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Analysis of total flavonoids present in some of the most consumed conventional and organic fruits and vegetables in southern Brazil

Análise de flavonoides totais presentes em algumas frutas e hortaliças convencionais e orgânicas mais consumidas na região Sul do Brasil

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Abstract

The flavonoids present in nature are related to a large variety of biological activities, such as anti-inflammatory and antioxidant action, and they have beneficial effects on health. The objective of this study was to analyze and compare the amount of flavonoids presented in the most consumed fruits and vegetables in southern Brazil, according to data of 2008/2009 Household Budget Survey (HBS). Fresh onions, tomatoes, bananas and apples, both from conventional and organic cultivations, were analyzed and submitted to dry heat cooking methods. Total flavonoid content was determined by the spectrophotometric method, using aluminum chloride. The results of the analysis for determination of total flavonoids correspond to the means ± standard deviation of three repetitions. The samples of apples and onions presented higher content of flavonoid. The cooking process affected the extraction of flavonoids in fruit by decreasing their levels, a fact explained by the presence of fibers, while it facilitated flavonoid extraction in the vegetables. Daily recommendation of flavonoids is not defined. However, as nutritionists know how food should be eaten and what their flavonoid levels are, they can prescribe and devise strategies and secure goals of intake, in order to promote the patient's health and hence also improve their social and economic condition.

Keywords: Flavonoids. Fruits. Vegetables. Organic Food. Crop Production. Food Handling.

Resumo

Os flavonoides existentes na natureza estão relacionados com grande variedade de atividades biológicas, como ação anti-inflamatória e antioxidante, apresentando efeitos benéficos à saúde. O objetivo deste estudo foi analisar e comparar o teor de flavonoides totais presentes em frutas e hortaliças mais consumidas na região Sul do Brasil, segundo dados da Pesquisa de Orçamento Familiar POF – 2008/2009. Foram analisados cebola, tomate, banana e maçã, de cultivo orgânico e convencional, *in natura,* e submetidos ao processo de cocção em calor seco na panela e micro-ondas. A determinação de flavonoides totais foi realizada por método espectrofotométrico, utilizando cloreto de alumínio. Os resultados das análises de determinação de flavonoides totais correspondem à média ± desvio padrão de três repetições. A maçã e a cebola apresentaram maior teor de flavonoides. O processo de cocção afetou a extração dos flavonoides nas frutas, diminuindo os teores, fato explicado pela presença das fibras. Já para as hortaliças, o processo facilitou a extração dos flavonoides. A recomendação diária de flavonoides não é definida, porém o profissional nutricionista, conhecendo como os alimentos devem ser consumidos e seus teores de flavonoides, pode prescrever e traçar estratégias e metas seguras de ingestão, para promoção da saúde do cliente, visando também a sua condição social e econômica.

Palavras-chave: Flavonoides. Frutas. Verduras. Alimentos Orgânicos. Produção Agrícola. Manipulação de Alimentos.

Introduction

Flavonoids are phenolic compounds that differ in their chemical structure, have 15 carbon atoms in the C6 – C3 – C6 form, based on the nucleus of two benzene rings, A and B, attached to a pyran ring, C (Figure 1).[1,2] They are in foods generally in the form of O-glycosides, with the sugar molecule bound in position 3 and in some cases in position 7. Glucose, galactose, rhamnose and fructose are the most commonly found sugars.[1]

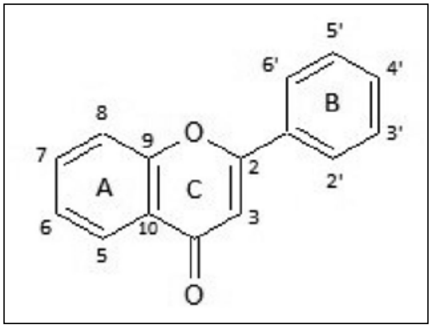

Figure 1. Basic structure of flavonoids.

According to Pereira & Cardoso,[3] flavonoids are secondary metabolites. More than 8,000 substances belonging to this group have been identified.[2] This variety of compound occurs due to a large combination of different sugars and hydroxyl (OH) as substituents in the basic chemical structure.[4] Flavonoids used in the human diet are subdivided into six classes: Flavanones, Flavonols, Flavones, Flavanols, Isoflavones and Anthocyanidins.[5,6]

Flavonoids are related to a great variety of biological activities, emphasizing the antioxidant, anti-inflammatory, anti-tumor, anti-allergic and antiviral ones, among others.[1,7,8] Flavonoids antioxidant action is due to its ability to sequester free radicals and chelate metal ions.[4] Flavonoids donate hydrogen atoms, thus protecting tissues from reactions caused by free radicals and lipidic peroxidation.[9]

Quercetin is the most abundant flavonoid in the diet. It presents significant anti-inflammatory action and antioxidant potential.[10] Average intake of flavonoids ranges from 26 mg to 1 g/day from the consumption of food sources such as fruits, vegetables, teas, wines, grains and seeds.[11] According to Kozlowska & Szostak-Wegierek,[12] average flavonoids consumption in Greek and Spanish populations ranges between 93 mg and 126.1 mg, respectively. In Japan, average consumption may reach 2 g due to high consumption of vegetables, soy and tea. Koehnlein et al.[13] have verified that flavonoids consumption from preparations and foods of vegetal origin in Brazilian diet was estimated at 374 mg/day. This study was carried out with 37 foods of plant origin from Brazilian population's food consumption and published in the 2008/2009 Household Budget Survey (HBS).[14]

According to the (2008/2009) HBS,[14] the fruits and potherbs most consumed by the population in southern Brazil are apple, watermelon, Dwarf Cavendish banana, orange, pear, papaya, onion, lettuce, tomato, cabbage, cassava and potato. Thus, the objective of the present study was to evaluate the amount of total flavonoids expressed in quercetin of some fruits and potherbs most consumed by the population in southern Brazil. Levels of flavonoids were evaluated in conventionally-produced and organic products *in natura* submitted to cooking process by dry heat in cooking pots and a microwave oven.

Materials and Methods

Material

The study was carried out in the Laboratory of Pharmacognosy and Chemistry II at the Autonomous University Center of Brazil (UniBrasil, in the Portuguese abbreviation) in the city of Curitiba, PR. Two fruits and two of the most consumed potherbs were selected in 2008/2009, according to data from the HBS.[10] They are: apple, Dwarf Cavendish banana, onion and tomato, which can be consumed *in natura*, post-cooking process and in microwave oven cooking. For analysis, three units of each food, conventionally-produced and organic, were used.

Method

Extraction of total flavonoids was performed according to Alves & Kubota,[15] with modifications. *In natura* samples and samples submitted to cooking process in dry heat in cooking pots and a microwave oven were used.

For preparation of the aqueous extract from the onion and tomato samples, both were washed in running water. Onion skins were removed and samples minced with a knife. Fifty grams of each chopped sample were weighed. The *in natura* onion sample was ground in a [Dutch technology company Koninklijke Philips N.V. (Koninklijke Philips N.V. of the Netherlands, Philips), (stylized as PHILIPS)] Walita brand blender, with 100 mL of purified water for 5 minutes. The *in natura* tomato sample was ground in a blender with 200 mL of purified water for 5 minutes. To obtain an aqueous extract, the resulting material was filtered through gauze and the liquid obtained was completed with purified water to 200 mL and homogenized. An aliquot of the aqueous extract was used for flavonoid determination.

The onion cooking process consisted of 50 g of chopped onions without oil on low heat for 4 minutes and 30 seconds. Other 50 g of sample were submitted to a microwave oven for 1 minute in the 100-watt power. For tomatoes, the cooking process consisted of 50 g of chopped tomatoes without oil on low heat for 2 minutes and 24 seconds. Other 50 g of sample were submitted to a microwave oven for 40 seconds in the 100-watt power. Afterwards, the onion and tomato samples submitted to the cooking process and microwave oven were also processed in the same way as the *in natura* ones to obtain an aqueous extract used for flavonoid evaluation.

To prepare the aqueous extract of the banana and apple samples, the banana peels were first removed, the apples were washed in running water, seeds were extracted and both samples were chopped with a knife. Fifty grams of each chopped sample were weighed. The *in natura* banana sample was ground in a [Dutch technology company Koninklijke Philips N.V. (Koninklijke Philips N.V. of the Netherlands, Philips), (stylized as PHILIPS)] Walita brand blender, with 200 mL of purified water for 5 minutes. The *in natura* apple was triturated in a blender with 100 mL of purified water for 5 minutes. To obtain an aqueous extract, the resulting material was filtered through gauze and the liquid obtained was completed with purified water to 200 mL and homogenized. An aliquot of the aqueous extract was used for flavonoid determination.

The banana cooking process consisted of braising without oil 50 g of chopped banana on low heat for 2 minutes. Other 50 g of sample were submitted to a microwave oven for 30 seconds in the 100-watt power. For the apple, the cooking process consisted of braising 50 g of chopped apple without oil on low heat for 2 minutes and 36 seconds. Other 50 g of sample were submitted to a microwave oven for 50 seconds in the 100-watt power. Then, the banana and apple samples submitted to the cooking process and microwave oven were also crushed in a blender with the same amount of water and filtered and the liquid obtained was used for the evaluation of flavonoids.

Two mL of 2% (m/v) aluminum chloride were mixed in 2 mL of the aqueous extracts. Absorbance was determined at 425 nm after ten minutes against a blank, consisting of a 2 mL solution of purified water with 2 mL of 2% (m/v) aluminum chloride. Total flavonoid content was determined using a standard quercetin curve with concentrations ranging from 5 to 50 µg/mL. All assays were performed in triplicate and expressed as mean ± standard deviation.[15]

Statistical analysis

Results from total flavonoid determination analyses corresponded to the mean ± standard deviation of three replicates and were compared by analysis of variance (ANOVA) followed by the Tukey's test to identify significant differences among means using the Sisvar software, where the means at the 5% level ($p < 0.05$) were considered expressive.

Results and Discussion

The calibration curve for determination of flavonoids obtained the equation of the straight line expressed by $y = 0.0333\,x - 0.0096$, where y is the absorbance and x is the concentration of quercetin em µg/mL, with $R^2 = 0.9997$. Aluminum chloride is a reagent used in UV-visible spectrometry for structural determination of flavonoids.[16] It is currently used for determination of total flavonoids in propolis and plants, using rutin or quercetin as standard.[15,17] In the reaction, the aluminum ion Al^{3+} is made complex with the flavonoid molecules of the sample, establishing the stable complex flavonoid-Al^{3+} of yellow color whose intensity is proportional to the flavonoid concentration present in the sample.[16] This methodology allows the dosage establishment of different flavonoids. Therefore, in the procedure performed in the present study the flavonoid quercetin was used as the standard and thus the results obtained were total flavonoids expressed in quercetin. Thus, other flavonoids, besides quercetin, are present in the absorption obtained.

Table 1 shows the average results of flavonoids present in potherbs (onion and tomato) and fruits (banana and apple), both conventionally and organically grown.

Table 1. Content of flavonoids present in potherbs and fruits, conventionally-produced and organic, *in natura*, acquired in the Brazilian city of Curitiba, PR, in 2015.

Potherbs/fruit	Flavonoid content Mean ± standard deviation (mg/100 g)
Conventionally-produced onion	10.0 ± 0.2 a3
Organic onion	11.8 ± 0.3 a4
Conventionally-produced tomato	3.3 ± 0.05 a1
Organic tomato	2.8 ± 0.05 a1
Conventionally-produced banana	8.7 ± 0.1 a2
Organic banana	8.8 ± 0.1 a2
Conventionally-produced apple	12.1 ± 0.1 a4
Organic apple	14.3 ± 0.3 a5

Letter a followed by an equal number means statistically equal results.

The organic onion presented higher levels of flavonoids compared to conventionally-produced onions. This fact can be justified by the non-use of pesticides in the crop. In this way, it produces higher concentrations of phenolic compounds, which act as defense agents against insects, microorganisms, bacteria and fungi.[18]

According to a study by Arbos et al,[19] the levels of phenolic compounds found in organic potherbs were higher than those found in conventionally-produced potherb cultures. Many factors must be considered in relation to organic foods nutritional quality, such as the type of production, the type of organic system used, external factors such as sunlight, rainfall and temperature, storage and transportation.[20] As for tomato, it has not presented a difference of flavonoid content between conventionally-produced and organic. This indicates that flavonoids present in tomatoes do not influence vegetable defenses as much as onions.

In the fruits analyzed, apples had higher levels of flavonoids in relation to bananas. And the organic sample had higher levels of compounds than the conventionally-produced sample. Bananas showed no difference between the organic sample and the conventionally-produced one, indicating, as in the case of tomatoes, that in bananas the flavonoids present influence the vegetable defense.

The species biodiversity results in basic components variations such as carbohydrates, proteins, lipids, dietary fiber, minerals, vitamins and also bioactive compounds[21] such as flavonoids. These are produced in response to environmental conditions. Therefore, it is possible to explain the difference in flavonoid contents of the same greens in different regions. Several studies have shown that there are many variations in flavonoid content[22] since its concentration may increase in response to stress from the environment, which can be caused by diseases, altitude, air pollution, nutrients, weather and ultraviolet (UV) radiation.[23]

Table 2 shows comparison results of potherbs flavonoid contents *in natura* and after cooking and microwave oven processes.

Table 2. Content of conventionally-produced and organic potherbs flavonoids obtained in the Brazilian city of Curitiba – PR, *in natura*, after cooking processes in 2015.

Process	Conventionally-produced onion mean ± SD (mg/100 g)	Organic onion mean ± SD (mg/100 g)	Conventionally-produced tomato mean ± SD (mg/100 g)	Organic tomato mean ± SD (mg/100 g)
In natura	10.06 ± 0.2 a1	11.80 ± 0.3 a1	3.36 ± 0.05 a1	2.86 ± 0.05 a1
Cooking	10.83 ± 0.05 a2	12.16 ± 0.2 a1	5.06 ± 0.05 a2	4.33 ± 0.1 a2
Microwave oven	11.0 ± 0.4 a2	12.40 ± 0.6 a1	5.73 ± 0.1 a3	4.50 ± 0.1 a2

Letter a followed by an equal number in the same column means statistically equal results.

In natura conventionally-produced onion presented lower value to the cooking process by dry heat in cooking pots and microwave oven, in which heating allowed better flavonoid extraction. In a study by Ioku et al.,[24] which has evaluated cooking methods for dosing flavonoids in onion, it was observed that after subjecting onions to a microwave oven during 1 minute there was increase in 1.5 times in quercetin total content, which is the main flavonoid present in human diets and found in onions, showing that the compounds were more easily extracted. However, when it was submitted to cooking in water, there was a significant loss of the compounds. This fact is justified because flavonoids migrate to the water used in cooking. It is verified that cooked or roasted onions present a better extraction of flavonols.[25]

In the present study, there was an increase in flavonoid content, since dry heat was used for analysis, facilitating extraction, not allowing it to be degraded or leached in the cooking water.[26] Organic onion *in natura* and subjected to cooking process by dry heat in cooking pots and a microwave oven presented the same result, which means that the process does not decrease flavonoids levels.

Conventionally-produced and organic *in natura* tomato presented lower flavonoids values in relation to the dry cooking process in cooking pots and a microwave oven, because in this case the heating process also allowed better extraction of flavonoids in the samples.

Table 3 shows the fruit flavonoid contents comparison result *in natura* and after the cooking and microwave oven processes. For conventionally-produced bananas, samples *in natura* and submitted to a cooking process in cooking pots presented higher flavonoid levels in relation to the sample submitted to the cooking process in a microwave oven. Organic bananas presented higher compounds contents in the *in natura* samples and the ones submitted to a microwave oven cooking process in relation to the sample submitted to the cooking process in cooking pots. For the apples it was observed that both conventionally-produced and organic ones had higher flavonoids values in the sample *in natura* in relation to the samples submitted to cooking in cooking pots and a microwave oven.

Table 3. Content of conventionally-produced and organic fruit flavonoids obtained in the Brazilian city of Curitiba – PR, *in natura*, after cooking processes in 2015.

Process	Conventionally-produced banana mean ± SD (mg/100 g)	Organic banana mean ± SD (mg/100 g)	Conventionally-produced apple mean ± SD (mg/100 g)	Conventionally-produced apple mean ± SD (mg/100 g)
In natura	8.70 ± 0.1 a2	8.8 ± 0.1 a2	12.13 ± 0.1 a3	14.30 ± 0.3 a3
Cooking	8.80 ± 0.1 a2	6.63 ± 0.4 a1	8.93 ± 0.05 a1	9.96 ± 0.3 a1
Microwave oven	7.86 ± 0.3 a1	8.06 ± 0.1 a2	11.43 ± 0.1 a2	10.93 ± 0.2 a2

Letter a followed by an equal number in the same column means statistically equal results.

In the present study, it was observed that the flavonoid extraction from fruits and potherbs was affected by the cooking processes employed. In the case of fruits, the dry heat influenced negatively, making it difficult to extract flavonoids, while in the potherbs, it influenced positively, facilitating the extraction. Possibly, the presence of fibers in the fruits made it difficult to extract flavonoids when they were heated. This heating promoted some trapping of flavonoids in fibers. Fibers are complex carbohydrates, which can be soluble and insoluble. Solubilities reduce gastrointestinal transit time and enteral absorption of cholesterol. They are represented by pectin in fruits or by gums in oats, barley and legumes.[27] One of the mechanisms that can explain the action of soluble fibers, such as pectin, would be for these to absorb water and form a gel in the intestinal lumen, acting to reduce the absorption of carbohydrates and decrease the resorption of bile acids.[28] Thus, as with carbohydrates and other nutrients, fibers make flavonoids less available for absorption.

Pectin is a fiber with high gelling power after heating and it is found in greater numbers in apples and bananas. Some studies show that the accumulation of pectin in fresh apples and bananas has a concentration of 0.5 – 1.6% and 0.7 – 1.2% of the fibers, respectively. Tomatoes have a lower concentration of 0.2 – 0.6% of the fiber.[29] The presence of higher fiber concentrations in bananas and apples justifies the fact that at least in some of the cooking processes there was a lower extraction of flavonoids since they may have been retained in the gelled fibers by heating.

In this research, heating potherbs allowed better extraction of flavonoids, since onions and tomatoes are not foods with high fibers content and these do not seem to interfere in the extraction. In this case, cooking softens these foods, favoring the extraction of flavonoids.

Thus, cooking fruits such as bananas and apples makes it difficult to absorb flavonoids in the intestinal tract, indicating that the ideal form of consumption of these fruits is *in natura*. As for the potherbs, it was verified that heating favors the extraction of flavonoids. Therefore, consumption of tomatoes and onions can be either *in natura* or under cooking.

According to a study by Arabbi et al.[30] in relation to the Brazilian population, the estimated intake of flavonoids ranged from 60 to 106 mg/day, with an average intake of 79 mg/day for women and 86 mg/day for men. This flavonoid intake was estimated according to food consumption, based on the dietary composition obtained from several dietary and eating habits pieces of research carried out and available in the country. Thus, flavonoid intake is very varied and can be explained by different eating habits. In another study carried out by Corrêa et al.,[31] it was verified that the Brazilian population consumes on average 138.92 mg/day of flavonoids. It was found that the intake of phenolic compounds such as flavonoids is low due to the insufficient consumption of fruits and potherbs. It is noteworthy that coffee and black beans are the main foods contributing to phenolic compounds consumption in the country.

Flavonoids daily intake is still poorly documented due to lack of data on flavonoid content in foods. The Food and Nutrition Board committee from the US National Academy of Sciences does not clarify the DRIs (Dietary Reference Intakes) of these compounds.[32] Knowledge about how potherbs and fruits should be consumed and about flavonoid levels present enables the nutritionist to prescribe and establish strategies and safe intake goals to promote patients' health.

Conclusion

The present study results demonstrate that there was a difference in onion and apple cultivation processes. It was observed that these samples presented higher flavonoid content. This fact is justified by these plants mechanism to produce flavonoids for their protection against environmental stresses. However, bananas and tomatoes did not present any difference between organic and conventionally-produced cultivation, indicating that flavonoids present possibly do not influence vegetable defenses as much as onions and apples.

With the research findings it was possible to verify that there was a difference in total flavonoid extraction from the foods *in natura* and submitted to a cooking process. In the case of potherbs, dry heat influenced, facilitating flavonoid extraction, while in fruits, it made it difficult. Possibly, the presence of pectin fiber in the fruits acted to decrease flavonoid extraction when this was heated. Therefore, fruits must be consumed *in natura* because dry heat makes flavonoid extraction and its absorption difficult in the digestive tract.

By means of knowledge of how foods should be consumed and about flavonoid content, nutritionists can prescribe and establish strategies and safe intake goals to promote patients' health, also taking into account their social and economic situations. This stresses the importance consuming food which is in fruit and potherbs groups with the aim of increasing flavonoid contribution in Brazilian diets. There are few data from the literature on the subject, which implies the need to analyze more foods and obtain more values on these compounds so that recommendations for safer prescription can be established in the future.

Contributors

Savi PRS has participated in the design, analysis, interpretation of data and final version of the article; Santos L, has participated in the design, analysis, interpretation of data and final version of the article; Biesek S, has participated in the study design of the essay writing and revision and final version; Macedo A, has participated in the analysis of the study; Lima CP has worked in all steps, from designing and projecting the study to revising the final version of the article.

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