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Chocolate and red wine – A comparison between flavonoids content

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ABSTRACT

The flavonoids and polyphenols found in four different types of chocolate (white, milk, 40% cocoa dark and 71% cocoa dark) and four varieties of red wine (Pinot-Noir, Cabernet Sauvignon, Merlot and Tannat) were evaluated. The best results were found using 71% cocoa dark chocolate (D71) (flavonoids = 21.6 ± 2.4 μmol of catechin equivalents/g and polyphenols = 62.9 ± 0.1 μmol of catechin equivalents/g) and Tannat wine (flavonoids = 5.4 ± 0.1 μmol of catechin equivalents/ml and polyphenols = 14.3 ± 1.1 μmol of catechin equivalents/ml) which were statistically different against other varieties. Vanillin flavour was shown to interfere with colour development in the spectrophotometric analysis of the flavonoids. The results indicated that 49 g of D71 dark chocolate has the same quantity of flavonoids as that of 196 ml of Tannat wine, which is the daily wine intake recommended to produce health benefits in an adult of 70 kg body weight.

1. Introduction

Flavan-3-ols or flavonoids, such as catechin, epicatechin and procyanidins, contribute to the major antioxidant activity of red wines in the prevention of LDL cholesterol oxidation (Teissedre, Frankel, Waterhouse, Peleg, & German, 1996). The amount of flavonoids in red wine depends on the grape variety, cultivation area, sun exposure, wine-making technique and wine age (Auger et al., 2004; Burns et al., 2000; Dell'Agli, Buscialà & Bosisio, 2004; Frankel, Waterhouse, & Teissedre, 1995).

Cocoa has also been described as being a good source of flavonoids, such as catechins. In this context, dark chocolate with high content of cocoa has been recognised as an important alternative antioxidant in the diet (Serafini et al., 2003). Recently, Counet, Callemien, and Collin (2006) identified the presence of *trans*-resveratrol in cocoa and chocolate. This stilbene derivative, present in red wines, is postulated as one of the compounds responsible for the French Paradox. Based on this, the present study was carried out to compare flavonoids content of different types of red wine and chocolate and to suggest a daily chocolate ingestion dose that could be taken as a reference. The evaluation of polyphenols content was carried out to show the importance of flavonoids in this group of antioxidants.

2. Materials and methods

2.1. Chocolate samples

A dark chocolate with 71% of cocoa (D71) was developed at the R&D Department of Florestal Alimentos S/A – Chocolates Neugebauer exclusively for this project. In previous tests, no statistically significant difference was found ($p < 0.05$) when D71 flavonoids content were compared with dark chocolates with 72% and 86% of cocoa (Pimentel, 2007).

Four types of chocolate, kindly provided by Florestal Alimentos S/A – Chocolates Neugebauer (Porto Alegre, Brazil), were compared: white chocolate (WC), milk chocolate (MC), dark chocolate with 40% cocoa (D40) and dark chocolate with 71% cocoa (D71). Three different lots of each kind of chocolate were collected directly on the production line ($n = 3$). The samples were kept in a cool and dry place at temperatures between 18 and 22 °C in order to guarantee their quality.

2.2. Red wine samples

Four varieties of red wine were provided by a local wine-manufacturer (Bento Gonçalves, RS, Brazil): Pinot-Noir, Cabernet Sauvignon, Merlot and Tannat. From each variety samples of three different lots produced in 2004 were collected ($n = 3$).

2.3. Determination of flavonoids content

Flavonoids were evaluated according to Lee, Kim, Lee, and Lee (2003). Red wine samples were treated as recommended and the

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absorbance was measured using a Hitachi U1100 Spectrophotometer against a blank at 510 nm. Some modifications were done for chocolate samples as the original technique was developed for cocoa powder. In the modified technique, 7.3 g of ground samples were dissolved in 200 ml of distilled water at 100 °C and mixed until the fat had melted. The mixture was then centrifuged in a Hermle Z323K refrigerated centrifuge for 5 min at 4 °C and 8500 g. The defatted supernatant was analysed in triplicate.

A standard curve was developed using a 1000 μM solution of (−)-catechin (Sigma–Aldrich, Deisenhofen, Germany) at intervals of 200 μM (−)-catechin concentration.

2.4. Determination of polyphenol content

2.4.1. Chocolate preparation

The technique described by Vinson, Hao, Su, and Zubik (1998) was used for all chocolate samples (WC, MC, D40 and D71).

2.4.2. Red wine preparation

Wine samples were prepared according to Vinson and Hontz (1995). Sulphite was eliminated using the technique described by Vinson, Proch, and Bose (2001) prior to polyphenols quantification.

2.4.3. Determination of total polyphenols

Total polyphenols content determination was performed according Vinson, Proch, et al. (2001) using a Hitachi U1100 Spectrophotometer against a blank at 750 nm. A standard curve was developed using a 1000 μM solution of (−)-catechin (Sigma–Aldrich, Deisenhofen, Germany) at intervals of 200 μM (−)-catechin concentration.

2.5. Fat content

Fat content of chocolate samples were measured by Soxhlet Extraction Method according to AOAC (1990) Official Method 963.15.

2.6. Determination of vanillin interference

Vanillin (0.1 g/l) solution was diluted with ethanol/water (1:4,v/v) and was used to test the interference of this ingredient on determination of total polyphenols and determination of flavonoids content. A standard curve was produced at intervals of 0.02 g/l vanillin concentration.

2.7. Statistical analysis

Statistical significance between the samples was examined using one-way-ANOVA. When appropriate, multiple comparisons were made using Tukey–Kramer corrections. Differences were considered significant with $p < 0.05$.

3. Results and discussion

3.1. Chocolate

Flavonoids and total polyphenols content for the different types of chocolate studied are presented in Table 1.

Results showed that 34% of polyphenols in dark chocolate D71 and D40 are from flavonoids group. White chocolate had the lowest relation flavonoids/polyphenols showing that it is not a good source when flavonoids are of concern. The highest polyphenols quantity was found in D71 (dark chocolate with 71% cocoa) could be attributed to the amount of cocoa solids on its formulation. As described by Miller et al. (2006), polyphenols are hydrophilic and tend to be found in the non-fat fraction of cocoa and chocolate. This could explain the significant difference found between MC and D40. Although they have almost the same quantity of cocoa, D40 has a higher percentage of cocoa solids than MC. According to Miller et al. (2006) there is a strong relation between the content of non-fat cocoa solids (NFCS) and the polyphenols. Nevertheless, flavonoids and polyphenols were found in WC (white chocolate) which does not have cocoa solids in its formulation. The WC result shown in Table 1 is different from the one obtained by Mursu et al. (2004) that quantified antioxidants in white chocolate using an HPLC method, where they found 0.3 mg of gallocatechin/100 g of chocolate, but did not find significant amounts of catechin and procyanidins in their samples. One explanation for the flavonoids and polyphenols found in WC could be the interference in the quantification method of a chocolate ingredient.

The WC ingredients are sugar, cocoa butter, whole milk powder, skimmed milk powder, soybean lecithin emulsifier and vanillin flavour. Vanillin has a phenol ring in its structure that could contribute to colour development with reagents used to quantify some compounds like catechins (Sun, Ricardo-Da-Silva, & Spranger, 1998) and flavanols (Sarkar & Howarth, 1976). A 0.1% maximum concentration was chosen because this is the quantity generally used in chocolate formulation. The result showed no interference in the determination of total polyphenols but a linear correlation ($r^2 = 0.9597$) between the amount of flavonoids and vanillin concentration.

According to the manufacturer, WC samples had 0.05% of vanillin. This vanillin amount contributes with 0.01 nm of colour in flavonoids determination which is much lower than the resultant absorbance of WC suggesting that this result does not justify the colour developed during the analysis. It is important to observe that flavonoids content is in accordance with the results reported by Lee et al. (2003) that showed antioxidant capacity when no epicatechin was detected.

Sugar is another ingredient that could contribute to the development of colour. According to Vinson, Proch, et al. (2001), sugars do not interfere with the acidic Folin reaction at concentrations found in high sugar foods like chocolate but they might interfere in the alkaline Folin method in high concentrations. Based on this, solutions with 28%, 44%, 52% and 54% of refined sugar cane concentrations were prepared to evaluate the sugar interference in the

Table 1
Comparative between different chocolate types.

Samples	Flavonoids (μmol of catechin equivalents/g)	Polyphenols (μmol of catechin equivalents/g)	Flavonoids/polyphenols (%)
Dark chocolate 71% (D71)	21.6 ± 2.4^a	62.9 ± 0.1^a	34
Dark chocolate 40% (D40)	17.2 ± 1.3^b	49.9 ± 1.8^b	34
Milk chocolate 34% (MC)	8.4 ± 0.7^c	39.2 ± 3.8^c	21
White chocolate 30% (WC)	3.4 ± 1.3^d	28.3 ± 2.6^d	12

Values in same column followed by different letters were significantly different $p < 0.05$.

Table 2
Comparative between different red wine varieties.

Samples	Flavonoids (μmol of catechin equivalents/ml)	Polyphenols (μmol of catechin equivalents/ml)	Flavonoids/polyphenols (%)
Tannat	5.4 ± 0.1[a]	14.3 ± 1.1[a]	38
Cabernet Sauvignon	4.8 ± 0.1[b]	13.3 ± 0.7[a]	36
Merlot	4.6 ± 0.0[c]	11.6 ± 1.1[a]	40
Pinot-Noir	4.0 ± 0.1[c]	8.4 ± 1.2[b]	48

Values in same column followed by different letters were significantly different $p < 0.05$.

method. Such sugar concentrations were chosen due to their amount found in each type of chocolate. In all cases, no interference was found (WC sugar concentration $r^2 = 0.1315$).

Although Miller et al. (2006) described flavonoids as hydrophilic, Auger et al. (2004) reported that flavonoids are the most lipophilic among natural antioxidants. Therefore, it may be considered that WC results could be derived from cocoa butter antioxidants. Despite the fact that samples were defatted before the determination of flavonoids and polyphenols, the interaction of cocoa butter with other chocolate ingredients, during chocolate manufacture, may contribute to final antioxidant results in WC. Charlton et al. (2002) showed the affinity between peptides and polyphenols demonstrating that peptides can be coated with polyphenols with increasing concentrations of this component. According to Serafini et al. (2003), the formation of secondary bonds between chocolate flavonoids and milk proteins seems to be one of the reasons why milk can inhibit the *in vivo* antioxidant activity of chocolate. As WC is rich in milk components, this polyphenols and peptides binding during chocolate manufacture can help to explain the obtained results.

The results suggests that there is some interference and over estimation of the results, the amount of polyphenols found in MC and D71 were lower than those reported by Vinson, Proch, and Zubik (1999) which found an average of 52.2 μmol catechin/g for MC and 126.0 μmol catechin/g for dark chocolate with a high cocoa content.

3.2. Red wine

The amounts of flavonoids and polyphenols in red wine are shown in Table 2. Pinot-Noir had the highest relation of flavonoids/polyphenols which represents a bigger percentage of flavonoids in the total amount of polyphenols measured. However, the amount of flavonoids in Pinot-Noir was 59% lower than Tannat variety which presented a flavonoids content statistically different compared to other varieties.

Gonzaléz-Neves et al. (2004) studied Tannat, Cabernet Sauvignon (CS) and Merlot grapes phenolic potential and their correspondent wine, in Uruguay. In this research the Tannat variety presented the largest, and statistically different ($p < 0.05$), polyphenols amount in grapes and the polyphenols, catechins and procyanidins amounts in the red wines studied. CS presented a higher, but not statistically different ($p < 0.05$), level of antioxidants than Merlot. As pointed out by Meyer, Yi, Pearson, Waterhouse, and Frankel (1997) regional and climatic differences influence the amount of antioxidants in wine. The evaluated wines were produced in the Brazilian southern region which is geographically near Uruguay thus presenting some soil and climate similarities that confirm these convergent results. Based on the obtained results the Tannat variety harvested in 2004, was chosen as the wine to compare against the chocolate D71 for polyphenols and flavonoids content.

3.3. Dark chocolate and red wine

According to Vinson, Teufel, and Wu (2001), consumption of 196 ml of 13° GL red wine per day is enough to significantly reduce the atherosclerosis risk in a 70 kg adult. Tannat variety was taken as reference with regard to antioxidant compounds, to estimate the chocolate amount to be eaten in order to ingest the same flavonoids amount as one portion of this wine (Table 3). Flavonoids are a polyphenol subgroup, so these results were chosen for comparison because they represent a better approximation for the catechins quantity in the sample. Total polyphenols results could be overestimated as other compounds like phenolic acids, lignans and stilbenes could also be measured by the method.

3.4. Chocolate and nutrition facts

As it could be observed in Table 3, in order to ingest the same flavonoids amount present in 49 g of D71 chocolate a 70 kg adult should eat 126 g of MC, which represents a 250% increase in chocolate quantity. The Brazilian legislation (ANVISA) and the United States Food and Drug Administration (FDA) recommend a ingestion of 2000 kcal/day for a 70 kg adult (Brasil, 2006; FDA, 2007). This quantity of MC represents 35% of the daily required calories. Another interesting point observed is the small difference, in grams, between what should be ingested from D71 and D40 which suggests that both samples can be used as an antioxidant source in a diet. Those amounts represent 14% and 16% of the daily calorie requirement, respectively.

Regarding fat content, the ANVISA recommendation is a maximum total fat ingestion of 55 g/day. According to FDA this maximum total fat ingestion is 65 g/day (Brasil, 2006; FDA, 2007). Using the results obtained from fat content analysis (Table 3) and the Brazilian legislation (which is more rigid than the American one), D40 was the chocolate with the lowest contribution to a dietary fat: 30% of the daily value, while D71 contributes with 38% of daily value and MC with 74%. These results raise another important discussion about the chocolate contribution in weight increase. Although chocolate makes a significant contribution to total fat ingested per day, some studies have shown that dark chocolate ingestion contributes to reduction in LDL cholesterol without weight gain (Mursu et al., 2004). According to Kubow (1996), this could be attributed to the chemical composition of cocoa butter. The fat structure changes the fat absorption mechanism and, as a result, a neutral effect of cocoa butter on LDL cholesterol is observed.

The cocoa contribution to the total calorie content can be calculated. Cocoa and sugar are the ingredients with the highest contribution to total calorie count. Whereas in MC 47% of the daily calories is derived from cocoa butter, for D40 it represents 43% and for D71 58% of the calories. As already mentioned, cocoa lipid absorption is lower than other fats (Kubow, 1996), thus a formulation with a higher percentage of cocoa butter could be better in healthy aspects than a formulation with the same amount of other fat types. Moreover, in a balanced dark chocolate formulation with high cocoa quantities the sugar amount is reduced, which could make only a minor contribution to weight gain.

Table 3
Chocolate quantity (g) that should be ingested to equal the same flavonoids quantity present in 196 ml of Tannat wine and their fat content.

Sample	g	% Fat	g of fat
Dark chocolate 71% (D71)	49	42.1	20.6
Dark chocolate 40% (D40)	61	27.4	16.7
Milk chocolate (MC)	126	32.2	40.6

4. Conclusions

Different types of chocolate present statistically different results with regard to flavonoids and polyphenols concentration. The highest flavonoids and polyphenols content were found in chocolate sample D71, as expected. An unexpected result was that quantities of these antioxidants were found in white chocolate (WC) which does not have cocoa liquor in its formulation, suggesting that other chocolate ingredients could be interfering in the method. Tannat variety presented the highest flavonoids values among the red wines studied. Considering similar absorption, 49 g of chocolate D71 should be eaten to provide an equivalent flavonoids amount to 196 ml of Tannat wine, which has been shown to reduce heart attack risk for an average adult. D40 results were very similar to D71, also making this formulation an important antioxidant alternative. Further studies should be conducted in order to evaluate these compounds functionality *in vivo* and to observe if the same amount of antioxidants, from different sources, would be able to offer similar health benefits.

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