Received March 2010
Revised May 2010
Accepted May 2010

Antioxidant activity, total phenolic compounds and flavonoids of mangoes coming from biodynamic, organic and conventional cultivations in three maturation stages

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Abstract

Purpose – Phenolic compounds, including flavonoids, are nutritionally important for their antioxidant activities and protective functions against disease risk caused by oxidative stress. These compounds are primarily found in fruits, and mangoes are an important source. This paper aims to address these issues.

Design/methodology/approach – In this work, the antioxidant activities of mangoes cultivated in three different ways were evaluated by their ability to capture free radicals using the DPPH radical.

Findings – The results showed that the biodynamic mangoes had highest antioxidant activity in mature-green and ripe fruits, while for those of organic origin the antioxidant activity was highest in unripe fruits. The organic mangoes also showed highest values of phenolic compounds at all maturation stages. The mangoes from conventional crops had lower values for all parameters evaluated in this study than the organically and biodynamically cultivated fruits.

Originality/value – This work brings an important contribution in the field of agriculture at a time when organic and biodynamic systems of cultivation are an alternative to the conventional system and that pollutes the environment and produces food that contains quantities of chemical contaminants that can damage the health of the consumer. The comparison in phenolic compounds content, flavonoids and antioxidant activity in biodynamic, organic and conventional systems is original and of great importance, showing that the ecological cropping systems are less harmful to the environment and promote improvements to the chemical composition of foods.

Keywords Agricultural products, Fruits, Organic food, Mango, Antioxidants, Biodynamic and organic farming

Paper type Research paper

The authors would like to thank CAPES for the financial support.

British Food Journal
Vol. 113 No. 9, 2011
pp. 1103-1113

0007-070X
DOI 10.1108/00070701111180319

1. Introduction

The practice of the conventional agriculture grew so that it did not consider the aggressions to the atmosphere, consuming in a systematic way an entire existent agricultural structure and constituted, and the food produced in agreement with your beginnings and practices, usually, they present residues of the chemical compounds (Santos and Monteiro, 2004).

In the organic agriculture they are used technologies that optimize the use of natural and socioeconomic resources, tends for objectives the self-sustaining, the maximizing of the social benefits, the minimization of the dependence of energies did not renew and the elimination of the pesticides employment and of other toxicant artificial inputs (Brazil, 1999). This agricultural system is based on natural methods for the effective control of pests and increasing soil fertility (Carpenter-Boggs *et al.*, 2000).

The agriculture biodynamic possesses a common base with the other organic production forms in what concerns the diversification and integration of the vegetable explorations, animals and forest (Darolt, 2002). The emphasis of this agricultural practice is in the relationships among the vegetable growth and the cosmos, the nature and the deep sense of the manuring, the vital balance among the farming and the livestock, the paper and the biological treatment of the vegetable and animal pests (Steiner, 2001).

The increasing demand for food produced without pesticides and less harmful to the environment is a worldwide trend that is reflected also in Brazil (Brazil, 2007). In this context, the development of sustainable agriculture such as organic and biodynamic agriculture, protect the environment and with the potential to strengthen the rural economy, which is a important challenge (Cristovão *et al.*, 2001).

Studies affirm that several factors as maturation stage, the soil, the climate, the cultivation system, among others can influence the chemical composition of fruits. In the last years, it has been having a growing interest in the study of the mango (*Mangifera indica* L.) due to your bioactive compounds, what turns the consumption of mangoes and derived products a healthy habit (Cao and Cao, 1999). The mango is all over the world one of the most important commercial cultures in production terms, commercialization and consumption. Brazil is between the great producing countries and mango exporters (FAO, 2004).

The world supply of mangoes in 2004 was approximately 26 million tonnes. Brazil, with an annual production, about 820 tons, is the ninth producer and second export with a share of 3.4 per cent of total supply (FAO, 2005). The mango cultivation is disseminated for almost the Brazilian territory, and the Northeast area concentrates most of the production, answering on average for 52 per cent, and Bahia is the state that more produces this fruit (Brazilian Development Bank, 2003). In the state of Bahia, mangoes are produced in the conventional, organic and biodynamic systems, mainly in the areas of Chapada Diamantina and in São Francisco Valley.

The high consumption of fruits and vegetables it has been associated to a lowest risk prevalence of degenerative chronic diseases. Such a protecting effect is related to the several antioxidant compounds presents in these foods (Kauer and Kapoor, 2001). The protecting effect exercised by these food it has been attributed to the presence of antioxidant compounds, among which stand out the phenolics compounds, secondary products of the vegetable metabolism (Rice-Evans *et al.*, 1996). These compounds exhibit great amount of physiologic properties, as, for instance, the property anti-allergenic, anti-inflammatory, antimicrobial and anti-thrombotic (Balasundram *et al.*, 2006).

Countless studies accomplished with phenolics compounds, especially the flavonoids, demonstrate the activity of they capture free radicals and your effects in the prevention of cardiovascular and circulatory illnesses risks (Ness and Powles, 1997; Stoclet *et al.*, 2004), cancerous (Wang and Mazza, 2002; Katsube *et al.*, 2003), in the diabetes and in the evil of Alzheimer (Hertog *et al.*, 1997; Abdille *et al.*, 2005). Some studies shown higher levels of these substances in organically grown products. Tomato fruits grown in organic agriculture present a higher content of flavonoids compared with conventional fruit cultivation (Slimestad and Verheul, 2009). Amor *et al.* (2008) found higher levels of total phenolics in sweet peppers originating from organic farming.

The objective of the present work was to evaluate the antioxidant activity of mangoes coming from three different cultivation systems in three different maturation stages and to esteem the antioxidant principal as the total phenolics compounds and flavonoids.

2. Material and methods

2.1 Samples

A total of 36 mango's tree fruits were analyzed (*Mangifera indica* cv. "Tommy Atkins") of each cultivation system, being 12 of each maturation stage, to know: ripe, "instead of" and unripe. The fruits were collected in the area of Chapada Diamantina, in the municipal district of Piatã – BA. This area was chosen because the farms produce mangoes in several different cultivation systems – biodynamic and organic, certificates, and conventional.

2.1.1 Prepare of the samples

The fruits were washed with water, droughts and peeled for the separation of the pulp. They were homogenized and stored, in recipients tightly closed, and taken to freezing in temperature of -18°C to the moment of the accomplishment of the analyses.

2.1.2 Extraction of the total phenolics compounds

The samples were submitted to tests seeking the maxim extraction of the phenolics compounds. For each 10 g of mango 20 mL were used of each one of the following solvents: water deionized, methanol, ethanol and acetone, as well as the following mixtures of solvents: 80 per cent methanol:20 per cent water deionized; 80 per cent acetone: 20 per cent water deionized; 50 per cent methanol: 50 per cent ethanol and acetone 30 per cent: água deionized 20 per cent. The recovery assays were performed with a gallic acid standard in known concentration. For the analyses 10 g of sample was used in 20 mL of acetone. For all the tests the samples were prepared in triplicates, triturated in a grinder of the type Ultra turrax® IKA® T18 BASIC regulated in 10.000 rpm by one minute and taken to the refrigerated centrifuge (Eppendorf® 5702R) with temperature from 5°C to 4,400 rpm. The supernatant was used as final sample.

2.2 Total phenolics compounds

The content of total phenolics compounds was certain using the reagent Folin-Ciocalteau (Singleton and Rossi, 1965), with which was prepared the reaction containing an aliquot of 0.5 mL of the extract phenolic, 2.5 mL of aqueous solution of Folin-Ciocalteau 10 per cent and 2.0 mL of carbonate of sodium 7.5 per cent. The mixture was maintained in heating to 50°C for 5 minutes and then accomplished the reading by spectrophotometry (spectrophotometer Femton 800XI) to an absorbance of 760 nm. The white was used as reference. For the quantification of the total phenolics the same procedure was accomplished using standard curve with solutions of acid gallic (GAE) ($R^2 = 0.9998$) and the results were expressed in mg $GAE.100g^{-1}$.

2.3 Flavonoids

For the determination of the content of flavonoids, the same extract was used obtained for the analysis of total phenolics compounds, in which, 1 mL of the final sample was transferred for a volumetric balloon of 10 mL previously containing 4 mL of deionized water. 0.3 mL of nitrite of sodium 5 per cent were added. After exact 5 minutes, 0.3 mL of chloride of aluminum 10 per cent were added and after 1 minute 2 mL of hydroxide of sodium 1M were added. The balloon volumetric was completed with deionized water and shaken manually. The absorbance was measured to 510 nm (spectrophotometer Femton 800XI) and the quantification done through a calibration curve built by the dilution of a solution standard of epicatechin (ECE) ($R^2 = 0.9995$) and the results were expressed in mg $ECE.100g^{-1}$ (Amor *et al.*, 2008).

2.4 Antioxidant activity

The antioxidant activity of the extracts was determined using the test DPPH (1,1-difenil-2-picrilidrazil) in agreement with the methodology described by Vinson *et al.* (2001). For the evaluation of the antioxidant activity, the prepared mango samples in acetone in the concentration of 200 $mg.mL^{-1}$ were submitted to the reaction with DPPH, in which 1 mL of the sample was mixed in 3 mL of the solution DPPH to 0,004% $m.v^{-1}$. The reduction of the radical of DPPH was measured through the continuous monitoring of the decline of the absorbance to 517 nm for 30 minutes, to the shelter of the light, even stable values of absorption. The absorbance was turned into percentage of the antioxidant activity. The values were calculated by lineal regression and the results were presented as average. The antioxidant activity of each sample (IC50) it is expressed as the final concentration in $\mu g.mL^{-1}$ of the present extract in the cuvette, requested for the initial concentration of DPPH to decrease in 50 per cent.

2.5 Statistical analysis

Tests of normality were accomplished to verify the data they followed Gaussian distribution. The used test was it of Anderson-Darling. In the cases in that the data had Normal Distribution; it was made the Analysis of Variance (ANOVA) to verify if there was difference existed among the averages of the treatments. When difference existed among the averages, the Test of Tukey was accomplished. However, when verifying that the data didn't follow a normal distribution, the Test of Kruskal-Wallis was used. In the existence of difference among groups, it was made a test of multiple comparisons no-parametric (Siegel and Castellan, 2006). In all the tests the level of 5 per cent of significance was adopted. The software was used MINITAB® 14 for the accomplishment of the analyses statistics.

3. Results and discussion

For the extraction of the phenolics compounds the acetone demonstrated to be the most appropriate solvent, after other solvents or mixtures of them they be tested. Using several solvents Ajila *et al.* (2007) they observed that the maximum extraction of phenolics compounds in mango peel it happened with acetone to 80 per cent, as well as Pereira *et al.* (2007), in study with mulberries, and Soares (2008), with apples.

The method of Folin-Ciocalteu adopted to quantify phenolics compounds it consists of the reduction of the reagent for these composed with the formation of a blue compound for the mixture of the acids fosfowolframic and fosfomolíbdic, that is reduced when rusting the phenolics compounds in half basic, originating blue oxides of wolframio (W_8O_{23}) and molybdenum (Mo_8O_{23}) (Moyer *et al.*, 2002). This complex one can be measured to 760 nm against the acid gallic as pattern (Imeh and Khokhar, 2002). The total contents of phenolics were certain as index of total phenolics compounds, represented in the Table I.

The organic mangoes, in all the maturation stage, presented the highest contents of phenolics compounds, followed for the biodynamics and conventional. All the analyzed samples suffered reduction in the phenolics content with moving forward of the maturation. The fruits of the conventional cultivation presented inferior values to the biodynamics and organic so much in the stage of unripe maturation as in the "instead of". For the ripe fruits, the biodynamics had lowest contents of these composed.

The data demonstrate a tendency for a highest content of total phenols under organic cultivation and biodynamic, corroborating with the hypothesis of Carbonaro and Mattera (Carbonaro and Mattera, 2001) and Asami *et al.* (2003), that organic food present highest content of endogenous phenol due to the cultivation method.

In the literature, several citations of phenolics contents exist for mangos that vary of 68 mg GAE.100g^{-1} to 266 mg GAE.100g^{-1} (Wu *et al.*, 2004). This variation can be due ace variety differences, climate, maturation, extraction method and agricultural system adopted. Padilha (2005) found in extracts methanolic (80 per cent) of mango "Tommy Atkins", 23.53 mg GAE.100g^{-1} of the total phenolics compounds in unripe fruits, and 22.14 mg GAE.100g^{-1} in ripe fruits. In works accomplished with pulp of mangoes of several varieties, Ribeiro *et al.* (2007), working with "Tommy Atkins", and Vasco *et al.* (2008), studying Ecuadorian mangoes, they concluded that these fruits presented approximate contents of 60 mg GAE.100g^{-1}. Melo *et al.* (2008), in extracts ketones of mangoes "sword" and "rose", they found 99.45 mg GAE.mL^{-1} and 84.15 mg.GAE.mL^{-1} of the total phenolics compounds, respectively.

Table I. Medium values with standard deviation in mg GAE.100g^{-1} of total phenolics compounds of mango "Tommy Atkins" of different cultivation systems to each maturation stage

	Total phenolics compounds (mg GAE.100g^{-1}) Maturation of the fruits		
Cultivation system	Unripe	"instead of"	Ripe
Biodynamic	126.03^{Aa} ± 4.01	122.58^{Ab} ± 2.19	63.48^{Ac} ± 1.01
Organic	147.37^{Ba} ± 2.61	130.52^{Bb} ± 2.00	102.67^{Bc} ± 1.36
Conventional	104.34^{Ca} ± 3.67	92.28^{Cb} ± 1.47	68.21^{Cc} ± 1.17

Notes: Same capital letters indicate that there is not significant difference ($p > 0.05$) among the cultivation systems (columns). Same small letters indicate that there is not difference among the stage of maturation of a same cultivation system (lines)

Caris-Veyrat *et al.* (2004) accomplished a study seeking to the comparison of the content of present antioxidant compounds in tomatoes cultivated in organic and conventionally systems. The results, expressed in humid base, they demonstrated highest phenolics compounds content for the organic tomato. Research accomplished in Brazil by Borguini (Borguini and Silva, 2007) it registered that coming tomatoes of organic system of production presented highest content of total phenolics compounds than the tomato produced by conventional cultivation.

For the flavonoids, the organic mangoes presented superior contents to the others just when they were unripe. In the stage "instead of" and ripe these mangoes demonstrated to possess lowest contents in relation to the biodynamics. The fruits biodynamics presented the highest contents in the stage "instead of" and ripe, while the conventional presented lowest contents in all the maturation stage. The unripe and ripe mangoes differed statistically ($p \leq 0.5$) for all the cultivation systems. The organic fruits presented similar statistically contents to the biodynamics in the stage "instead of". When evaluated only one cultivation system to the three maturation stage it can be noticed that the fruits biodynamics, organic and conventional presented values similar statistically in the unripe stage and "instead of" and these differed of the ripe. A reduction of the flavonoids was observed during the ripening (see Table II).

Lima *et al.* (2007) found contents of total phenolics compounds (58 mg GAE.g^{-1} and 63 mg GAE.g^{-1}) and flavonoids (6.1 mg.g^{-1} and 3.3 mg.g^{-1}) with significant difference ($p \leq 0.5$) among the conventional and organic cultivation of extracts ketones of unripe mangoes.

The results found in this work they demonstrate that variations exist among the analyzed fruits, in what concerns the total phenols and the flavonoids content in mangoes biodynamics, organic and conventional.

The activity of the different extracts of mangoes in capturing free radicals was expressed in percentile of maximum inhibition of the free radicals in comparison with the percentile of inhibition of the acid gallic (Table III) and as final concentration of the extract necessary to inhibit the oxidation of the radical DPPH in 50 per cent – IC50 (Table IV). The percentile of maximum inhibition of the free radicals of extracts of mango as compared to gallic acid standard estimates the antioxidant potential of these extracts. The IC50 demonstrates this potential in a measure (μg.mL-1) of bioactive compounds able to scavenge 50 per cent of free radicals. The antioxidant substances present in the extracts react with DPPH that is a stable radical, and it turns into 2,2-difenil-1-picril hydrazine. The discoloration degree indicates the antioxidant activity of the extract. An extract that

Table II. Medium values with standard deviation in mg ECE.$100g^{-1}$ of flavonoids of mango "Tommy Atkins" of different cultivation systems to each maturation stage

	Flavonoids (mg ECE.$100g^{-1}$) Maturation of the fruits		
Cultivation system	Unripe	"instead of"	Ripe
Biodynamic	$25.19^{Aa} \pm 1.54$	$16.41^{Aa} \pm 0.79$	$15.96^{Ab} \pm 1.05$
Organic	$27.23^{Ba} \pm 2.05$	$15.71^{Aa} \pm 0.75$	$15.59^{Bb} \pm 1.32$
Conventional	$24.31^{Ca} \pm 0.83$	$10.49^{Ca} \pm 0.38$	$10.45^{Cb} \pm 0.77$

Notes: Same capital letters indicate that there is not significant difference ($p > 0.05$) among the cultivation systems (columns). Same small letters indicate that there is not difference among the stage of maturation of a same cultivation system (lines) presents high capacity in capturing free radicals possesses low value of IC50. This way, a small amount of extract is capable to reduce of the initial concentration of the radical DPPH in 50 per cent. The antioxidant activity of the extracts can be attributed to the ability of capturing free radicals through the donation of hydrogen, because the mentioned extracts present a reasonable content of phenolics compounds.

According to Atkinson *et al.* (2006), agronomic practices interfere with growth and chemical composition of plants and can use them to manipulate the growth, yield and optimize the production of bioactive compounds in fruits. Researches accomplished by Vasco *et al.* (2008), through the method DPPH, reported that Ecuadorian mangoes possessed 84 per cent of potential sequestrant of free radicals. Azevedo (2006) worked with conventional mangoes in three maturation stage and concluded that the unripe fruits reached 44.41 per cent of inhibition, already the "instead of" and the ripe ones inhibited 29.99 per cent and 25.95 per cent, respectively.

Unripe organic mangoes captured the highest amount of free radicals among analyzed them, followed for the biodynamics and conventional. Among the fruits "instead of" and ripe, the mangoes biodynamics presented the best result. The conventional mangoes were inferior to the others in all the maturation stage. However, it is observed that, in elapsing of the maturation process, the mangoes suffer a notable decrease in your antioxidant activity.

As demonstrated in the determination of total phenolics compounds, the extracts mangoes of the organic presented highest content in relation to the extracts mangoes of

Cultivation system	AA(%) Maturation of the fruits		
	Unripe	"instead of"	Ripe
Biodynamic	$87.27^{Aa} \pm 0.18$	$84.40^{Ab} \pm 0.45$	$77.81^{Ac} \pm 0.43$
Organic	$92.94^{Ba} \pm 0.27$	$79.68^{Bb} \pm 0.34$	$75.67^{Bc} \pm 0.30$
Conventional	$77.62^{Ca} \pm 0.82$	$74.88^{Cb} \pm 0.12$	$72.24^{Cc} \pm 1.03$

Notes: Percentage of inhibition of gallic acid = 99.98; same capital letters indicate that there is not significant difference ($p > 0.05$) among the cultivation systems (columns). Same small letters indicate that there is not difference among the stage of maturation of a same cultivation system (lines)

Table III. Maximum antioxidant activity, (percentile of inhibition) of the mango extracts 200 mg.mL^{-1}

Cultivation system	IC50* μg.mL^{-1} Maturation of the fruits		
	Unripe	"instead of"	Ripe
Biodynamic	$152.25^{Aa} \pm 8.58$	$184.07^{Ab} \pm 1.42$	$226.54^{Ac} \pm 5.70$
Organic	$130.82^{Ba} \pm 2.73$	$225.32^{Bb} \pm 1.75$	$270.87^{Bc} \pm 4.53$
Conventional	$192.64^{Ca} \pm 3.13$	$236.25^{Cb} \pm 2.77$	$289.81^{Cc} \pm 5.32$

Notes: Same capital letters indicate that there is not significant difference ($p > 0.05$) among the cultivation systems (columns). Same small letters indicate that there is not difference among the stage of maturation of a same cultivation system (lines). *Obtained values of the lineal regression possess level of trust of 95 per cent. IC50 is defined as the amount of antioxidants able to reduce the initial concentration of DPPH for 50 per cent

Table IV. Capacity of the mango extracts in capturing free radicals (DPPH) biodynamic and conventional systems. The organic green mangoes were superior in capacity to capture free radicals. The extracts of biodynamic mangoes presented highest levels of flavonoids and lowest IC50 for fractions "instead of" and ripe. For the extracts of biodynamic unripe mangoes, the phenolics content was 14.48 per cent lowest than the organic and 20.79 per cent highest than the conventional. These results suggest a highest contribution of the antioxidant capacity of the flavonoids, since these fruits present lowest content of compositions total phenolics and highest content of flavonoids in relation to the organic mangoes.

Several authors have been demonstrating that a great positive relationship exists between the content of total phenolics and the antioxidant activity of fruits and vegetables (Abdille *et al.*, 2005; Vinson *et al.*, 1998; Kauer and Kapoor, 2002). Besides the presence in the extracts of other phytochemicals, the chemical structure of the active component has influence on the effectiveness of the natural antioxidant, once the position and the number of hydroxyl in the molecule of the total phenolics compounds are an important factor for this activity. According to Shahidi *et al.* (1992), the ortho-dihydroxylation contributes significantly to the antioxidant activity of the compound. Thus, the antioxidant capacity of an extract can not be explained solely based on their content of total phenolic compounds, because the characterization of the structure of the active compound, it is necessary (Heinonen *et al.*, 1998).

These results are important to support all other appeals to the consumption of organic products. Organic producers must seek certification to ensure all requirements for organic production. The information of higher content of compounds with antioxidant activity the consumer may feel motivated to change eating habits to consuming these products.

4. Conclusions

In the present study the maturation stage influenced significantly in the contents of phenolics compounds, flavonoids and in the antioxidant activity of these compounds. In the stage of unripe maturation the fruits presented highest antioxidant activity and declined with the progress of the maturation in all the cultivation systems.

In the unripe organic fruits the antioxidant activity, the phenolics compounds and flavonoids were superiors significantly to the other fruits. In the biodynamics mangoes "instead of" and ripe, the superiority of the flavonoids seems to have a positive influences on their antioxidant activity, even with lowest contents of total phenolics compounds in relation to the organic mangoes.

The conventional fruits presented results inferior of phenolics compounds, flavonoids and lowest antioxidant activity in relation to the organic cultivations and biodynamics.

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