

Phenol Antioxidant Quantity and Quality in Foods: Vegetables

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Fruits and vegetables in the diet have been found in epidemiology studies to be protective against several chronic diseases. Epidemiological evidence suggests that flavonoid consumption in the diet is protective against heart disease. Phenols in 23 vegetables have been measured by extraction with and without acid hydrolysis to determine the percent of conjugated and free phenols. Phenols were measured colorimetrically using the Folin–Ciocalteu reagent with catechin as the standard. The extracts' antioxidant quality was assayed by the inhibition of lower density lipoprotein oxidation mediated by cupric ions. Vegetables had antioxidant quality comparable to that of pure flavonols and were superior to vitamin antioxidants. The phenol antioxidant index, measuring both the quantity and the quality of antioxidants present, was used to evaluate 23 vegetables. Isolated lower density lipoproteins from plasma spiked with two vegetable extracts were enriched with phenol antioxidants and showed decreased oxidizability. The average per capita consumption of vegetable phenols in the United States was estimated to be 218 mg/day of catechin equivalents. This is 3 times higher than the recommended intake of vitamin antioxidants.

Keywords: Phenols; antioxidants; vegetables; lipoprotein oxidation

INTRODUCTION

Increased consumption of fruits and vegetables is associated with a lower risk of degenerative diseases that come with aging such as cancer, cardiovascular disease, cataracts, and brain and immune dysfunction (Ames et al., 1993). The risk of macular degeneration (Seddon et al., 1994) and stroke (Gillman et al., 1995) is diminished in people consuming large amounts of fruits and vegetables. Men who ate 10 or more servings of tomato products a week were found to develop prostate cancer less often than men whose intake was low (Giovanucci et al., 1995). Over 170 epidemiological cancer studies have been reviewed and consistently showed that there is a lower risk with increasing intake of fruit and vegetables (Block, 1992).

It is generally assumed that the vitamin and provitamin antioxidants in these foods (ascorbic acid, tocopherols, and carotenoids) account for the beneficial effects. However, the consequences of dietary intakes of these antioxidants are difficult to separate by epidemiological studies from other important constituents such as the flavonoids (Ames et al., 1995). Currently there are two published epidemiological studies inversely relating the consumption of five flavonoids to the risk of heart disease. The first investigated elderly Dutch men (Hertog et al., 1993a), and the second was a 7 country, 16 cohort study (Hertog et al., 1995). A more recent study in Wales showed that the intake of five flavonoids was not beneficial for heart disease risk (Hertog et al., 1997). These conflicting results show the problems and limitations associated with epidemiologi-

cal evidence and the difficulties of measuring only five flavonoid compounds in the diet.

Flavonoids are ubiquitous in vascular plants, and >4000 of these compounds have been identified (Harborne, 1988). In 1994 there were almost 1000 citations in *Chemical Abstracts* under the heading of flavonoids and polyphenols. Flavonoids include flavones, flavonols, flavanones, and derivatives and conjugates thereof. In addition, other phenolic and polyphenolic compounds are present in plants such as cinnamic acid derivatives, for example, chlorogenic acid, and isomers of flavones known as isoflavones. In this study the broader classification of phenols, which encompasses the flavonoids and other compounds, will be used. The mechanism for the protective effect of these phenol antioxidants has been recently reviewed (Kinsella et al., 1993). Many of these phenols have been found to be more powerful antioxidants than vitamins C, E, and β -carotene using an in vitro model for heart disease, namely the oxidation of lower density lipoproteins (Vinson et al., 1995a). Thus, vegetable consumption may provide protection against oxidative stress that is a pathogenic mechanism of both carcinogenesis and atherosclerosis (Ames et al., 1993).

The average daily intake of flavonoids in the United States in 1971 was estimated to be 1 g/day as quercitrin by adding up the contribution of individual compounds found in foods assayed by thin-layer chromatography and spectrophotometry (Kuhnau, 1976). Using HPLC analysis Dutch investigators found the average intake of five flavonoids (quercetin, kaempferol, myricetin, luteolin, and apigenin) in the Dutch diet to be 23 mg/day (Hertog et al., 1993b) and 12.9 mg/day in the United States (Hertog et al., 1995). We have thus decided to measure the quantity of total phenols and phenol antioxidants in vegetables and to determine the average daily intake in the United States using current USDA per capita consumption data. Epidemiological studies

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Table 1. Total Phenol Content of Vegetables Based on Dry and Wet (Fresh) Weight^a

vegetable	total phenols ^b ($\mu\text{mol/g}$)				free phenols, dry wt ($\mu\text{mol/g}$)	% conjugated ^c
	dry wt	rank	wet wt	rank		
asparagus	40.2 \pm 13.3	4	2.8 \pm 0.6	9	25.8 \pm 12.5	33.5 \pm 16.3
bean (snap)	17.8 \pm 0.9	16	1.5 \pm 0.1	16	8.01 \pm 1.9	55.4 \pm 8.4
bean (kidney)	35.9 \pm 8.2	5	31.6 \pm 5.2	1	31.9 \pm 5.6	43.7 \pm 1.3
bean (pinto)	31.9 \pm 5.7	8	28.6 \pm 5.6	2	21.5 \pm 3.5	41.5 \pm 4.5
beet	53.4 \pm 7.6	1	8.2 \pm 3.5	4	45.2 \pm 0.4	23.2 \pm 9.9
broccoli	40.6 \pm 22.3	3	3.6 \pm 0.8	7	17.5 \pm 3.8	41.5 \pm 4.5
cabbage	19.2 \pm 13.5	14	1.8 \pm 1.4	11	9.5 \pm 2.9	60.6 \pm 4.9
carrot	15.3 \pm 7.3	20	1.6 \pm 0.8	15	4.7 \pm 1.7	59.9 \pm 29.9
cauliflower	20.9 \pm 8.8	13	1.8 \pm 0.2	12	11.7 \pm 4.4	43.3 \pm 3.1
celery	13.6 \pm 7.7	22	1.2 \pm 0.9	19	7.0 \pm 5.5	53.5 \pm 13.4
corn	19.1 \pm 0.1	14	4.9 \pm 0.3	5	13.9 \pm 0.2	27.3 \pm 0.8
cucumber	15.5 \pm 9.9	19	0.4 \pm 0.8	23	4.4 \pm 4.3	74.8 \pm 12.4
garlic	34.3 \pm 11.0	7	12.9 \pm 2.2	3	5.2 \pm 3.1	85.6 \pm 4.5
lettuce	16.9 \pm 8.1	17	0.8 \pm 0.4	22	8.4 \pm 1.9	46.9 \pm 13.9
mushroom	22.5 \pm 3.7	12	1.5 \pm 0.1	17	21.9 \pm 5.3	27.6 \pm 6.8
onion (red)	41.0 \pm 9.9	2	4.0 \pm 1.2	6	11.4 \pm 7.6	79.6 \pm 15.2
onion (yellow)	22.9 \pm 1.4	11	2.4 \pm 0.5	10	4.7 \pm 3.8	79.4 \pm 16.5
pepper (bell)	26.1 \pm 6.0	10	1.6 \pm 0.4	14	16.4 \pm 1.2	35.2 \pm 13.1
potato	5.9 \pm 3.9	23	1.2 \pm 0.7	20	3.5 \pm 1.6	57.9 \pm 6.5
squash (green)	17.6 \pm 8.6	18	0.9 \pm 0.3	21	5.6 \pm 0.1	64.0 \pm 16.9
spinach	27.6 \pm 13.4	9	1.7 \pm 0.4	13	13.4 \pm 9.8	54.6 \pm 13.4
sweet potato	13.7 \pm 6.2	21	3.1 \pm 1.1	8	5.5 \pm 4.6	87.1 \pm 5.3
tomato	18.9 \pm 11.7	15	1.3 \pm 0.6	18	9.5 \pm 7.0	53.5 \pm 9.0

^a Free phenol content based on dry weight. Mole percent of phenols as conjugates. ^b Total phenols assayed in the hydrolyzed sample. ^c Percent conjugated value will be the same for the wet and dry samples.

suggest that flavonoids reduce heart disease risk and also breast cancer risk (Ingram et al., 1997). This study is aimed at the evaluation of the quantity and quality of the phenolic antioxidants in the vegetables, which may be important phytochemicals for the prevention of age-related degenerative diseases.

METHODS

Sample Preparation. Vegetables were obtained fresh from two or three local supermarkets, cleaned, and chopped into small pieces. Onions and potatoes were processed after removal of the dead and dry skins. The samples were blended under liquid nitrogen in a high-speed blender (Hamilton Beach Silex professional model) for 1 min. A weighed portion was then lyophilized overnight (Virtis 10-324), and the dry weight was determined. The lyophilisate was then ground to a fine powder in a mortar and pestle and kept at -20°C until used.

Extraction and Hydrolysis. A modification of a published method was used for extraction and hydrolysis (Hertog et al., 1992). A weighed portion (50–500 mg) of lyophilisate was mixed with 5 mL of 50% methanol/water and heated at 90°C in a plastic screw-capped tube with intermittent shaking for 2 h to determine the unconjugated (“free”) phenols present. Another weighed sample was heated with 5 mL of 1.2 M HCl in 50% aqueous methanol for 2 h at 90°C to measure the unconjugated plus conjugated (“total”) phenols. The extracts, each done in duplicate, were then filtered with a $0.45\ \mu\text{m}$ filter and stored at -20°C until assay.

Analysis. The phenol content in the extracts was measured by the Folin–Ciocalteu reagent (Sigma Chemical Co., St. Louis, MO) using catechin (Sigma) as a standard. A measure of the quality of antioxidants, the IC_{50} value, which is the concentration of phenols in the extract to inhibit 50% of the oxidation of lower density lipoproteins (LDL + VLDL), was determined as previously described (Vinson and Hontz, 1995). The phenol antioxidant index (PAOXI), a combined measure of quantity and quality of antioxidants, was used for comparison purposes. It is calculated by dividing the total phenol concentration of the vegetable ($\mu\text{mol/kg}$) by the IC_{50} (μM). The average daily per capita consumption of the total phenol antioxidants in vegetables (fresh weight) was then calculated as catechin equivalents using amolecular weight of 290 for the phenols present. The most recent vegetable consumption data

from 1994 was used in the calculations (U.S. Department of Agriculture, Economic Research Service, 1995).

Ex Vivo Spiking. Plasma was spiked with the hydrolyzed and neutralized extract of several vegetables (50 μM) and equilibrated for 1 h at 37°C . The LDL + VLDL was isolated and oxidized with cupric ion (Vinson et al., 1995b). The kinetics of conjugated diene formation was determined at 234 nm.

RESULTS AND DISCUSSION

Table 1 shows the results of phenol analysis of 23 commonly consumed vegetables. The average intra-assay precisions were 7.6% for the free phenol assay and 8.6% for the total phenols. Simple carbohydrates such as fructose and lactose showed no response as interferences in the Folin assay. Similarly, solid-phase separation of the phenols in several vegetable extracts showed $\leq 8\%$ Folin-active substances in the water wash of the solid phase after initial absorption of the phenols. Thus, phenols are the source of the antioxidants in the Folin assay.

Interestingly, beets had the highest dry weight concentration of total phenols followed by red onion, broccoli, and kidney beans. The potato had the lowest phenol content of the 23 vegetables. Assuming an average molecular weight of 290 for the phenols, this corresponds for yellow onion and celery to 6641 and 3944 mg/kg of dry weight, respectively. Hertog et al. (1992) found a total of 5076 and 2145 mg/kg, respectively, of the five flavonoids they assayed in these two vegetables. The lower values of Hertog et al. are to be expected as only five phenols were considered versus total phenols in our assay. Tomatoes were found to contain as much as 203 mg/kg quercetin wet weight in the acid-hydrolyzed extract (Crozier et al., 1997), and our method measured 368 mg/kg of total phenols. Potatoes contained up to 0.53 mg of chlorogenic acid/kg of wet weight (Friedman, 1997), and our method found 1.16 mm/kg of total phenols. Interestingly, the HPLC assay of potatoes found no flavonoids at all in potatoes (Justesen et al., 1997), which is not surprising

Table 2. Antioxidant Quality (IC₅₀) and Total Phenol Antioxidant Index (PAOXI) of Vegetables

vegetable	IC ₅₀ (μM)		total ^a PAOXI × 10 ⁻³			
	free	total ^a	dry wt	rank	wet wt	rank
asparagus	0.58	0.28	144	1	10.0	5
bean (snap)	0.33	0.27	65.9	4	5.7	6
bean (kidney)	1.10	0.58	61.8	5	54.4	1
bean (pinto)	0.85	0.65	49.4	6	44.3	2
beet	1.08	1.54	34.6	10	5.3	8
broccoli	0.87	0.88	46.1	8	4.1	10
cabbage	1.50	1.16	16.6	21	1.6	21
carrot	0.49	0.69	22.1	17	2.33	15
cauliflower	0.84	0.79	26.4	15	2.22	16
celery	0.57	0.73	18.6	18	1.67	19
corn	1.69	1.77	10.8	22	2.76	13
cucumber	0.55	0.92	16.8	20	1.08	23
garlic	0.80	0.41	83.7	3	31.5	3
lettuce (head)	0.67	0.60	28.2	14	1.27	22
mushroom	0.61	0.75	30.0	13	2.03	17
onion (red)	0.46	0.38	101	3	10.5	5
onion (yellow)	0.52	0.20	115	2	11.9	4
pepper (bell)	0.64	0.61	42.9	9	2.69	14
potato	0.53	0.25	23.6	16	4.64	9
squash (green)	1.05	0.55	32.2	11	1.62	20
spinach	0.78	0.86	32.1	12	1.98	18
sweet potato	0.53	0.75	18.3	19	4.09	11
tomato	1.05	0.39	48.5	7	3.25	12

^a Total phenols analyzed in the hydrolyzed extract.

because they did not measure chlorogenic acid, the major phenol present in potatoes.

We measured wet weight phenol content of the vegetables, which is the way foods are purchased and eaten. Any vegetable processing that adds water will of course decrease the wet weight phenol content. Kidney beans and pinto beans were by far the best source of phenol antioxidants, as measured in terms of fresh weight. This can be ascribed to the beans' high phenol concentration and because they contain <10% water by weight. Garlic was a distant third. Red onions contain more phenols than yellow onions because they contain additional phenolic anthocyanins, which are also responsible for the reddish color. This difference was also found by HPLC analysis of quercetin, the major flavonol in the onions (Crozier et al., 1997). A recent study measured the oxygen radical absorbance capacity (antioxidant quantity) of vegetables using a nonphysiological substrate (Cao et al., 1996). They found that garlic had the greatest antioxidant content and spinach was 3rd; we found garlic 3rd and spinach 13th.

There was no correlation with the mole percent of conjugated phenols and total phenol concentration in the vegetables analyzed in Table 1. For instance, beets that had the highest dry weight phenol concentrations had the lowest percent conjugated, only 23.2%. Absorption of flavonoids from the human diet was long considered to be negligible since only free aglycons were thought to be absorbed from the gut wall, and no enzymes are present to split the β-glycosidic bonds (Kuhnau, 1976). However, they can be hydrolyzed in the large intestine by the bacterial flora enzymes and be absorbed (Bokkenhueser et al., 1987). Quercetin from onions, which is mostly present in the conjugated form, is well absorbed in humans (Hollmann et al., 1996).

The quality of the phenol antioxidants was determined by the IC₅₀ values shown in Table 2. The IC₅₀ values are the same for both wet or dry weight phenol of a vegetable. A low IC₅₀ indicates strong antioxidants in a vegetable. Yellow onions had the best antioxidants

based on hydrolyzed (total) polyphenols, and snap beans had the strongest antioxidants when free phenols were considered. Potato contained the lowest quantity of total and free dry weight phenols, yet had the second best antioxidant quality based on total phenols.

There was no trend or significant difference in IC₅₀ when comparing the hydrolyzed or unhydrolyzed fractions. Normally the hydrolyzed extract would be expected to contain the better quality antioxidants because it is generally found that glycosides of phenols are poorer antioxidants than the aglycons using two *in vitro* models (Vinson et al., 1995a; Afanasev et al., 1990). However, recently Wang et al. (1997) have found that glycosylation raised, lowered, or did not affect the oxygen radical absorbing capacity of individual anthocyanins. In addition, there may be unanticipated synergism or antagonism when complex mixtures such as the vegetable extracts are used. Comparing the IC₅₀ of vegetables extracts with beverages, we have found that they are similar. In fact, the IC₅₀ for the best vegetable antioxidant, yellow onion, was slightly better than that of brewed green tea, the best beverage antioxidant (0.20 versus 0.23 μM, respectively, unpublished results). Vegetable extracts had IC₅₀ values comparable to those of pure flavonols such as quercetin (0.224 μM) and rutin (0.512 μM) (Vinson et al., 1995b). Most of the vegetables had much lower IC₅₀ values (range = 0.20–1.77 μM) than the vitamins C, E, and β-carotene: 1.45, 2.40, and 4.30 μM, respectively (Vinson and Hontz, 1995).

The phenol antioxidant index (PAOXI) is a combined measure of the quality and quantity of antioxidants present in the vegetables. The vegetable with the largest fresh weight PAOXI as seen in Table 2 was kidney bean, at 54.4 × 10³, followed by pinto bean at 44.3 × 10³ and garlic at 31.5 × 10³. On a dry weight basis asparagus has the highest PAOXI at 144 × 10³, followed by yellow onion at 115 × 10³, red onion at 101 × 10³, and garlic at 83.7 × 10³.

There have been only a few studies that evaluated the antioxidant content in vegetables. A very recent study used the autoxidation of linoleic acid and added vegetable extracts but measured only three vegetables (Furuta et al., 1997). Another Japanese study used the Folin assay for fresh vegetable extracts and measured their activity using β-carotene bleaching coupled with the oxidation of linoleic acid (Tsushida et al., 1994). They found a correlation of antioxidant activity with Folin phenol content with a correlation coefficient of 0.77. This correlation suggests that, although the vegetables may contain other antioxidants such as proteins, ascorbate, and the carotenoids, these do not contribute significantly to the antioxidant activity in their model. In our model, this assumption is corroborated by the much higher IC₅₀ values of these vitamin and provitamin antioxidants compared with the individual phenols present in the vegetables as well as the vegetable extracts (Vinson et al., 1995a).

The results of the spiking study with two vegetable extracts are shown in Figure 1. As can be seen, the spinach and sweet potato extracts increased the lag time (time point where the rate of oxidation increases sharply) to 105 (17% increase) and 128 min (42% increase), respectively, compared with the control of 90 min. This indicates that the phenols in the vegetables are able to enrich the lipoproteins by binding with them and subsequently protect them from oxidation. We have previously observed an increased LDL + VLDL lag time

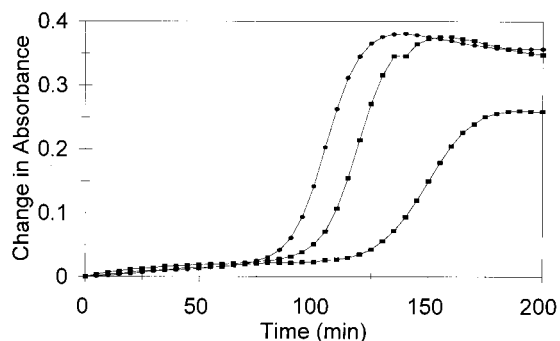


Figure 1. Ex vivo spiking of hydrolyzed vegetable extracts in plasma and the effect on the cupric ion oxidation of isolated LDL + VLDL as measured by conjugated diene formation at 234 nm: (●) control; (■) spinach; (▼) sweet potato.

Table 3. Per Capita Consumption of Vegetable Phenol Antioxidants in the United States

vegetable	total phenols, ^a fresh wt (mg/kg)	per capita consumption ^b		phenol consumption (mg/day)
		fresh wt (g/day)	rank	
tomato	368	113	2	41.7
corn	1418	26.1	4	37.0
bean (pinto)	8294	3.3	15	30.2
potato	283	175	1	27.6
onion ^c	920	21.5	5	19.8
garlic	3738	2.5	17	9.4
carrot	467	14.5	6	6.8
broccoli	1053	6.3	12	6.7
lettuce (head)	220	28.0	3	6.2
bean (kidney)	9164	0.6	22	5.5
sweet potato	890	5.8	13	5.1
cabbage	528	9.4	8	4.9
bean (snap)	447	9.2	9	4.2
pepper (bell)	476	7.7	11	3.7
cucumber	287	12.4	7	3.6
celery	354	8.1	10	2.9
beet	2365	1.1	20	2.6
mushroom	440	4.9	14	2.1
cauliflower	507	2.2	18	1.2
asparagus	809	1.2	19	1.0
squash (green)	258	3.0 ^d	16	0.8
spinach	493	1.0	21	0.5

^a Total phenols in the hydrolyzed extract as catechin equivalents. ^b Based on total of fresh and frozen vegetable consumption. ^c Average of red and yellow varieties. ^d Estimated (Gary Lucier, USDA, personal communication).

after spiking plasma with pure polyphenols (Vinson et al., 1995b). Others have demonstrated similar results with tocopherol both by ex vivo spiking in plasma and following tocopherol consumption (Jialal et al., 1995).

In Table 3 are listed the total phenols by fresh weight and the per capita consumption of vegetables. The average milligrams per day of phenols from vegetables was calculated as catechin equivalents with a molecular weight of 290, which is similar to that of quercetin (302), the most prevalent flavonol in vegetables (Hertog et al., 1993b). This is only an estimate because the molecular weights of pure polyphenols vary from gallic acid at 170 to tannins at >500. The vegetables are listed in decreasing order of phenols consumed per day. The top five vegetables, tomato, corn, pinto bean, potato, and onion, account for 70% of the total daily phenol consumption.

Dry beans are greatly underutilized vegetables in that they have a high content of phenol antioxidants (1st and 2nd for kidney beans and pinto beans) but are very low in the ranking of per capita consumption, 22nd and

15th, respectively. Beans provide fiber and phytoestrogens such as isoflavones that have been shown to be beneficial for both heart disease and cancer (Adlercreutz, 1996). Garlic was also underutilized; it was 2nd in total phenol content but only 17th in per capita consumption. Garlic has been found to have hypolipidemic and antithrombotic properties (Agarwal, 1997) and thus would be an excellent food to consume for health benefits. Using data from USDA Handbook 8-11, one serving of pinto beans would provide 806 mg of phenols, beets 166 mg, sweet potato 120 mg, corn 113 mg, tomato 47 mg, broccoli 44 mg, and potato 39 mg. This is calculated for fresh vegetables and will probably be lower following cooking because microwaving and boiling decreased the phenol content of onions and tomatoes (Crozier et al., 1997).

Previous investigation showed that onion was the leading vegetable source of the five flavonoids measured in the U.S. diet (Hertog et al., 1995), whereas in our survey it was fifth. Tomato was the leading source of phenols in the diet in our survey. The Dutch group found that the total dietary consumption of the five flavonoids was 12.9 mg for U.S. railroad workers (Hertog et al., 1995). Our total from fresh vegetables alone is 218 mg and is similar to the value of 241 mg found by Kühnau (1976).

Evidence is slowly mounting that the phenols in foods are appreciably absorbed by humans. Consumption of isoflavonoids in beans has been shown to significantly increase isoflavonoid excretion in humans (Hutchins et al., 1995). Two studies have shown absorption of phenols from vegetables. A Dutch group found that the quercetin in onions was still detectable in the plasma 48 h after ingestion of 215 g of fried onions that provided 64.2 mg of quercetin equivalents (Hollman et al., 1996). Most recently, a Danish group found that 500 g of broccoli/day ingested for 12 days produced detectable kaempferol in the urine, 52–57 ng/mL. The daily dose of kaempferol was 12.5 mg (Nielsen et al., 1997). Our spiking studies indicate that phenols in vegetables, once absorbed, can enrich the LDL and prevent them from oxidizing. This effect provides one mechanism for the beneficial effect of vegetables for heart disease.

Our measurements suggest that even average consumption of vegetables contributes a greater amount of antioxidants (218 mg of phenols) in the diet than the sum of the recommended daily value of vitamins C, E, and β -carotene—72 mg/day (National Research Council, 1989). Unfortunately, only 9% of Americans eat five servings of fruits and vegetables per day as recommended by the National Cancer Institute and the National Research Council (Block et al., 1992; Block, 1992). A recent report found that fewer than 50% of children and adolescents eat one serving of fruit/day and fewer than 30% eat one serving of vegetables (Krebs-Smith et al., 1996). We believe that a greater consumption of vegetables and fruits will provide health benefits due to the nutrient content but also because of the phenol antioxidants present. Currently we are measuring these substances in fruits, beverages, and other foodstuffs.

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