) and non-paramateric test for unpaired and paired data (Mann–Whitney U test, Wilcoxon signed rank test and Kruskal–Wallis test). Statistical significance was set at $p < 0.05$ and analysis was performed with SPSS 8.0 for Windows statistical software (SPSS Inc., Chicago. IL, USA).

3. Results

3.1. Phytochemical content in broccoli

The carotenoid and tocopherol content in cooked broccoli at the start and at the end of the intervention study is shown in Table 2. Compared to the start of the study, higher values were found at the end of the storage period regardless of the use of MAP, although these changes did not reach statistical significance for any of the nutrients

Table 2
Carotenoid and tocopherol content (mg/100 g) (mean, $CI_{95\%}$) in the broccoli throughout the dietary intervention study

	Lutein (mg/100 g)	β-Carotene (mg/100 g)	α-Tocopherol (mg/100 g)	γ-Tocopherol (mg/100 g)
Control (no MAP)[a]				
Start	1.31 (1.17, 1.44)	0.79 (0.70,0.89)	2.83 (2.26, 3.40)	0.67 (0.42, 0.92)
End	1.44 (1.35, 1.53)	0.83 (0.77, 0.89)	3.69 (2.47, 4.92)	0.74 (0.57, 0.90)
MAP[a]				
Start	1.78 (1.74, 1.82)	1.22 (1.09, 1.35)	3.48 (2.84, 4.13)	0.73 (0.73, 0.74)
End	1.93 (1.84, 2.02)	1.24 (1.19, 1.28)	4.62 (4.31, 4.94)	0.84 (0.79, 0.90)

[a] No significant between concentrations at start-end (Mann–Whitney U test).

assessed. Due to this and because of "in vitro" digestion studies performed in our lab showed that the recovery and isomerization degree of carotenoids and tocopherols did not change with storage time under these conditions (Granado, Olmedilla, Herrero, Pérez-Sacristán, & Blázquez, 2005), average values were used to calculate the amounts supplied to the volunteers during the intervention study (Table 3). As shown in Table 3, significant differences were observed in the content of lutein and β-carotene between untreated and MAP broccoli.

3.2. Human intervention study

Baseline values for the compounds of interest at the start of each intervention period are shown in Table 1 and there was no statistical difference in the initial concentrations of both periods. Dietary compliance was excellent since none of the participants reported to have consumed dark green leafy vegetables or fortified foods during the intervention periods. The most frequently consumed fruit and vegetables included orange/mandarin (fresh or juices) and potatoes, respectively, although on average they accounted for less than 0.25–0.30 servings/person/day, while other relevant carotenoid sources (i.e. eggs, lettuce, meals containing ketchup and tomato sauce) were scarcely ingested (<0.05 servings/ person/day). Other fruits and vegetables consumed were apple, pear, kiwi, pineapple, strawberries, melon (honeydew), mushrooms, cabbage (white and red), aubergine and squash (white flesh).

Serum changes upon consumption of broccoli for 7-days are shown in Table 4. Within each period, net increments in serum concentrations were observed for all the compounds, except for β-carotene, although statistical significance was only reached for lutein, regardless of the type of broccoli consumed (control or MAP). Also, net increments and the final concentrations of carotenoids and tocopherols achieved in serum showed no significant differences according to the type of broccoli consumed (Table 4).

Because of the relatively high baseline concentrations of β-carotene in some participants, some of them showed a virtually negative response ($n = 5$ with control broccoli; $n = 3$ with MAP broccoli). When we re-analysed the data excluding these volunteers, a significant increase in serum concentrations of β-carotene was observed upon ingestion of both types of broccoli (mean 0.029 μmol ($CI_{95\%}$ 0.003, 0.055) and 0.051 μmol ($CI_{95\%}$ 0.025, 0.077) for control and MAP broccoli, respectively) and there was no difference in the net increment nor in the final serum levels achieved regarding the type of broccoli consumed.

Due to the different amounts of lutein and β-carotene supplied with each type of broccoli, we also calculated the serum response of lutein and β-carotene per μmol of carotenoid supplied. Again, no significant differences in the changes were obtained according to the type of broccoli consumed (0.021 μmol versus 0.020 μmol for lutein ($p = 0.82$) in the whole group; 0.010 μmol versus 0.012 μmol for β-carotene ($p = 0.69$) in volunteers with positive net increments). Similarly, increments of α-tocopherol and γ-tocopherol in serum (per μmol ingested) were not significantly different according to the type of broccoli consumed (0.02 versus 0.06 for α-tocopherol ($p = 0.65$); 0.027 versus 0.025 for γ-tocopherol ($p = 0.75$)).

Finally, no significant changes were observed for total cholesterol, c-HDL, c-LDL triglycerides or retinol in serum throughout the intervention study, regardless of the type of broccoli consumed.

Table 3
Carotenoid and tocopherol content in broccoli (mean, $CI_{95\%}$) and estimated daily intake from broccoli

	Lutein (mg/100 g)	β-Carotene (mg/100 g)	α-Tocopherol (mg/100 g)	γ-Tocopherol (mg/100 g)
Control (no MAP)	1.37 (1.20, 1.55)	0.81 (0.73,0.90)	2.83 (2.26, 3.40)	0.67 (0.42, 0.92)
MAP	1.77[a] (1.64, 1.90)	1.15[a] (1.05,1.25)	3.69 (2.47, 4.92)	0.74 (0.57, 0.90)
Estimated intake (mg/day)[b]				
Control (no MAP)	2.4–3.1	1.4–1.8	4.5–6.8	0.8–1.8
MAP	3.2–3.8	2.1–2.5	4.9–9.8	1.1–1.9

[a] $p < 0.01$, difference in the average content of both types of broccoli assayed (Mann–Whitney U test).
[b] Values considering the content ($CI_{95\%}$) and a consumption of 200 g broccoli/day.

Table 4
Serum responses of tocopherols and carotenoids (net increment from baseline concentrations; mean, $CI_{95\%}$) upon broccoli consumption and effect of minimal processing

	Lutein (μmol/L)	β-Carotene (μmol/L)	α-Tocopherol (μmol/L)	γ-Tocopherol (μmol/L)
Control (no MAP)	0.10[a] (0.07, 0.13)	−0.007 (−0.047, 0.034)	0.28 (−1.01, 1.58)	0.086 (−0.144, 0.316)
MAP	0.12[a] (0.09, 0.16)	−0.068 (−0.089, 0.075)	1.02 (−0.95, 3.00)	0.091 (−0.168, 0.349)
Effect of MAP (p value)[b]	0.38	0.12	0.65	0.89

[a] $p \leqslant 0.001$, within-subject difference upon broccoli consumption (Wilcoxon signed rank test).
[b] Differences in net increments according to the type of broccoli consumed (Kruskal–Wallis test).

4. Discussion

The present study investigated the effect of modified-atmospheres packaging of broccoli on the serum response of carotenoids and tocopherols in apparently healthy volunteers. Broccoli was chosen as a relevant dietary source of carotenoids and tocopherols and because of the food industry interest in the comercialization of plant foods using this and other emerging technologies. Also, the level of consumption was set at achievable dietary levels (i.e. 200 g/day) since it is compatible with a balanced diet and current dietary recommendations, and also considered sufficient to provoke a relevant impact on the serum status of these phytochemicals when consumed on a regular basis.

Overall, the significant increase of lutein in serum upon broccoli consumption is consistent with other human studies using green vegetables as carotenoid sources (Novotny, Kurilic, Britz, & Clevidence, 2005; Van Het Hof, Brouwer et al., 1999; Van Het Hof, Tijburg, Pietrzik, & Weststrate, 1999), a fact observed regardless of the post-harvest storage treatment of the broccoli consumed (control versus MAP). On the contrary, and independently of the type of broccoli consumed, the lack of a significant effect in serum β-carotene levels on a group level was somewhat unexpected since increments in serum β-carotene have been reported upon green vegetable consumption (Novotny et al., 2005; Van Het Hof, Brouwer et al., 1999; Van Het Hof, Tijburg et al., 1999). Although the absence of significant changes of β-carotene in serum is consistent with that reported upon consumption of low-vegetable diets (Van Het Hof, Brouwer et al., 1999) and the lack of post-prandial response after acute ingestion of MAP-stored lettuce (Serafini et al., 2002), first-pass metabolism at intestinal level (conversion of β-carotene into retinol) may have also contributed since an increase in isotopically labelled retinol has been reported upon consumption of green vegetables containing labelled β-carotene (Novotny et al., 2005).

In the present study, the lack of a significant increase on a group level may be related, at least in part, to the lower amount of broccoli provided (and the dose of β-carotene supplied) compared to previous studies (Novotny et al., 2005; Van Het Hof, Brouwer et al., 1999) as well as the relatively high levels of β-carotene in some volunteers at the entrance of the study. In fact, some participants showed a net decrease upon broccoli intake which may be better explained by an insufficient consumption of β-carotene to compensate their habitual intake rather than to a lack of response (i.e. non-responders) given the high serum β-carotene concentrations they showed at baseline. Supporting this fact is the finding that when these volunteers were excluded from the analysis, a significant increase in serum β-carotene was observed, regardless of the type of broccoli consumed.

After consumption of both types of broccoli, small increments were observed for tocopherols in serum although they did not reach statistical significance, even after correction for total cholesterol concentration (i.e. α-tocopherol/cholesterol ratio). Based on our calculations, the consumption of 200 g/day of broccoli provided, at least, 30% of the recommended daily intake for α-tocopherol (Institute of Medicine, 2000). However, this amount (4.5–9.8 mg/day) is lower than those usually tested in human bioavailability studies, both using foods or capsules (Borel et al., 1997; Traber & Sies, 1996), and thus it may have been insufficient to provoke significant changes in serum. Comparatively, the daily amount of α-tocopherol supplied was 4–5 times that of γ-tocopherol but, although increments in serum α-tocopherol were of greater magnitude (as expected), when serum increments were adjusted for the amount supplied (per μmol ingested), no differences were observed in the serum response of both isomers. Moreover, the percentage of change for both vitamers in serum accounted for 1–4% and 16–18% of the basal concentrations for α-tocopherol and γ-tocopherol, respectively, regardless of the type of broccoli consumed. In this sense, given the lack of biodiscrimination between both vitamers at intestinal level and the protein-mediated incorporation of α-tocopherol into nascent very-low density lipoproteins in the liver (Traber & Sies, 1996), these relative changes were somewhat expected and also are consistent with data on γ-tocopherol responses in serum (Cooney, Custer, Okinaka, & Franke, 2001) and thus, the present findings support the lack of effect of the MAP on the *in vivo* bioavailability of these compounds.

The effect of new packaging materials and emerging technologies (i.e. MAP, high-pressure, pulsed electric fields) on the bioavailability of nutrients and phytochemicals have been studied in a few commercial foods (i.e. spinach, lettuce, orange juice) (Gil, Ferreres, & Tomás-Barberán, 1999; Jacobson, Nielsen, & Sjoholm, 2004; Sánchez-Moreno et al., 2003, 2004; **?**) with conflicting results. MAP-storage has been reported to affect negatively the content of several phytochemicals (including β-carotene and vitamin C) and antioxidant capacity both in spinach

(Gil et al., 1999) and lettuce (Serafini et al., 2002) although it has been reported that differences in packaging materials and the modified-atmospheres developed inside the packaging (i.e. O_2 and CO_2 proportions) affect differently the food constituents in broccoli (i.e. aroma profile and the concentration of sulphur-containing compounds) (Jacobson et al., 2004). Compared with fresh lettuce acute intake, the consumption of MAP-stored lettuce failed to improve the plasma levels of these nutrients and plasma total antioxidant capacity in humans (Serafini et al., 2002) while, on the contrary, the consumption of orange juice processed with high-pressure and pulsed electric fields effectively improved serum status of vitamin C and markers of antioxidant status in humans, indicating that these preservation methods retain the content, bioavailability and biological properties of food components (Sánchez-Moreno et al., 2003, 2004).

Consistent with these findings, we did not find significant changes in the carotenoid and tocopherol content after storage for up to 9 days nor in the serum responses upon consumption, indicating that minimal processing (plus modified-atmosphere packaging) of broccoli does not affect significantly the stability of carotenoids and tocopherols in the food nor modify the *in vivo* bioavailability of these phytochemicals. Nevertheless, the conflicting results reported with other foods (i.e. lettuce) suggest potential effects of other factors related to the food matrix, packaging materials and/or the atmospheres developed inside the packaging (i.e. O_2 availability), and indicate the need for more studies to assess the effect of minimal processing (i.e. MAP) on the bioavailability of relevant phytochemicals and micronutrients.

In summary, modified-atmosphere packaging does not affect significantly the *in vivo* bioavailability of carotenoids and tocopherols from broccoli, supporting its convenience for use by the food industry and consumers. In addition, the present study supports the efficacy of the regular consumption of both fresh and MAP broccoli to improve the serum status of lutein, β-carotene and γ-tocopherol in young healthy subjects.

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