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Grapefruit Juices Impair the Bioaccessibility of β-Carotene from Orange-Fleshed Sweet Potato but Not Its Intestinal Uptake by Caco-2 Cells

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ABSTRACT: Among various factors influencing β-carotene (Bc) bioavailability, information on interactions between carotenoids or other micronutrients such as flavonoids during a meal that contains different plant-derived foods is quite limited. Because orange-fleshed sweet potato (OFSP) is an important Bc-rich staple food, a source of vitamin A in developing countries, this study focused on the effect of citrus fruit juice carotenoids and flavonoids on Bc bioaccessibility from OFSP. In vitro digestion coupled with the Caco-2 cell culture model was used to evaluate the bioaccessibility and cellular uptake of Bc from OFSP in the presence of pink grapefruit (pGF) or white grapefruit (wGF) juices. The addition of grapefruit juices significantly decreased the bioaccessibility, by up to 30%, but not the cellular uptake of Bc from boiled OFSP. Lycopene, but more probably naringin, present in grapefruit juices was suspected to be responsible for the inhibitory effect of the citrus juices on Bc bioaccessibility. This inhibition was apparently due in part to competition for incorporation between Bc and naringin into mixed micelles during in vitro digestion. In contrast, Bc uptake from dietary micelles was not impaired by naringin.

KEYWORDS: *naringin, lycopene, citrus juices, micellarization, in vitro digestion*

INTRODUCTION

In a normal meal containing plant-derived foods, β-carotene (Bc) is necessarily ingested with macro- and micronutrients that can affect its bioavailability.[1] More precisely, the biological activity of Bc from food is highly dependent on its bioavailability, including bioaccessibility and intestinal uptake. An in vitro digestion model to screen the bioaccessibility of carotenoids from food was developed by Failla's research group[2] and is now widely used by scientists. In this model, bioaccessibility is evaluated by the efficiency of carotenoid micellarization (i.e., transfer into micelles). The model can be coupled to Caco-2 cells to evaluate carotenoid uptake by human intestinal cells.[2,3] Carotenoid bioaccessibility and intestinal uptake depend on an array of factors, including the food matrix in relation to processing/cooking and the presence of macronutrients such as fat and fibers or microconstituents in the food.[1,4,5] Among microconstituents, other carotenoids may also affect Bc bioavailability. Interactions between Bc and other carotenoids during absorption and postabsorptive metabolism have been shown in animal and human supplementation studies.[6,7] Interactions could occur during micellar transfer and/or cellular uptake. Tyssandier et al.[8] observed that the addition of lutein or lycopene to an oil emulsion decreased Bc micellarization. Conversely, Thakkar et al.[9] found that high lutein concentrations enhanced the efficiency of Bc micellarization. Interactions between carotenoids during their transport through Caco-2 cell monolayers was investigated by During and Harrison.[10] These authors observed that interactions during uptake occurred between carotenes (nonpolar carotenoids), that is, Bc with lycopene and Bc with α-carotene. On the other hand, other microconstituents, such as polyphenols, could also influence carotenoid absorption or bioaccessibility. Reboul et al.[5] showed that lutein absorption was markedly affected by naringenin. Furthermore, it was demonstrated that quercetin increased the level of hepatic Bc in mice fed a supplemented Bc plus quercetin diet.[11] However, the majority of these studies have investigated these interactions with standard molecules but not through a complex food matrix environment.

Orange-fleshed sweet potato (*Ipomoea batatas* (L.) Lam, OFSP) appears to be an interesting matrix model for studying the effect of micronutrients on Bc bioaccessibility. As it contains a high amount of Bc (80−90% of the total carotenoids), its consumption contributes to preventing vitamin A deficiency in developing countries (particularly sub-Saharan Africa).[12−14] However, the bioaccessibility of the

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all-trans Bc of this staple food remains relatively low[15,16] for unknown reasons.

As citrus fruits contain a diverse range of carotenoids and specific flavonoids, these foods represent a good model because they are a source of micronutrients susceptible to interaction with OFSP Bc bioavailability. Furthermore, citrus fruits are commonly consumed in Africa and used in household food preparations, whereas banana or apples are considered luxury products.[17] Indeed, 3% of world citrus production comes from Africa, and particularly Nigeria (3.7 million t of citrus fruit/year; FAO 2004), and although there are many constraints, a good local and regional market expanded.[18]

The present study was designed to assess the effect of grapefruit juices on the bioaccessibility of Bc from boiled OFSP and to identify micronutrients that may compete with Bc. In a second part, the Bc cellular uptake was evaluated using the in vitro digestion procedure coupled with the Caco-2 cell culture model.

MATERIALS AND METHODS

Chemicals and Standards. Pepsin (porcine), α-amylase (porcine), bile extract (porcine), pancreatin (porcine), taurodeoxycholate (TDC), and pyrogallol were purchased from Sigma-Aldrich (St Quentin Fallavier, France). Extraction solvents were of RPE-grade from Carlo-Erba (Val de Reuil, France). Carotenoid standards (Bc, β-apo-8′-carotenal, lycopene) and naringin were purchased from Extrasynthèse (Genay, France) for HPLC use. Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/L glucose and trypsin–EDTA (500 and 200 mL/L, respectively) was purchased from Bio Whittaker (Fontenay-sous-Bois, France). Fetal bovine serum (FBS) was purchased from Biomedia (Issy-les-Moulineaux, France), and nonessential amino acid and penicillin/streptomycin were from Gibco BRL (Cergy-Pontoise, France).

Sample Preparation. OFSPs (*I. batatas* Lam.) from Israel were purchased in a local market in Montpellier, France. OFSPs were peeled and cut in equal-sized pieces. Samples were boiled in tap water (OFSP/water; 2:1; w/v) in an uncovered pot. The boiling time was around 18 min. Boiled OFSP was then mixed with a thermomix (Thermomix TM 31 Vorwerk) to obtain a homogeneous purée. Finally, samples were placed in amber bottles and stored under nitrogen at −20 °C until analysis. Pink grapefruit (pGF) and white grapefruit (wGF) (*Citrus paradisi* Macf) juices were commercial 100% pure juices purchased in a local market in Montpellier, France. Fruit juices were stored in amber bottles and kept frozen at −20 °C until analysis.

In Vitro Digestion. The in vitro digestion procedure was similar to that described in our previous study[19] with the addition of an oral phase to be closer to physiologic digestion. Samples (5 g) of OFSP purée alone or with 30 mL of fruit juices were subjected to simulated oral, gastric, and small intestinal phases of digestion. The purée/fruit juice ratio was a trade-off between the potential quantities ingested during a meal (around 5 g of purée and 30 mL of fruit juices) and the experimental constraints (medium had to remain relatively liquid to be filtered). Samples were mixed with a saliva solution (6 mL) with a composition close to physiologic conditions. This was prepared by dissolving in 100 mL of ultrapure water 0.5208 g of $NaHCO_3$, 0.0878 g of NaCl, 0.0478 g of KCl, 0.044 g of $CaCl_2.H_2O$, 0.1044 g of K_2HPO_4 mucin (0.216 g), and porcin α-amylase (200 units/mL).[20] The pH was adjusted to 7.0, and the mixture was incubated for 10 min at 37 °C in a shaking water bath. Then gastric (pH 4, pepsin, 37 °C, 30 min) and duodenal (pH 6, bile and pancreatin, 37 °C, 30 min) digestions were simulated (note that the quantity of bile exact was adjusted to the quantity of food to have always a bile concentration of 6 mg/mL in the final digested volume). Micelles were separated by centrifugation (48000*g* for 4 h at 10 °C using a Beckman JA21rotor). Note that after in vitro digestion of boiled OFSP, the recovery of *all-trans* and 13-*cis* Bc was at least 90%. The aqueous fraction was collected and filtered through a 0.22 μm filter (Millipore). Aliquots were stored at −20 °C under nitrogen until analysis. In the case of standard addition, lycopene was solubilized in tetrahydrofuran (THF), and naringin was used as crystalline powder. Both were added in NaCl (0.9%) containing pyrogallol (12.6 mg/mL) solution at the beginning of the digestion phase. Lycopene was added in the proportion found in fruit, and naringin was added to obtain a final saturated solution.

Micellarization Tests. *Interaction Tests.* Equimolar quantities (0.017 μmol) of Bc solubilized in petroleum ether and lycopene solubilized in dichloromethane were placed in an amber bottle. The solvent was evaporated under nitrogen. Then, and when necessary, a crystalline form of naringin was added to obtain a final saturated solution. Five milliliters of 100 mg/mL of bile extract was then added. After 17 h of incubation at 37 °C on a magnetic stirrer at 600 rpm, the samples were centrifuged at 1700*g* for 5 min and filtered (0.2 μm, Millipore filter), and 3 mL was extracted as described above according to the digested sample extraction conditions.

Solubilization Tests. Bile or taurodeoxycholate (TDC) solution was prepared in a Tris buffer (12.11 g/L, pH 6.0). Increasing concentrations of bile or TDC were added to naringin. The solutions were mixed and incubated for 17 h at 37 °C on a magnetic stirrer. Samples were centrifuged at 1700*g* for 5 min and filtered (0.2 μm, Millipore filter) to collect the micellar phase. Naringin was extracted as described above.

Carotenoid Extraction from Foods and HPLC Analysis. Carotenoid extraction from food was carried out according to our previous study.[19] Fruit juice (20 g) or boiled OFSP (5 g) was extracted with ethanol/hexane (4:3, v/v). β-Apo-8′-carotenal was added as an internal standard. Carotenoid extracts were dissolved in 1 mL of 50:40:10 (v/v/v) mixture of dichloromethane, methyl *tert*-butyl-ether (MTBE), and methanol and analyzed by HPLC. Carotenoid extraction from digested food was also performed as described by Dhuique-Mayer et al.[19] An aliquot of digested sample (10 mL) or a sample resulting from the micellarization test (3 mL) was extracted three times with 10 mL of hexane and 5 mL of ethanol spiked with 100 μL of β-apo-8′-carotenal as recovery standard. The pooled hexanic extracts were evaporated and redissolved in 500 μL of mobile phase (250 μL of dichloromethane and 250 μL of an 80:20 (v/v) mixture MTBE and methanol). Carotenoids were analyzed by reverse-phase HPLC using an Agilent 1100 system (Massy, France). Carotenoids were separated along a C_{30} column (250 × 4.6 mm i.d., 5 μm YMC (EUROP GmbH), mobile phases included H_2O as eluent A, methanol as eluent B, and MTBE as eluent C. The flow rate was set at 1 mL/min, the column temperature was set at 25 °C, and the injection volume was 20 μL. A gradient program was performed: the initial condition was 0–5 min, 40% A, 60% B; 5–10 min, 20% A, 80% B; 10–60 min, 4% A, 81% B, 15% C; 60–71 min, 4% A, 11% B, 85% C; 71–72 min, 100% B; and back to the initial condition for reequilibration. Carotenoid quantification was achieved by establishing calibration curves with *all-trans* Bc and lycopene with five concentrations, and the correlation coefficients ranged from 0.994 to 0.998, respectively. The 13-*cis* Bc was expressed as equivalent *trans*-form.

Naringin Extraction and Analysis. The extraction method was the same as the procedure described by Mouly et al.[21] Naringin was determined by the HPLC system that consisted of an Agilent 1100 model (Massy, France) mounted with an RP 18e Licrospher 100 (5 μm) column (250 mm × 4.6 mm i.d.) (Merck KgaA, Darmstadt, Germany). The isocratic solvent system was water/acetonitrile/THF/acetic acid (80:16:3:1; v/v/v/v). Detection was set at 280 nm. The flow rate was set at 1 mL/min. Naringin quantification (solubilized in DMF/water 2:1, v/v) was achieved using a calibration curve with correlation coefficients of 0.997.

Measurement of Carotenoid Uptake by Intestinal Cells. Caco-2 clone TC7 cells were a gift from Dr. M. Rousset (U178 UNSERM, Villejuif, France). Cells were cultured in the presence of DMEM supplemented with 20% of heat-inactivated FBS, 1% of nonessential amino acid, 1% of streptomycin, and 2% of L-glutamine (complete medium) as previously described.[19] For each experiment, cells were seeded at a density of 1.5×10^6 cells/75 cm^2 flask (Becton Dickinson, le Pont-de Chaix, France). Cells received 15 mL of

Table 1. Carotenoids and Naringin Content in Boiled OFSP and Grapefruit Juices[a]

	carotenoid content (μg/g)			
	Bc			
food	*all-trans*	13-*cis*	Lyc	Nar (μg/g)
OFSP	75.69 ± 2.99	7.98 ± 0.37		
pGF	3.12 ± 0.04	0.16 ± 0.01	16.49 ± 0.40	287.21 ± 2.53
wGF	nd	nd	nd	553.98 ± 13.08

[a]Data are the mean ± SD of three independent experiments. OFSP, orange-fleshed sweet potato; pGF, pink grapefruit juice; wGF, white grapefruit juice; Bc, β-carotene; Lyc, lycopene; Nar, naringin; nd, not detected (i.e., <0.005 μg/g).

complete medium for 14 days. For the last 7 days, the complete medium was replaced by FBS-free medium. Media were changed every day for 21 days to obtain confluent differentiated cell monolayers. The experiment design was as follows: Carotenoid-rich micelles derived from the in vitro digestion were used at 1:3 dilution according a previous study.[19] At the beginning of each experiment, cell monolayers were washed with 2 mL of phosphate-buffered saline (PBS). Cell monolayers were incubated with 15 mL of diluted micelles for 2 h at 37 °C. Media and cells were harvested after the incubation period. Cell monolayers were scraped and collected in 2 mL of PBS. All of the samples were stored at −80 °C under nitrogen before carotenoid extraction and HPLC analysis. Aliquots of cell samples were used to estimate protein concentrations with a bicinchoninic acid kit (BCA kit, Montluçon, France). Carotenoid extractions from Caco-2 cells were carried out as reported by Dhuique-Mayer et al.[19]

Statistical Analysis. Results are expressed as mean values with their standard deviation from at least three independent experiments. The variance homogeneity was evaluated by the Bartlett test. Statistical significance was determined using a one-way analysis of variance (ANOVA) with posthoc Fisher's tests for multiple comparisons using XLStat software. Results obtained in the naringin with bile extract solubilization test were analyzed using a Pearson correlation test. Differences with $P < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Effect of Grapefruit Juices on the Bioaccessibility of Bc from Boiled OFSP. The present study was designed to assess the effect of citrus grapefruit juices on the bioaccessibility of OFSP purée Bc. Grapefruit juices were selected for their special carotenoid profile and flavonoid content (Table 1). The Bc content in boiled OFSP was 75.7 μg/g for *all-trans* Bc and 8 μg/g for 13-*cis* Bc. The percentage of 13-*cis* Bc represented around 10% of the total Bc content. pGF was selected for its carotenoid profile, in which the major carotenoid was lycopene. Furthermore, it contained a very low amount of Bc as compared to OFSP (only 4%). wGF did not contain a detectable level of carotenoids. Hence, it was interesting to include it in our study to have a matrix in which carotenoids would not compete with Bc. These citrus juices also contained a major flavanone glycoside: naringin. The naringin content was about 2-fold higher in wGF than in pGF.

The bioaccessibility of Bc from boiled OFSP was relatively low and reached 3.1 and 15.9% for *all-trans* and 13-*cis* isomers, respectively (Table 2). The low bioaccessibility of Bc from boiled OFSP observed in our experiment was in agreement with recent literature.[15,16] The addition of grapefruit juices affected the bioaccessibility of both Bc isomers (Table 2). The percentage of Bc micellarization significantly ($P < 0.05$) decreased in the presence of grapefruit juices (pink and white). pGF reduced the bioaccessibility of *all-trans* and 13-*cis* Bc to 2.1 and 10.0%, respectively. These values corresponded to approximately 68 and 63% of the control. The addition of wGF drastically reduced the Bc isomers, and only 0.4 and 4.3% of *all-trans* and 13-*cis* isomers, respectively, were found in micelles.

Table 2. Bioaccessibility of Bc from Boiled OFSP in the Presence of Grapefruit Juices[a]

	bioaccessibility (%) (ref/control%)[b]	
	all-trans Bc	13-*cis* Bc
OFSP	3.1 ± 0.3 a (100.0)	15.9 ± 2.2 x (100.0)
OFSP + pGF[c]	2.1 ± 0.2 b (67.7)	10.0 ± 0.8 y (62.8)
OFSP + wGF[c]	0.4 ± 0.3 c (12.9)	4.3 ± 0.1 z (27.0)
OFSP + pGF/2 + wGF/2[c]	0.8 ± 0.2 d (25.8)	4.7 ± 1.1 z (29.6)

[a]Data are the mean ± SD, $n = 6$ independent simulated digestions of each conditions. Significant differences ($P < 0.05$) are represented by different letters. OFSP, orange-fleshed sweet potato; pGF, pink grapefruit juice; wGF, white grapefruit juice; Bc, β-carotene. [b]Percentage of Bc micellarized in reference to the control (OFSP). [c]pGF = 30 mL, wGF = 30 mL, pGF/2 = 15 mL, wGF/2 = 15 mL.

Similarly, the bioaccessibility of *all-trans* Bc was significantly lower ($P < 0.05$) when pGF and wGF were added simultaneously to boiled OFSP, suggesting a half-effect of each grapefruit juice.

With regard to these results, the present study suggests that grapefruit micronutrients, such as lycopene and naringin, or both, could be responsible for the inhibitory effect of fruit juices on OFSP Bc bioaccessibility. The possibility that additional polyphenols may be contributing to the lower bioaccessibility of Bc in OFSP is not probable because in grapefruit juices, naringin represents around 68% of the polyphenols, each other were in very low concentrations.[22] Moreover, compounds expected to interfere in bioaccessibility or in micellarization could have a relative lipophilicity compared with other soluble compounds. Interaction of flavonoid with surfactant has been reported by Bushra Naseem et al.[23]

Effect of Lycopene on Bc Micellarization. To evaluate the possible inhibitory effect of pGF lycopene on OFSP Bc micellarization, *simulated* digestion of boiled OFSP was conducted with the addition of lycopene standard instead of pGF. Standard lycopene was added in equivalent amount determined in 30 mL of pGF (the volume of juice used for in vitro digestion). The data in Table 3 show an inhibitory effect of pure lycopene on OFSP Bc micellarization. More precisely, the addition of lycopene during in vitro digestion significantly reduced ($P < 0.05$) the bioaccessibility of OFSP *all-trans* Bc and, to a lesser extent, OFSP 13-*cis* isomer bioaccessibility. Note that this inhibition was close to that observed when pGF was introduced into boiled OFSP (percentage of the control value). Nevertheless, a micellarization test was carried out to obtain further evidence on the interaction between these two carotenoids and to avoid the matrix effect of OFSP food on Bc micellarization. This test consisted of incubating equimolar amounts of standard carotenoids in the presence of bile extract. The micellarization of Bc alone was adjusted to 100% (Figure 1). The addition of lycopene significantly ($P < 0.05$) reduced

Table 3. Bioaccessibility of Bc from Boiled OFSP in the Presence of Lycopene and Naringin Standards[a]

	bioaccessibility (%) (ref/control %)[b]	
	all-trans Bc	13-*cis* Bc
OFSP	3.1 ± 0.3 a (100)	15.9 ± 2. x (100)
OFSP + Lyc	2.3 ± 0.1 b (74.2)	13.2 ± 2.0 y (83.0)
OFSP + Nar	1.3 ± 0.1 c (41.9)	10.5 ± 1. z2 (66.0)

[a]Data are the mean ± SD, n = 3 independent simulated digestions of each condition. Significant differences ($P < 0.05$) are represented by different letters. OFSP, orange-fleshed sweet potato; Bc, β-carotene; Lyc, lycopene; Nar, naringin. [b]Percentage of Bc micellarized in reference to the control (OFSP).

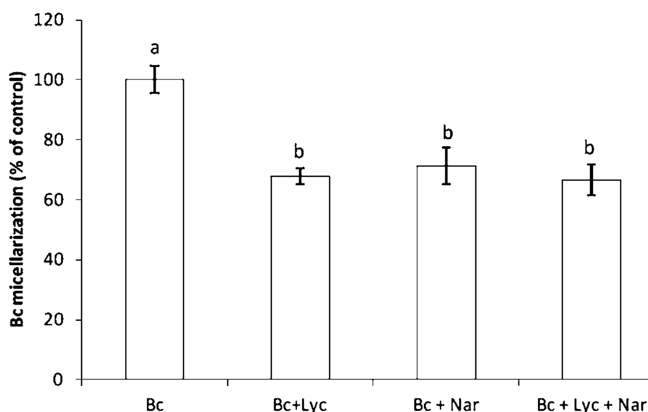

Figure 1. Micellarization of Bc standard (*all-trans*) in the presence of lycopene and/or naringin standard: percentage of Bc micellarized in reference to the control (Bc alone). Data are the mean ± SD, $n = 3$ independent micellarization tests of each condition. Significant differences ($P < 0.05$) are represented by different letters. Bc, β-carotene; Lyc, lycopene; Nar, naringin.

Bc micellarization by around 68%. Thus, the lycopene standard added to either the in vitro digestion procedure or to the micellarization test significantly decreased the bioaccessibility of OFSP *all-trans* Bc. This suggested that the inhibitory effect of pGF on Bc micellarization could be in part due to the presence of lycopene. It has already been shown that the incorporation of Bc in mixed micelles can be impaired by other carotenes such as lycopene.[8] More generally, similar effects were observed on a chylomicron[7] and on liver[24] Bc responses in the presence of lycopene. The most likely mechanism for this interaction was competition between carotenoids for incorporation into mixed micelles during digestion. As suggested by Tyssandier et al.,[8] this interaction was due to the hydrophobicity of both Bc and lycopene and their localization in the core of the lipid droplets.

Effect of Naringin on Bc Micellarization. Our results have shown a higher inhibitory effect of wGF as compared to pGF. Because the wGF matrix did not contain carotenoids, we hypothesized that flavanone glycoside, present at higher concentration in wGF than in pGF, could be responsible for this effect. Naringin is the major flavanone glycoside in grapefruit. When naringin standard was added instead of wGF in the in vitro digestion model, the bioaccessibility of *all-trans* Bc was significantly reduced ($P < 0.05$). A similar trend was observed for the 13-*cis* isomer (Table 3). Moreover, the result of the micellarization test (Figure 1) showed a similar effect: the micellarization of Bc dropped to 71% in the presence of naringin. These data confirmed the possible interaction between Bc and naringin. To elucidate the mechanism of this interaction, we next examined the possibility that some of the naringin was micellarized in the aqueous fraction of digesta because of the low solubility of naringin in water. Note that Walsh et al.[25] suggested that some polyphenols are incorporated into mixed micelles during small intestinal digestion. Therefore, in our experiment, increasing concentrations of bile extract were incubated with naringin (sufficient amount to have a saturated solution in water). The results (Figure 2) showed that the percentage of naringin solubilized in

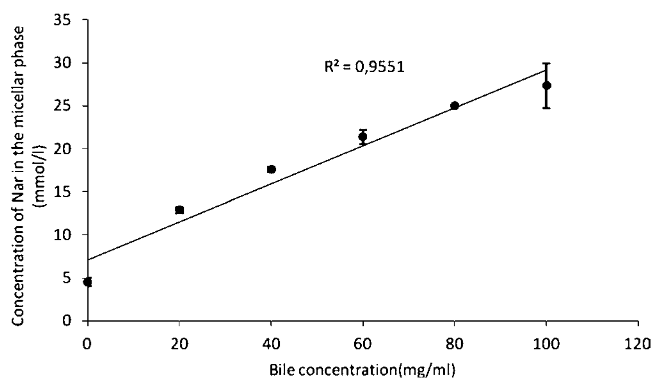

Figure 2. Solubilization of naringin in the presence of increasing bile concentration. Data are the mean ± SD ($n = 3$ independent experiments).

the bile salt aqueous phase increased with the concentration of bile extract. The naringin concentration in the aqueous fraction was around 4.5 ± 0.5 mM when bile extract was omitted. This concentration increased significantly ($P < 0.01$) with the bile concentration, to reach approximately 27.3 ± 2.6 mM. The amount of naringin in the aqueous fraction was positively correlated ($R = 0.98$, $P < 0.005$) with the concentration of bile extract.

To complete this first experiment and demonstrate the actual micellarization of naringin, another experiment was carried out using only TDC instead of a mixture of bile extract. The critical micellar concentration (CMC) of this bile salt, that is, the concentration of bile salts above which micelles form and almost all additional salt added to the system goes to micelles, was around 2.5−3 mM.[26] The results (Figure 3) showed that solubility of naringin was relatively high in the absence of bile

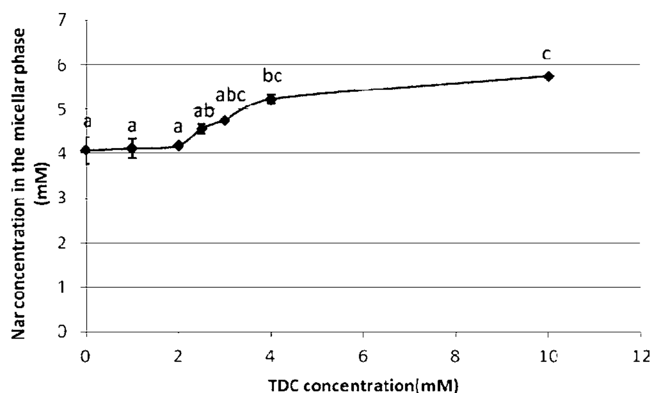

Figure 3. Solubilization of naringin in the presence of an increasing taurodeoxycholate bile salt. Data are the mean ± SD ($n = 3$ independent experiments). Significant differences ($P < 0.05$) are represented by different letters.

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extract and increased in a concentration-dependent manner by approximately 35% once the CMC of TDC was exceeded. It is likely that this increase was due to the solubilization of naringin in micelles, which occurred when the TDC concentration reached the CMC. These data confirmed the hypothesis that naringin could be incorporated into mixed micelles during small intestinal digestion. Therefore, naringin could compete with Bc for the incorporation in mixed micelles. Another mechanism by which naringin could inhibit Bc micellarization concerns its ability to absorb at the interface of oil in water lipid droplets.[27] It has been previously suggested that the presence of carotenoids at the surface of the lipid droplets could interfere with the transfer of Bc from these droplets to micelles.[8] Consequently, naringin may have exerted the same effect. This absorption possibly impairs triglyceride lipolysis by pancreatic lipase and, hence, the transfer of Bc from lipid droplets to mixed micelles. Therefore, the inhibition of OFSP Bc micellarization by pGF was probably due, in part, to the presence of naringin. In agreement with this hypothesis, the inhibitory effect was higher in the presence of wGF due to the higher naringin concentration. Interestingly, Veda et al.[28] observed that some citrus fruits, such as lime juice, enhanced Bc bioaccessibility from fruits or vegetables (carrot or pumpkin). The authors suggested that the high citric acid content could explain this effect by loosening of the matrix, rendering Bc more bioaccessible. However, lime species are very different from grapefruit species, in which citric acid is very low compared to lime juice.[29] Moreover, *Citrus aurantifolia* lime juice does not contain the flavanone naringin.[22]

Combined Effect of Naringin and Lycopene on Bc Micellarization. Although pGF contains lycopene and naringin, its effect on Bc bioaccessibility was less marked than that of wGF (Table 2). The micellarization test results illustrated in Figure 1 show that naringin and lycopene had similar effects on Bc micellarization (67.9 ± 2.6 and 71.2 ± 6.2%, respectively). Surprisingly, in the presence of both molecules, this effect was not accentuated (66.6 ± 5.1%). We concluded that there was no additive effect of these molecules on Bc micellarization. These results supported the hypothesis that lycopene had a minor effect compared to the naringin. Indeed, the reduction of Bc micellarization was correlated (R = 0.98, $P < 0.05$) with the amount of naringin in the different in vitro digestion conditions.

Effect of Grapefruit Juices on Cellular Uptake of Bc. Bc uptake from micelles generated during in vitro digestion from boiled OFSP and boiled OFSP plus grapefruit juices was examined to complete the bioaccessibility study. After 2 h of incubation, the filtered and diluted fraction of digested boiled OFSP was added to monolayers of differentiated Caco-2 cells. *all-trans* Bc (around 20% of the medium) was preferentially absorbed as compared to 13-*cis* (20 vs 10%, respectively) (Table 4). These results agreed with a previous study on other staple foods and obtained with micelles generated during simulated digestion.[30] Moreover, the higher absorption of the *all-trans* form as compared to the 13-*cis* form was already observed in a previous study with synthetic or physiologic micelles.[31,32]

No differences were observed in the uptake of micellarized OFSP Bc when grapefruit juices were added to boiled OFSP. Note that cellular uptake was expressed as a percentage of absorbed carotenoid in recovered scraped cells representing apparent uptake from medium ,and it was similar in each condition (20% of medium). On the other hand, micellar

Table 4. Cellular Uptake and Cellular Content of Micellarized Bc Isomers Generated from in Vitro Digestion[a]

	Bc			
	cellular uptake (%)		cellular content (pmol/mg prot)	
	all-trans	13-*cis*	*all-trans*	13-*cis*
OFSP	20.2 ± 5.0 a	9.9 ± 1.1 a	9.1 ± 0.3 a	3.5 ± 0.1 a
OFSP + pGF	22.0 ± 2.2 a	10.6 ± 1.9 a	6.4 ± 0.7 b	1.8 ± 0.3 b
OFSP + pGF + wGF	20.5 ± 0.9 a	7.8 ± 0.8 a	1.4 ± 0.5 c	0.2 ± 0.1 c

[a]Caco-2 cells were incubated with micelles obtained from digested boiled OFSP, mixed boiled OFSP and pGF, or mixed boiled OFSP and pGF + wGF (OFSP + pGF/2 + wGF/2). Cellular uptake was expressed as a percentage of absorbed carotenoid in recovered scraped cells (cells content + apical content = 100%) representing apparent uptake. Cellular content refers to the amount of Bc absorbed by cells. Note that Bc content in micellar phase was different for each condition: for *all-trans* Bc, 142 ± 11.2, 61.2 ± 2.6, and 34.5 ± 1.4 nM; for 13-cis Bc, 100.0 ± 13.6, 38.1 ± 3.9, and 38.7 ± 3.9 nM. The concentrations of naringin were 52.8 ± 1.7 and 74.6 ± 2.5 μM, respectively. Lycopene was absent from the cell culture medium due to the low micellarization of this carotenoid. Data are the mean ± SD, n = 3 independent experiments. Significant differences ($P < 0.05$) are represented by different letters in columns. OFSP, orange-fleshed sweet potato; pGF, pink grapefruit juice; wGF, white grapefruit juice; Bc, β-carotene.

aqueous fraction resulting of in vitro digestion containing different amounts of Bc implied that intracellular contents were different in each condition. Thus, Bc was not detected in the OFSP plus wGF condition due to the initially low Bc bioaccessibility of this carotenoid. For the condition OFSP plus pGF, only trace amounts of lycopene were detected in micelles due to its poor micellarization compared with other carotenoids.[33] Therefore, in our experiments this carotenoid could not interfere with Bc uptake despite a significant reduction in Bc absorption in the presence of lycopene with synthetic micelles observed in a previous study.[31] By contrast, almost 100% of grapefruit juice naringin was recovered in the aqueous micellar fraction of the digesta. We could therefore conclude that these naringin concentrations did not impair Bc uptake in our conditions. This lack of effect of naringin on Bc uptake by Caco-2 was confirmed by co-incubating OFSP digesta with pure naringin. Again, no inhibitory effect on Bc uptake was observed (25.1 ± 2.1% as compared with 31.8 ± 2.7%). Reboul et al.[5] found that naringenin, the aglycone of naringin, impaired lutein uptake. The difference between naringin and naringenin is therefore probably due to the higher hydrophobicity of the latter.

Simulated digestion coupled with the Caco-2 cells appeared to be representative of the overall bioavailability. In these conditions, the cellular accumulation of *all-trans* Bc was proportional to the quantity present in micelles generated during simulated digestion (Table 4). Therefore, in decreasing order, the bioavailable amounts of Bc from mixed vegetables were boiled OFSP alone > boiled OFSP plus pGF > boiled OFSP + pGF/2 + wGF/2 > boiled OFSP + wGF. These observations were closer to those obtained in the previous study of Tyssansier et al.,[34] who found that Bc absorption from a single vegetable was greater when the food was administered alone than when it was coadministered with a second carotenoid-rich vegetable.

In conclusion, our results suggested that the presence of pGF or wGF had a negative effect on the "bioavailability" (bioacessibility + cellular uptake) of Bc from OFSP. Naringin present in grapefruit juices was largely responsible for the inhibitory effect of grapefruit juices on Bc bioaccessibility. The present findings also suggested that the interaction between naringin and OFSP Bc was mainly due to competition for micelle incorporation. In contrast, Bc uptake from dietary micelles was not impaired by grapefruit juice microconstituents. Further investigations are needed to verify the in vitro results of this work by in vivo experiments in animal or human intervention studies.

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ABBREVIATIONS USED

OFSP, orange-fleshed sweet potato; pGF, pink grapefruit; wGF, white grapefruit; Bc, β-carotene; TDC, taurodeoxycholate; CMC, critical micellar concentration.

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