

## Dietary Intake of Total and Citrus Flavonoid of Individuals with Self-Reported Diseases

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### Abstract

**Title:** Dietary intake of total and citrus flavonoid of individuals with self-reported diseases.

**Background:** This work estimated the intake of total and citrus flavonoids by comparing groups of individuals with and without self-reported diseases, and identified the main dietary sources of flavonoids.

**Methods and Findings:** A cross-sectional observational study was conducted with 122 individuals of both genders, which answered questions about social-demographic data, health status, lifestyle, diet, disease, etc., and were divided in a self-reported diseases group and a without self-reported diseases group. The intake of total and citrus flavonoids was evaluated using the 24-hour recalls and the main dietary sources of flavonoids have been identified. Citrus, onion, beans, lettuce, banana, apple, arugula and chocolate were the most important dietary sources of flavonoids. The total flavonoids average intake was  $37.67 \pm 40.05$  mg/day, citrus flavonoid average intake was  $37.35 \pm 36.78$  mg/day and hesperidin, narigenin and eriodictyol intake were  $28.11 \pm 23.00$  mg/day,  $4.54 \pm 4.22$  mg/day and  $1.30 \pm 1.29$  mg/day, respectively. The intake of flavonoids of the self-reported diseases group was significantly higher ( $p \leq 0.05$ ) than the without self-reported diseases group.

**Conclusions:** Orange, orange juice and lemon were the main flavonoids sources, responsible for the total dietary flavonoid intake. The individuals with self-reported diseases showed to be more concerned about health since they diet, consume more dietary supplements, drink less alcoholic beverages and consume more citrus and total flavonoids than the individuals without self-reported diseases ( $p \leq 0.05$ ).

**Keywords:** Flavonoid intake; Citrus flavonoids; Flavonoid sources; Self-report disease; Orange juice

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### Introduction

The health status of a population is influenced by many factors, such as age, genetic, environmental, social conditions, and especially eating habits [1]. Some habits can negatively affect health, such as smoking, alcohol, drugs, stress and sedentary lifestyle. Among the positive habits, balanced diets, physical activity, leisure and restful sleep (6-8 hours/day) can be found [1]. Positive habits contribute to a healthier life as they improve quality and life expectation of individuals, being able to prevent the emergence of diseases as the non-transmissible chronic diseases.

Fruits and vegetables are important components of a healthy diet, together with low levels of fat, sugar and sodium. They are rich sources of vitamins and minerals, dietary fiber and many beneficial non-nutrient substances including mainly flavonoids and other antioxidants. The suitable intake of fruits and vegetables contributes to guarantee most of these essential nutrients. At least 400 g should be consumed a day for an overall health [2]. The increase of fruit and vegetable consumption has been associated to the reduction of risk of non-communicable diseases and others [2-4].

Flavonoids are secondary metabolites produced by plants. All of them are phenolic compounds with antioxidant activity, able to stabilize and/or prevent oxidative stress of cellular membranes. Flavonoids are mainly found in fruits, vegetables and some grains [5]. Citrus flavonoids, also called flavanones, are characteristics of Citrus. They are found in different parts of citrus fruits at different concentrations [6].

The most consumed fruit juice in the world is orange juice, representing 40% worldwide juice consumption [7]. Brazil is the main orange juice producer and exporter in the world, accounting for 61% of the global orange juice production in 2016 [8,9]. The state of São Paulo is responsible for 50% of the Brazilian orange juice [9]. Orange juice is an important source of antioxidant compounds in diet, like flavonoids, carotenoids and ascorbic acid. Hesperidin, hesperitin, naringin, naringenin, narirutin and eriodictyol are orange juice flavanones, also presenting antioxidant activity [6,10-13].

The consumption of flavonoid has been associated with the prevention of some diseases in addition to different beneficial effects on human health. Studies have shown that flavonoids exert anti-inflammatory action, and prevent cancer and obesity, among others diseases. Moreover, orange juice flavonoids reduce total cholesterol and blood pressure, reducing risk of coronary heart disease [6,14-17].

There are few studies on flavonoid consumption. For some decades, studies have been conducted to estimate the consumption of flavonoids in diet of specific populations, but data are still scarce and inconsistent. In the United States flavonoid consumption was estimated in 268 mg/day [18], while in Spain it was 313.26 mg/day [19], in China 165.6 mg/day [20] and in Brazil 60 to 106 mg/day [21]. As well as orange, one of the major dietary sources of flavonoid in the world, also apple, tea and onion have been reported as important dietary sources of flavonoids [19-25].

The content of total and individual flavonoids in food is influenced by many factors as cultivation system, agricultural practices, and soil and climate conditions, among other factors, which can interfere in the flavonoid amount and thus in the estimation of the consumption. The Brazilian diet is diverse, consisting of different foods from different regions and habits related to the large extension of the country. In this work diet consumed by adults from São Carlos, a city of the southeast region of the state of Sao Paulo, the most populated of the country, was chosen. It is important to consider that there are few studies assessing the intake of total flavonoid in Brazilian diet and none about citrus. Furthermore there is no Brazilian database of flavonoid food content. So, the calculation to estimate the intake of flavonoids had to be based on international database.

This work aimed to estimate the intake of total and citrus flavonoids of adults by comparing a group of individuals with and without self-reported diseases, and to identify the main dietary sources of flavonoids.

## Methods

### Sampling and food intake

A cross-sectional observational study was conducted with adults of both gender attended in a clinical laboratory of São Carlos, a medium size city of the state of São Paulo, in the southeastern region of Brazil. São Carlos city is an important region concerning orange cultivation. The clinical laboratory analysis was chosen for data collection, in order to interview adults concerned about health, which seek to prevent diseases or who are sick.

The sample was calculated based on the prevalence of chronic diseases of the Brazilian population, according to the Brazilian Ministry of Health survey [26], which estimated that about 40% of the total adult population has at least one chronic non-communicable disease. Considering a margin of error of 10% and a 95% confidence, 122 individuals were interviewed.

The participants were randomly recruited at the entrance and waiting area, answered questions of the identification protocol about social-demographic data, health status, lifestyle and weight and height during the interview. Volunteers also answered questions about diet, disease, smoking, drinking alcohol and physical activity. In order to compare flavonoid intake, participants were divided in a self-reported diseases group and a without self-reported diseases group. The group of individuals with self-reported diseases included participants who declared any kind of non-transmissible chronic diseases, respiratory, renal, hepatic, intestinal, and autoimmune disease, among others. The group of individuals without self-reported diseases did not declare any disease. The intake of total and citrus flavonoids (hesperidin, naringenin, eriodictyol) of the respondents was evaluated using the 24-hour recalls, in three different and non-consecutive days including one day of the weekend. Questions about the meals during the day and the amount of food intake (household measures) were done considering the food intake of the day before. Also participants answered questions about the use of dietary supplements and medicine. The first 24-hour recall was applied at the clinical laboratory, by a trained researcher, and the two other 24-hour recalls were conducted by phone in unannounced different days. Data were collected from May to July 2014. The study was approved by the Ethics Committee on Human Research (protocol no. 661 489) of the School of Pharmaceutical Science, São Paulo State University-UNESP, Araraquara, SP. All the participants signed an informed consent before answer the identification protocol.

### Food Sources and Dietary Flavonoid Intake

The kinds of food consumed according to the three 24-hour recalls data and present in the Database of the Flavonoid Content of Selected Foods [27], were used to establish the flavonoid food sources and then estimate the total and citrus flavonoid intake. This Database [27] is considered the most used and complete database in estimating flavonoid intake. The Database [27] contains 506 food items, providing contents of individual flavonoids from five classes (flavonols, flavones, citrus flavonoids (flavanones), flavan-3-ols and anthocyanidins). Data of food

consumption from all the three 24-hour recalls were converted into intake of total and citrus flavonoids using the Nutwin® nutrition software, which was supplied with the flavonoid food content provided by the Database [27].

Individual flavonoid intake was determined multiplying total weight of food intake (g) by the flavonoid content (mg/g) of each kind of food. Total flavonoid intake was calculated by the summation of the individual flavonoids intake of all kinds of food reported in the three the 24-hour recalls, and citrus flavonoid intake the sum of hesperetin, naringenin and eriodictyol.

## Statistics

Frequency and percentage were obtained for social-demographic data. For total and citrus flavonoid intake, calculated from the three 24-hour recalls, mean and standard deviation of the total of participants and of the individuals with and without self-reported diseases were estimated. Social-demographic data were associated with the diseases using Chi-square or Fisher exact test. A repeated measure design was used to compare consumption of the groups, considering a generalized linear model with gamma distribution, due to the asymmetry of the data distribution adjusted for possible confounding variables. A 5% significance level was adopted. All statistical analyses used SAS for windows, v.9.3 (SAS Institute Inc, 2012).

## Results

### Characterization of the population

From the total of adults, 105 (86.1%) were female and 17 (13.9%) male (mean age of  $43.03 \pm 9.95$ ). Among the respondents, most were married (78.7%), earn five or fewer minimum salaries a month (65.6%) and had completed high school (80.3%) (**Table 1**). In order to attain the nutritional status of the respondents, BMI (weight/height<sup>2</sup>) was calculated based on the reported weight and height. In this study 42.6% of the population was eutrophic (BMI 18.5 to 24.9 kg/m<sup>2</sup>), 34.4% were overweight (BMI 25.0 to 29.9 kg/m<sup>2</sup>) and 23.0% were obese (BMI >30.0 kg/m<sup>2</sup>) (28).

The prevalence of individuals with some disease was estimated in 57%. The self-reported diseases group was constituted by seventy respondents and fifty two respondents did not declare any disease. Individuals who have not agreed to answer the questions of the identification protocol were excluded from the research. The self-reported diseases group had lower educational level ( $p \leq 0.009$ ) than that without self-reported diseases group (**Table 1**).

**Table 2** shows lifestyle data including diet, use of dietary supplements, drinking alcohol, smoking habits, and practice of physical activity. Among the interviewers, 25.4% reported to follow a diet and 22.1% reported consuming some type of dietary supplement. The group with self-reported diseases had a higher number of individuals following a specific diet ( $n=25$ ) and who consumed dietary supplements ( $n=23$ ) than the individuals without self-reported diseases. The drinking alcohol was reported by 33.6% of the respondents, with lower numbers of individuals ( $n=14$ ) consuming alcoholic beverage in the self-reported disease

group than the group without self-reported diseases. 5.7% of the respondents reported smoking, and in both groups a low number of smokers were observed. Regarding physical activity, 52.4% of the individuals were active, and 75% of them practiced physical activity at least three times a week or more.

### Flavonoid dietary sources

According to the three 24-hour recalls 206 foods were consumed, 66 of which were in the Database [27]. Eight foods were considered flavonoid food sources, based on the frequency of consumption equal or higher than 1%, amount of consumption equal or higher than 50 g/day and level of flavonoid equal or higher than 4 mg/100 g. Frequency of consumption was calculated according how many times the consumption of each food was mentioned in the 24-hour recalls.

The major dietary sources of flavonoids of the individuals and their flavonoid levels (mg/100g) were, respectively, citrus (79.2%), arugula (47.1%), onion (25.5%), apple (15.8%), chocolate (15%), banana (13.7%), beans (7.3%) and lettuce (4.6%). Orange, orange juice and lemon were identified as the main source of citrus flavonoids of the respondents' diet.

### Flavonoids intake

**Table 3** shows the dietary intake of total individuals and those with and without self-reported disease according to the 24-hours recalls. The dietary intake of total flavonoids was estimated in  $37.67 \pm 40.05$  mg/day. The average intake of citrus flavonoids of the 122 participants was  $37.35 \pm 36.78$  mg/day, and hesperetin, naringenin and eriodictyol intake were  $28.11 \pm 23.00$  mg/day,  $4.54 \pm 4.22$  mg/day and  $1.30 \pm 1.29$  mg/day, respectively. Our results showed that citrus flavonoids are the main source of total flavonoids in the participants' diet.

The comparison of the flavonoids intake between groups was estimated (**Table 3**). Total flavonoids intake of individuals from the group with self-reported diseases was 39.31 mg/day, higher than that of the group without self-reported diseases, 31.65 mg/day. There was statistical difference in total flavonoid intake among groups ( $p < 0.0001$ ). Individuals of the group with self-reported diseases also had higher consumption of citrus flavonoids (flavanones) than those from the group without self-reported diseases, 38.51 mg/day and 33.50 mg/day, respectively ( $p < 0.0003$ ). Hesperetin was the most important citrus flavonoids for both groups, representing 68.9% and 86.5% of the flavonoid intake for individuals with and without self-reported diseases, respectively, while naringenin and eriodictyol intake accounted together for 15.2% and 16%, respectively. The intake of hesperetin ( $p < 0.5227$ ), naringenin ( $p < 0.2268$ ) and eriodictyol ( $p < 0.4875$ ) did not statistically differ between groups.

## Discussion

The prevalence of individuals with some disease (57%) was higher than that reported in the Brazilian Ministry of Health survey (40%) [26], suggesting that it is possible to consider the studied sample as representative of the population of the city of São Carlos, since a larger margin of error was established.

**Table 1:** Social-demographic data of total individuals, and those with and without self-reported diseases.

Variables	Categories	Without SRD <sup>a</sup> (n) <sup>b</sup>	With SRD <sup>a</sup> (n) <sup>b</sup>	Total (%)	p
Gender	Female	42	63	105 (86.1)	0.146
	Male	10	7	17 (13.9)	
Civil status	Married	39	57	96 (78.7)	0.391
	Single	13	13	26 (21.3)	
Education	< high school	3	21	24 (19.7)	0.001
	high school	49	49	98 (80.3)	
Income	≤ 5 MS <sup>c</sup>	31	49	80 (65.6)	0.233
	> 5 MS <sup>c</sup>	21	21	42 (34.4)	

SRD<sup>a</sup>: Self-reported diseasesn<sup>b</sup>=Individuals numberMS<sup>c</sup>: Minimum salaries**Table 2:** Lifestyle data of total individuals, and those with and without self-reported diseases.

Variables	Categories	Without SRD <sup>a</sup> (n) <sup>b</sup>	With SRD <sup>a</sup> (n) <sup>b</sup>	Total (%)	p
Diet	Yes	6	25	31 (25.4)	0.0044*
	No	46	45	91 (74.5)	
Dietary supplements	Yes	4	23	27 (22.1)	0.0009*
	No	48	47	95 (77.9)	
Alcohol drinking	Yes	27	14	41 (33.6)	0.0002*
	No	25	56	81 (66.4)	
Smoking	Yes	3	4	7 (5.7)	0.9897
	No	49	66	115 (94.3)	
Physical activity	Yes	33	31	64 (52.4)	0.0621
	No	19	39	58 (47.5)	
Frequency (days/week)	≥ 3	13	25	48 (75.0)	0.475
	≤ 3	6	10	16 (25.0)	

SRD<sup>a</sup>: Self-reported diseasesn<sup>b</sup>=Individuals number

\*p ≤ 0.05

**Table 3:** Total and citrus flavonoid intake (mg/day) of total individuals and individuals with and without self-reported diseases.

Flavonoid intake <sup>a</sup> (mg/day)	Total individuals <sup>b</sup> (mean ± SD)	With SRD <sup>b</sup> (mean ± SD)	Without SRD <sup>b</sup> (mean ± SD)	p-value
Total flavonoid	37.67 ± 40.05	39.31 ± 8.75	33.50 ± 10.88	<0.0001*
Citrus flavanoids	37.35 ± 36.78	38.51 ± 21.74	31.65 ± 14.53	0.0003*
Hesperitin	28.11 ± 23.00	28.98 ± 12.66	26.53 ± 9.45	0.5227
Naringenin	4.54 ± 4.22	4.54 ± 1.93	4.18 ± 0.95	0.2268
Eriodictyol	1.30 ± 1.29	1.30 ± 1.05	1.17 ± 0.92	0.4875

<sup>a</sup>: Values adjusted for energy and age<sup>b</sup>: SRD: self-reported diseases

\*p ≤ 0.05

The results showed that the self-reported diseases group had lower educational level than the without self-reported diseases group. Several studies have been shown that there is an association between prevalence of diseases in adults and educational level [28-32]. Gronner *et al.* [33] estimated that metabolic syndrome prevalence in adults, characterized by risk factors for cardiovascular disease and diabetes such as hypertension, dyslipidemia, among others, were significantly associated with the low educational level in women (p ≤ 0.05). It has also been reported that the higher the prevalence of obesity and diabetes among US adults the lower the educational level [34].

The higher number of individuals following a diet in the group with self-reported diseases suggests that those who have some

disease had been probably instructed by doctors or nutritionists to consume a healthy diet in order to improve health status and prevent the development of new diseases. Regarding the consumption of dietary supplements, multivitamin supplements, vitamin D, calcium, omega 3 and amino acids were the most cited among the volunteers. Among volunteers that reported drinking alcohol, beer was the most consumed beverage, with higher frequency of consumption at weekends. Diet, dietary supplements and alcohol consumption were the variables that showed statistically significant differences (p ≤ 0.05) between the groups with and without self-reported diseases. These results suggest that individuals from the self-reported diseases group are more concerned about health once they follow a diet, consume dietary supplements and drink less alcohol. The



smoking habit follows the national trend of decreasing number of Brazilian smokers [35]. Regarding physical activity, most of the active individuals follow the WHO recommendation on the practice of physical activity at least three times a week. This result is considered positive for the health of individuals once to obtain benefits from the physical activity the weekly frequency of practice is a determining factor [36].

Regarding flavonoid intake, citrus flavonoids, mainly from orange and orange juice, were primarily responsible for the total flavonoid intake of the diet showing that the population who lives in an orange producer region consumes the fruit and juice frequently. Some studies reported that orange is the main dietary source of citrus flavonoids, mainly those performed in citrus producer countries, where there is higher availability of orange. Fruits and fruit beverages are considered important dietary sources of citrus flavonoids. Also apple, tea and onion have been mentioned as important dietary sources of flavonoids [20-25]. The main dietary sources of flavonoids reported in an US survey of adult's flavonoid consumption were tea, wine, beer, citrus and apple [24]. Another survey to estimate phytonutrients intake identified persimmon, soy, onion, mandarin, grapefruit and nuts as the main flavonoid food source of the Korean's diet [25].

Food frequency questionnaires and a flavonoid database of Dutch foods were used by Sampson *et al.* [22] to estimate the USA flavonoid intake of adults, aged between 40 and 75 years, in 20 mg/day, similarly to our results, which dietary intake of total flavonoids was  $37.67 \pm 40.05$  mg/day. Arabbi [21] estimated from 60 to 106 mg/day the flavonoid intake of Brazilians using food frequency recalls and dietary surveys of men and women aged 17 to 88 years. Chun *et al.* [37], Zamora *et al.* [19] Li *et al.* [20] and Bai *et al.* [24] reported total flavonoid intake higher than ours, ranging from 165.6 to 344.83 mg/day. In Spain, flavonoid intake of adults participants in a cohort study related to nutrition and cancer, aged 35 and 64, was 313.26 mg/day of total flavonoids, with the dietary intake being assessed through personal interviews about the dietary history of individuals [19]. A sectional study on cancer prevention conducted in the United States with 70 year-old adults using semi quantitative food frequency questionnaires estimated flavonoid intake in 268 mg/day [18]. Bai *et al.* [24] used the USDA flavonoids database and three 24-hours recalls to estimate the American flavonoid intake in 344 mg/day. Different habits of consumption including tea, red wine, berries and soy could explain the higher intake of total flavonoids as observed in Spain, USA, Australia and China, when compared to our results, whose population does not frequently consume such kinds of food.

When compared to other studies our results of citrus flavonoids showed some differences. The USA intake of citrus flavonoids was estimated in  $14.4 \pm 0.6$  mg/day according to Chun *et al.* [37] and in  $22.38 \pm 1.79$  mg/day regarding to Bai *et al.* [24], lower than our values. Citrus flavonoids were the second highest contributors to total dietary flavonoids in the USA and the Mediterranean countries, where citrus flavonoid intake was estimated in  $50.54 \pm 47.15$  mg/day [19,24-38], higher than ours. Our results showed

that citrus flavonoids are the main source of total flavonoids in the participants' diet. Lee *et al.* [25] estimated dietary intake of hesperitin of the Korean population in  $25.4 \pm 3.2$  mg/day, mainly from orange and lemon. Australian children and adults consumed hesperitin ranging from 2.97 mg/day to 5.89 mg/day, naringenin from 0.92 mg/day to 3.48 mg/day and eriodictyol from 0.02 to 0.04 mg/day [39], similarly to our results.

Differences in the intake of flavonoids among studies can be due to differences in eating habits, dietary flavonoid sources and databases, and calculation procedures [24-35]. Despite the importance of flavonoids for health, recommended daily intake of flavonoids has not been established yet [40].

The intake of citrus and total flavonoid from individuals of different groups also suggests that those who already present some disease are more aware of the importance of a healthy diet, rich in fruits and vegetables, to prevent or treat diseases such as the chronic diseases. The practice of healthy diets has been increasingly recommended by health professionals, since their importance to the maintenance of health of individuals and disease prevention. Awareness campaigns of healthy diet, nutrition education programs, among other actions, have been developed by Brazilian public agencies aiming to encourage individuals to eat better and to be more concerned about the quality of food. These kinds of action generally take place in public places such as health centers, hospitals and clinics. In our case the individuals with self-reported diseases, which attend these places, should have been influenced by public actions, because of the increase of consumption of fruits and vegetables. The beneficial health effects obtained by the consumption of citrus and total flavonoids have been shown in many studies [14-17]. The increase in the consumption of dietary sources of flavonoids can help to reduce the high prevalence of sick adults in the Brazilian population.

It should be mentioned that there are several limitations in the present study. It is the first study, a cross-sectional one, to estimate citrus flavonoids intake in Brazil, which will be deepened in the near future. The studied sample was representative of a developed middle city (240 thousand habitants) from the state of São Paulo. The study of a more representative sample of the population would be quite appropriate, as well as the use of biochemical tests to identify individuals with and without diseases instead of the self-reported diseases. Also, the results may have been influenced by previous advices from health professionals about healthy habits, including flavonoid intake, and even because the interviews were carried out in a clinical lab where people could already have a diagnostic. Another limitation was the use of an American database to estimate flavonoid intake, as it has been said previously. It would be more suitable to use the flavonoid contents of Brazilian foods to estimate the total and citrus flavonoid intake of the studied population. Moreover the American database contains a rather limited number of foods currently consumed in Brazil. It should also be mentioned that, as with all dietary surveys, the use of the 24-hour recall to estimate the dietary intake could cause inaccuracy in the flavonoid and food intake assessed. It is commonly observed in studies which

used a self-reported dietary assessment, the misreporting, like underreporting or over-reporting of food intake. The 24-hour urine collection was established like the gold standard to estimate nutrient intake, although, this is unworkable in most of the studies. Finally, in order to minimize these issues about accuracy of 24-hour recalls and have a good representativeness of individual's diet; three 24-hour recalls were applied, as recommended.

## Conclusion

The intake was characterized by a diversified source of flavonoids. Citrus, onion, beans, lettuce, banana, apple, arugula and chocolate were the major dietary sources of flavonoids of

the individuals. Orange and orange juice and lemon were the main flavonoids sources. The dietary intake of total and citrus flavonoids was estimated in 37.67mg/day and 37.35mg/day, respectively. The individuals with self-reported diseases showed to be more concerned about health since they diet, consume more dietary supplements, drink less alcoholic beverages and consume more citrus and total flavonoids than the individuals without self-reported diseases ( $p \leq 0.05$ ).

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