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Antioxidant Potential of Flours from Cereals, Tubers, Beans and Seeds Chemical Profile of *Curcuma longa* Flour

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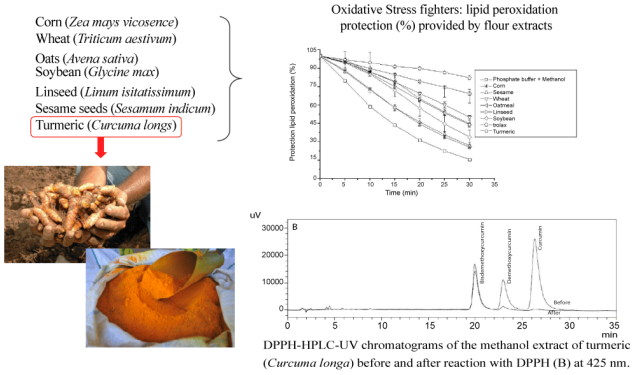
Abstract

Phenolic compounds have been reported to prevent diseases resulting from oxidative stress. Cereals, tubers, beans and seeds are sources of phenolic compounds and other bioactive compounds, so, the aim of this study was to evaluate the antioxidant capacity of methanol extracts of several edible flours. Determinations of the total phenols content and antioxidant capacity by RSA-DPPH•, CUPRAC, FRAP assays and lipid peroxidation inhibition tests were performed. Turmeric showed the best results with respect to antioxidant capacity across all methods used in this study. HPLC analysis revealed the presence of three phenolic compounds in the methanolic extract of turmeric: bisdemethoxycurcumin, demethoxycurcumin and curcumin. DPPH-HPLC was employed and showed that demethoxycurcumin and curcumin were the compounds with the highest antioxidant power toward DPPH•. In summary, all the flours had shown antioxidant capacity, with turmeric flour, the one with better performance. These flours can be used to balance the effects of oxidative stress, when introduced in the human and animal diets and aggregate value to the local production of these products.

Graphic Abstract



DPPH-HPLC-UV chromatograms of the methanol extract of turmeric (*Curcuma longa*) before and after reaction with DPPH (B) at 425 nm.

Keywords: Antioxidant capacity; *Curcuma longa*; *Zea mays* viçosence; *Sesamum indicum*; HPLC-DPPH; Curcuminoids

Introduction

Oxidative Stress is defined as an imbalance between oxidants, mainly reactive oxygen species (ROS) and reactive nitrogen species (RNS), and antioxidants, in favor of the oxidants, leading to a disruption of redox signaling and control, and / or molecular damage [1-5].

ROS are generally taken to encompass the initial species generated by oxygen reduction as well as their secondary reactive products, which include free radicals such as the superoxide anion $O^{2\bullet-}$, the hydroxyl ($HO^{\bullet}$), peroxyl ($RO^{2\bullet}$), alcoxyl ($RO^{\bullet}$) and hydroperoxyl ($HO^{2\bullet}$) ones, and non-radical species such as singlet oxygen ($^1O_2$), H_2O_2 and hydrochlorous acid (HOCl), among others. RNS include free radicals like nitric oxide ($NO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$), anions like peroxynitrite ($ONOO^-$), as well as non-radicals such as nitrous oxide (HNO_2), nitryl chloride (NO_2Cl) and alkyl peroxynitrites (ONOOR) [6]. ROS are important in regulating normal cellular processes, but deregulated ROS contribute to the development of various human diseases, including cancer, diabetes, cardiovascular disease, neurodegenerative and gastrointestinal diseases and more [1,4,5,7-12].

An intricate exogenous and endogenous antioxidant defense system contributes to the fight against the destructive effects of reactive

oxygen and nitrogen species (RONS). These compounds are classified as enzymatic and non-enzymatic. Among the enzymatic compounds are SODs, catalase and GSH-Px, although heme oxygenase-1 and redox proteins (thioredoxin, peroxiredoxins and glutaredoxins) can also be cited [13,14]. The non-enzymatic antioxidants include low molecular weight compounds such as vitamins (vitamins C and E), β-carotene, uric acid and GSH which is a tripeptide (L-γ-glutamyl-L-cysteinyl-L-glycine) that contains a thiol group (sulfhydryl) [5,10,13,14]. Among the non-enzymatic compounds, plant polyphenols, mainly flavonoids, have played important roles [15]. They have been implicated in diverse functional roles, including plant resistance against microbial pathogens and animal herbivores such as insects (antibiotic and antifeeding actions), in protection against solar radiation, reproduction, nutrition, and growth, along with anti-lipid peroxidation, anti-tumor, anti-platelet, anti-ischemic, anti-allergic, anti-inflammatory, and anti-bacterial actions [5,11,16]. Phenolic compounds have also been reported to prevent diseases resulting from oxidative stress by a variety of mechanisms [15,17].

Cereal grains, seeds, legumes and tubers are good sources of phenolic compounds and other bioactive compounds [18-20]. One of the more cultivated cereals worldwide is corn (*Zea mays*) [21]. It is a grain rich in phenolic acids, with a predominance of ferulic acid (90%) [18]. Wheat (*Triticum aestivum*) is a cereal of major human consumption. Its antioxidant activity is more related to wheat sprouts, which contain a very high level of organic phosphates and a potent blend of antioxidant compounds such as enzymes, reducing glycosides and polyphenols that have shown a remarkable reduction capacity against reactive oxygen species [22,23]. Another cereal that stands out regards human consumption is oats (*Avena sativa*). It is often used like flour due to the high content of soluble dietary fiber. Research on this grain has reported the presence of antioxidant compounds on the outside of the grain, such as tocopherols, phytic acid, sterols and phenolic compounds [24,25].

Other foods highlighted in recent years are seeds such as linseed (*Linum usitatissimum* L.) and sesame (*Sesamum indicum*). Linseed is the seed of the flax plant, and used as a raw material for producing oil and bran. It has been of special interest for being rich in essential fatty acids ω-3 and ω-6, but also contains chemical constituents like fiber and phenolic compounds [26,27]. Sesame has significant relevance in the production of oils. These oils are characterized by their resistance to oxidative rancidity, attributed to the presence of secondary metabolites that exhibit antioxidant activity, such as α-tocopherol and lignans [28,29].

Of the legumes, soybean (*Glycine max*) stands out for possessing bioactive compounds such as isoflavones, chlorogenic acid, caffeic acid, ferulic acid, tannins and proanthocyanidins that can be associated with reduced risk of cancer with a hormonal basis such as breast and prostate cancer, as well as cardiovascular disease [30,31].

Turmeric (*Curcuma longa*) is a tuber widely used as a spice in food preparations for flavor and color, and has shown beneficial effects on health, mainly for its antioxidant properties [32,33]. These activities have been attributed to phenolic compounds present in the turmeric rhizomes, known as curcuminoids, these being curcumin, demethoxycurcumin and bisdemethoxycurcumin [34]. Many studies have demonstrated antioxidant, anti-inflammatory and antiviral activity for *Curcuma longa* [35–38], however, up to our knowledge; studies on inhibition of lipid peroxidation and DPPH•-HPLC of its flour were not reported in the literature.

As such, the aim of this study was to evaluate the antioxidant capacity of flours obtained from corn (*Zea mays viçosence*), wheat (*Triticum aestivum*), oats (*Avena sativa*), soybean (*Glycine max*), linseed (*Linum isitatissimum*), sesame seeds (*Sesamum indicum*) and turmeric (*Curcuma longa*), as a potential characteristic to fight against reactive species.

Materials and Methods

Standards and reagents

Soy phosphatidylcholine, DPPH• (α,α-diphenyl-β-picrylhydrazyl radical), Folin-Ciocalteu reagent, ammonium acetate, trolox®, TPTZ (2,4,6-tripyridyl-s-triazine), hydrochloric acid, ferric chloride, copper chloride, neocuproine, AAPH (2,2′-azobis (2-amidinopropane) dihydrochloride), acetonitrile, curcumin, demethoxycurcumin and bisdemethoxycurcumin were purchased from Sigma Aldrich (Steinheim, Germany). Acetic acid and gallic acid (GA) were supplied by Vetec Química Fina Ltda (Rio de Janeiro, Brazil). Sodium acetate was procured from CRQ (Cromato Produtos Químicos Ltda). Anhydrous sodium carbonate was supplied by Reagen Químibras Indústrias Químicas S.A (Brazil). Methanol and ethanol were supplied by Dinâmica Química Contemporânea Ltda. Monobasic sodium phosphate, dibasic sodium phosphate, EDTA (ethylenediaminetetraacetic acid) were from Acros Organics (Brazil) and the fluorescent fatty acid analogue 4,4′-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-S-indacene-3-undecanoic acid (C11-BODIPY581/591) was purchased from Molecular Probes (Ontario, Canada). All the reagents were of analytical grade and the stock solutions and buffers were prepared with Milli-Q purified water.

Sample preparation and extraction

The samples sesame (*Sesamum indicum*), linseed (*Linum isitatissimum*), oat (*Avena sativa*) and wheat (*Triticum aestivum*) were purchased from local stores of Maceió (Casa de Produtos Naturais – Erva Doce e Doce Ervas); samples of soybean (Glycine max) from Luiz Eduardo Magalhães-BA; samples of turmeric powder (*Curcuma longa*) were purchased from the Cooperaçafrão located in Mara Rosa-GO; and samples of corn (*Zea mays viçosence*) were purchased from the Setor de Melhoramento Genético de Plantas (SMGP), located in Rio Largo-AL. The samples were dried in an air circulation oven at 60°C for 48 h, except turmeric that had already been purchased in powder form. All samples were ground on a blade mill and then sieved (sieve particle size - 35 ABNT). For soybean, linseed, oat and corn samples, the seed coat, pericarp, germ and endosperm were used to obtain the flour, while for wheat and sesame samples, only the germ was used. The flour obtained was placed in amber glass and stored under refrigeration.

The crude extracts were then prepared from the flours. Extracts were prepared using methanol, in a Soxhlet apparatus heated at 60°C, until the extracts became completely colorless. The solvents were eliminated using a rotary evaporator at 50°C, the extract residues stored in nitrogen and the extracts stored in amber glass under refrigeration (5°C).

Determination of the total phenolic content

Total phenolic content (TPC) of the methanolic extracts obtained from the flours was determined using Folin-Ciocalteu reagent, as described by Cicco et al. [39], with minor modifications. The extracts

were dissolved in methanol (375 mg L^{-1}), and were added to test tubes, as described: 0.12 mL of the extracts' solutions (15 mg L^{-1}), 0.3 mL of the Folin-Ciocalteu reagent (0.2 M) and 2.4 mL of sodium carbonate 5% solution (40 g L^{-1}), to obtain a final concentration of 15 mg L^{-1} and then added to test tubes. The mixture was shaken and heated at 40°C in a water bath for 20 min. The tubes were then cooled rapidly and the developed color was read at 767 nm in a UV-Vis spectrophotometer (model Mutispec-1501, Shimadzu, Japan). The concentration of phenolic compounds was estimated using a calibration curve traced with gallic acid (GA) in methanol (10^{-4} mol L^{-1} to 10^{-3} mol L^{-1}) as a polyphenol reference, in triplicate. The results are expressed as mg of GA equivalents/g of extract (mg GAE g^{-1}).

Radical scavenging activity of the α,α-diphenyl-β-picrylhydrazyl radical (RSA-DPPH•)

The antioxidant capacity of flour samples were measured in terms of their radical-scavenging ability (RSA), using the DPPH• method [40], with minor modifications. The extract (150 mg L^{-1}) and DPPH• (0.04 mg L^{-1}) were dissolved in methanol. Each methanol extract (0.30 mL, 15 mg L^{-1}) was mixed with 2.7 mL of DPPH• solution (32 μg mL^{-1}) to give a final sample concentration of approximately 15 mg L^{-1}. The mixture was homogenized and stored in the dark, prior to analysis. Spectroscopic evaluation of antioxidant activity was performed at 767 nm in a UV-Vis spectrophotometer (model Mutispec-1501, Shimadzu, Japan).

The percentage of DPPH radical-scavenging activity (RSA%-DPPH•) of each sample was calculated as follows:

$$\% RSA = (1 - AC / AD) \times 100$$

Where AC is the absorbance of the solution when the extract was added at a particular concentration after 30 min, and AD is the absorbance of the DPPH• solution [41], all the analyses were performed in triplicate.

The IC_{50} (half maximal inhibitory concentration) or I% (percentage of inhibition) was calculated graphically using a calibration curve in the linear range by plotting the extract concentration vs. the corresponding scavenging effect, according to Oliveira et al. [42], using the following equation:

$$I\% = [(Ab_{s0} - Ab_{s1}) / A_0] \times 100$$

Where Ab_{s0} is the absorbance of the control and Abs1 the absorbance in the presence of the test compound.

FRAP assay

The FRAP assay was performed according to the method described by Rufino et al. [43], which is based on the reduction of a ferric tripyridyl triazine complex to its dark blue ferrous form, in the absence and presence of antioxidants.

In brief, the FRAP reagent is prepared by mixing 2.5 mL of a solution of TPTZ (10 mmol L^{-1} in 40 mmol L^{-1} HCl), 2.5 mL of $FeCl_3$ (20 mmol L^{-1} in deionized water) and 25 mL of acetate buffer (0.30 mol L^{-1}, pH 3.6). The extracts were dissolved in methanol (510 mg L^{-1}). Each methanol solution (15 mg L^{-1}) (0.09 mL) was mixed with 0.27 mL of deionized water and 2.7 mL of FRAP reagent and incubated at 37°C for 30 min, resulting in a final concentration of 15 mg mL^{-1} of extract. The absorbance of the reaction mixture was measured at 595 nm and a calibration curve was prepared with trolox® (0.15 to 30 μmol L^{-1}). The results are expressed as Trolox Equivalent Antioxidant Capacities ($TEAC_{FRAP}$), in μmol of Trolox g^{-1} of dry extract.

CUPRAC (copper reducing antioxidant capacity) assay

The CUPRAC assay was performed according to the method described by Ozyurek et al. [44], which are based on the reduction of Cu (II) to Cu (I) by the combined action of all the antioxidants (reducing agents) in a sample. One milliliter each of copper chloride ($CuCl_2$) (1×10^{-2} mol L^{-1}), neocuproine (7.5×10^{-3} mol L^{-1}) and ammonium acetate buffer solutions (NH_4Ac) (1.0 mol L^{-1}, pH = 7.0) were placed in a test tube. Then, 0.5 mL of the extract solution (15 mg L^{-1}) and 0.6 mL of H_2O Milli-Q were added to the initial mixture to reach a final volume of 4.1 mL. The tubes were stoppered, and after 1 h, the absorbance at 450 nm was recorded against a blank reagent. The extract solutions were prepared in methanol at a concentration of 50 μg mL^{-1}. The calibration curve was prepared with Trolox (0.6 to 73 μmol L^{-1}). The results are expressed as the total antioxidant capacity in μmol of Trolox g^{-1} of extract.

Lipid Peroxidation Measurements

Preparation of unilamellar vesicles

Unilamellar vesicles of soy phosphatidylcholine (1 mM) were prepared by extrusion (100 nm pore diameter membrane, at 25°C) in 10 mL of phosphate buffer (50 mM, pH 7.4) with the additional incorporation of 0.1 μM of the peroxyl-sensitive fluorescent probe C11-BODIPY581 / 591 as described by Oliveira et al. [42]. The particle size was confirmed at around 100 nm by Nanotrac-Zetatrac; model NPA151-31A-0000-D30-10M.

Lipid peroxidation measurements

Fluorescence measurements were carried out at 37°C using a RF-5301PC spectrofluorophotometer (Shimadzu, Japan). In a 1 mL-quartz cuvette, 0.2 mL of unilamellar vesicle suspension, 0.6 mL of phosphate buffer pH 7.4, and 0.1 mL of the extract in methanol (15 mg L^{-1}) or positive controls (Trolox - 10 μM mL^{-1}) were mixed. Methanol and phosphate buffer were used as negative controls. The reaction was initiated with the addition of 0.1 mL of AAPH (100 mM). The fluorescence decay (λ_{exc} = 580 nm, λ_{em} = 600 nm) was continuously monitored during 30 min.

Identification and quantification of compounds by HPLC

The identification and quantification of phenolic compounds were carried out for the methanol extract of *Curcuma longa*. The HPLC system was a Shimadzu (VP series, Kyoto, Japan) system controller (CBM-20A), pump (LC-20AT vp), a column oven (CTO-20A/C), Shimadzu VP-ODS c18 column (250 L × 4.6 mm), UV/VIS detector (SPD-M20A) and computer software (LC-solution).

The chromatographic conditions were adapted from Malasoni et al. [45]. A reverse-phase HPLC assay was carried out using an isocratic system with a flow rate of 1.5 mL min^{-1}, a column temperature of 33°C, a mobile phase of acetonitrile, water and acetic acid (40:60:1, v/v/v), and a detection wavelength of 425 nm. The injection volume was 20 μL. The extracts were dissolved in acetonitrile (1 mg mL^{-1}) and filtered through a 0.45 μm nylon membrane prior to HPLC injection. The total chromatographic analysis time was 35 min per sample.

For the quantification of the compounds, stock solutions of curcumin, demethoxycurcumin and bisdemethoxycurcumin in acetonitrile were prepared separately at a concentration of 500 µg/mL. Standard solutions were prepared by diluting the stock solutions with acetonitrile to obtain concentrations of 15.62, 31.25, 62.5 and 125 µg mL^{-1} of curcumin, demethoxycurcumin and bisdemethoxycurcumin, separately, to obtain the calibration curve.

DPPH•-HPLC experiment

In brief, 25 µL of the diluted sample (4 mg/mL) was mixed with 80 µL of DPPH• solution (0.4 mg mL^{-1}) and then made up with methanol to a final volume of 250 µL. The mixture was incubated, at 37°C, for 30 min, and then passed through a 0.45 µm filter and subjected to HPLC analysis. The chromatographic conditions used were the same as described in the previous section. The peaks related to compounds with higher RSA activity will be reduced or disappear after the reaction.

Statistical analysis

The results correspond to the average of three replications and were expressed as mean ± SD (standard deviation). The data were analyzed by analysis of variance (ANOVA), followed by Tukey's test to detect significant differences between treatments. Statistical analysis was performed using SAEG 9.1 (System for Statistical Analysis, MG, and Brazil). The Pearson's correlation test was used to determine the correlations between the means. A probability value of $p < 0.05$ was considered statistically significant.

Results and Discussion

Determination of total phenolic content (TPC) and antioxidant capacity

Table 1 displays the values obtained by the Folin-Ciocalteu method and the antioxidant capacity methods for methanol extracts of the different samples. For the total phenolic content, the turmeric extract had the highest value, 119 mg GAE/g of dry extract, indicating a higher phenolic content compared to the other flours, which ranged between 31 mg GAE/g to 53 mg GAE/g of dry extract, with the linseed sample showing the lowest value. Wheat, corn and sesame flour showed statistically similar values at $p \leq 0.05$, of around 53, 49 and 42 mg GAE/g of dry extract, respectively, displaying the next best results after the turmeric extract. Samples of soybean and oats showed statistically similar values to corn and sesame flours.

Methanol extracts	TPC (mg GAE[1] g^{-1} dry extract)	DPPH• (RSA% – DPPH•[2])	IC50 DPPH• (µg/mL)	FRAP TEACFRAP3 (µ molTrolox g^{-1})	CUPRAC TEACCuprac3 (µ molTrolox g^{-1})
Turmeric	119 ± 2^{a}	31.5 ± 0.8^{a}	33.5	945 ± 60^{a}	1738 ± 51^{a}
Wheat	53 ± 6^{b}	10.7 ± 4.1^{b}	-	126 ± 6^{b}	856 ± 67^{b}
Corn	49 ± 2^{b}	13.5 ± 0.5^{b}	-	99 ± 1^{bc}	583 ± 44^{cd}
Sesame	42 ± 3^{bc}	10.6 ± 0.1^{b}	-	153 ± 14^{b}	481 ± 14^{d}
Soybean	37 ± 4^{cd}	12.7 ± 2.0^{b}	-	102 ± 6^{bc}	597 ± 14^{cd}
Oatmeal	35 ± 4^{cd}	2.3 ± 1.4^{c}	-	83 ± 2^{bc}	652 ± 49^{c}
Linseed	31 ± 3^{d}	5.7 ± 0.7^{c}	-	43 ± 1^{c}	325 ± 74^{e}

Table 1: TPC, DPPH• (RSA% and IC50), FRAP and CUPRAC for methanol extracts of flours, [1]Gallic acid equivalents. [2]Percentage of DPPH radical-scavenging activity after 30 min. [3]TEAC: Trolox Equivalent Antioxidant Capacity (mmol TE / g dry extract). Mean values followed by the same letters in each column do not differ significantly at $p \leq 0.05$ by the Tukey test.

Hirun et al. [46] studying methanolic extracts of *Curcuma longa* under different drying conditions, found values between 24.72 mg and 113.10 mg GAE g^{-1} to total phenolic content using the Folin-Ciocalteu method, where the highest value found was similar to that found in this study for the methanol extract of *Curcuma longa* at 119 mg GAE g^{-1} of dry extract.

Cereals such as wheat, corn and oatmeal showed levels of phenols, 53, 49 and 35 mg GAE / g of dry extract, respectively. Perez-Jimenez and Saura-Calixto [47] investigated the antioxidant capacity of aqueous-organic extracts (acidic mixture of methanol / water 50:50 v/v, pH 2) of cereals. They found values between 0.21 mg and 3.44 mg GAE g^{-1} of extract, with wheat flour showing the highest value, while the oat flour extract showed a value of 1.95 mg GAE g^{-1}, values well below the values obtained for the methanol extracts of cereals, corn and wheat and oatmeal samples, for the current study.

For the DPPH• method, which measures the radical scavenging ability of the samples, turmeric reached values of around 31.5% at the studied concentration (final concentration of 15 mg L^{-1}), followed by the extracts of corn, soy, wheat and sesame flour, which had values of 13.5, 12.7, 10.6 and 10.7%, respectively, while the extracts of linseed and oat flour had the lowest percentages at 5.7 and 1.6%, respectively. The calculation for IC_{50} in relation to the inhibitory capacity against DPPH• indicates the sample concentration required to reduce the radical by 50%. In Table 1, it can be observed that with the exception of turmeric, no flour was able to reduce the DPPH radical by 50%. In turn, turmeric showed an IC_{50} value of approximately 33.5 µg mL^{-1}. Hirun et al. [46], in addition to verifying the total phenolic content of methanol extracts of dry *Curcuma longa* flour under different vacuum conditions, also verified the IC_{50} value using the DPPH• method, with values varying between 23.41 µg mL^{-1} and 59.56 µg mL^{-1}, which is consistent with this study considering that the methanol extract of *Curcuma longa* was the only sample that reached the IC_{50} at a concentration of 33.5 µg mL^{-1}. For scavenging capacity of radicals by the DPPH• method, many studies with similar samples did not indicate the IC_{50} values, and the values that were shown for the method were generally for different concentrations than the data reported in this study, hampering comparisons.

Regards the values for antioxidant capacity by FRAP and CUPRAC methods (Table 1), the turmeric sample stands out, showing a value of 945 µmol and 1738 µmol TEAC g^{-1} of dry extract for FRAP and CUPRAC, respectively, being much higher values than observed for the other samples, which ranged between 43 µmol and 153 µmol TEAC g^{-1} of dry extract for FRAP, and 325 µmol - 856 µmol TEAC g^{-1} of dry extract for CUPRAC. Data for FRAP and CUPRAC methods are less common in the literature for many of the samples analyzed in the present study and comparisons with values from some studies became complicated by way of the results being expressed differently, without using a calibration curve. Wojdyło et al. [48] verified the antioxidant activity of various plants including *Curcuma longa* using the FRAP method where values ranged between 13.8 µmol and 2133 µmol TE

100 g^{-1} of dry extract, with *Curcuma longa* showing a value of 62.6 µmol TE 100 g^{-1} of dry extract, a value much below the present value (945 µmol TE g^{-1}) of dry extract. Tufan et al. [49] studied the antioxidant activity of different cereals, including wheat and oats, using the CUPRAC method, and found values well below the results of this study. For wheat, the values were 4.31, 2.18 and 3.25 and for oats 7.51, 1.44 and 3.83 µmol TE g^{-1} of dry extract-for aqueous, ethanol and methanol extracts, respectively, while for the present study the values ranged from 325 µmol to 1738 µmol TE g^{-1} of dry methanol extracts.

Figure 1 shows lipid peroxidation protection (%) versus time using a negative control, liposome plus C11-BODIPY581/591, AAPH and phosphate buffer containing methanol. Trolox was used as the positive control to compare the extracts obtained from the flours. The negative control showed a sharp decay as a function of time, indicating that there was a radical attack on the liposomes. With the addition of trolox as the positive control, the decay was minimal, approaching 30 min with 82% protection. The methanol extracts of corn and sesame flours showed low protection at the end of 30 min, 25% and 26% respectively, however, up to 10 min, they showed a protection value above 70%. The best results were found for wheat, oats, linseed and soybean flour, which maintained a protection above 70% for up to 15 min, and after 30 min of monitoring the extracts showed a 50, 44, 43 and 33% protection, respectively. Turmeric was the sample with the highest percentage of protection against lipid peroxidation, above 69% for up to 30 min of monitoring.

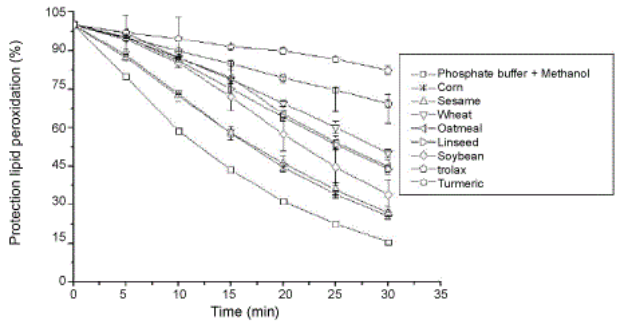


Figure 1: Lipid peroxidation protection (%) provided by flour extracts, a positive control (trolox) and a negative control (phosphate buffer and methanol). PB (phosphate buffer), liposome plus C11-BODIPY581 / 591 were added in all cases.

Oliveira et al. [42], also applied the method of lipid peroxidation inhibition for acerola, passion fruit and pineapple residue extracts, and found a greater protection value for the acerola and passion fruit extracts and a steeper decay of protection for the pineapple extract. However, the pineapple extract showed a protective value of about 80% for up to 15 min of monitoring, thereby resembling the applied positive control (trolox) and the results of this study where wheat, oats, linseed, soybean and turmeric flours showed protection above 70% up to 15 min.

Although the results for total phenolic content, DPPH, FRAP and CUPRAC assays indicated better values for the methanol extract of turmeric, the results for the other flours deserve attention, with regards to the results of lipid peroxidation protection [50], as seen by the result that for up to 10 min of monitoring, they all showed a protection value greater than 70%.

Intergroup comparisons of the results were made considering the highest activity as 100%, in each experiment (each column), in order to normalize the data (Figure 2). The proportions are shown as a percentage for each extract studied. From these results, it is possible to make an intergroup observation. Among all the extracts studied, turmeric stands out for most of the methods, in relation to the other samples.

As turmeric flour had shown the best results with respect to antioxidant capacity across all methods used in this study, for this reason, identification and quantification of the phenolic compounds using HPLC and DPPH•-HPLC analysis was carried out for the methanol extract of turmeric.

Identification and quantification of the phenolic compounds of turmeric (*Curcuma longa*) using HPLC and antioxidant screening by DPPH-HPLC analysis Figure 3A shows chromatograms for the methanol extract of *Curcuma longa*. Three compounds were identified in the extract: bisdemethoxycurcumin, demethoxycurcumin and curcumin, being found at retention times of 20, 23 and 27 min under the conditions studied. The chromatographic conditions used promoted good separation of compounds, which allowed the quantification of the compounds.

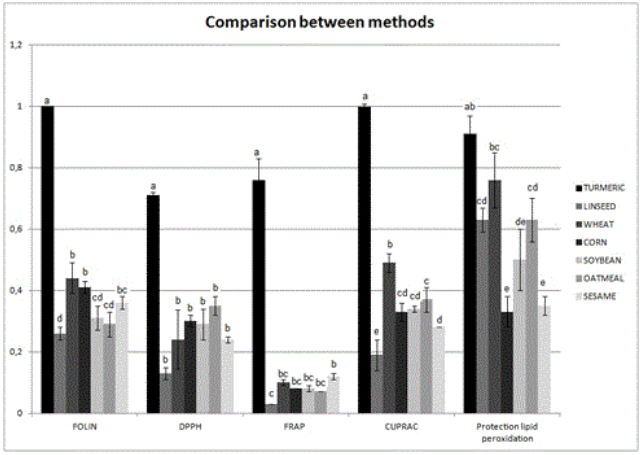


Figure 2: Normalized activities of the extracts in relation to each antioxidant test. The highest activity was considered 100% and the other values correspond to a relative percent of activity. Within each column, averages denoted with the same letter were not significantly different by this test ($p < 0.05$).

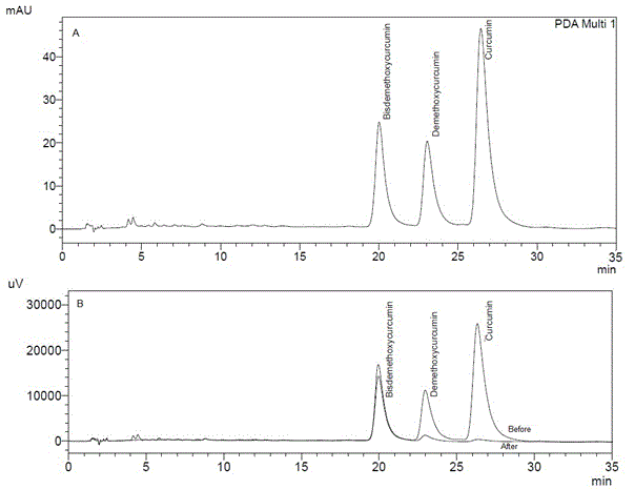


Figure 3: HPLC-UV chromatogram of the methanol extract of turmeric (*Curcuma longa*) (A) and DPPH-HPLC-UV chromatograms of the methanol extract of turmeric (*Curcuma longa*) before and after reaction with DPPH (B) at 425 nm.

Quantification was made, using a calibration curve for each compound to yield the linear regression equation for each curcuminoid and their respective coefficients for determining the peak areas (Table 2). The concentrations varied for the three compounds; there was a greater concentration of curcumin in relation to the other compounds, showing a concentration of 48.43 mg g^{-1} dry extract, whereas bisdemethoxycurcumin and demethoxycurcumin had concentrations of 20.17 mg and 15.42 mg g^{-1} dry extract, respectively.

Compounds	Linear regression equation for peak area	R^2	Concentration of the sample (mg g^{-1} dry extract)
Bis-demethoxycurcumin	y = 97776 × -508 278	0.9994	20.17
Demethoxycurcumin	y = 98196 × -378 701	0.9999	15.42
Curcumin	y = 105570 × -2E +06	0.9989	48.43

Table 2: HPLC analysis of the methanol extract of turmeric (*Curcuma longa*).

The turmeric extract showed varying concentrations for the three compounds, but a higher concentration of curcumin in relation to other compounds. Karioti et al. [51] analyzed *Curcuma longa* compounds extracted with hydro-alcoholic solutions (60%, v/v): the dried plant material was macerated for a minimum period of 21 days, obtaining a tincture. Similarly to the present study, were identified and quantified the same three curcuminoids: bisdemethoxycurcumin, demethoxycurcumin and curcumin, showing retention times of 27.9, 28.8 and 29.8 min, respectively, under the conditions used by the authors. The authors also show different concentrations for the three curcuminoids and a higher concentration of curcumin. Concentrations of curcuminoids for the current study were higher compared to the study by Karioti et al. [51], where the authors found concentrations of 0.38, 0.27 and 0.46 mg/ml of bisdemethoxycurcumin, demethoxycurcumin and curcumin, respectively, in the hydro-alcoholic extract.

The HPLC-UV chromatogram of the methanol extract of *Curcuma longa* before and after reaction with DPPH• at 425 nm is shown in Figure 3 (B). The chromatogram showed decrease in absorbance values of two compounds after reaction with the DPPH•. The highest reductions were for compounds demethoxycurcumin and curcumin, respectively. The curcumin decreased by 99% of its area in relation to the chromatogram before the reaction and the demethoxycurcumin decreased by 90% of its area in relation to the chromatogram before the reaction, indicating which are the compounds that act as better antioxidants in further reaction with DPPH•. In turn, the bisdemethoxycurcumin was the least altered, with a reduced area of only 13%, indicating a much lower antioxidant activity, in relation to the two other curcuminoids.

Conclusion

The present work has shown that the studied flours have good total phenolic content and antioxidant capacities, being the turmeric flour the one with better performance in the majority of the methods. All flours protected against lipid peroxidation about 70% for up to 10 min of monitoring and the turmeric was the sample with the highest percentage of protection, above 69% until 30 min. So, the turmeric (*Curcuma longa*) proved to have the highest antioxidant potential. The quantification of the phenolic compounds in turmeric, using HPLC-UV analysis, shows the higher amount of curcumin in relation to other curcuminoids. DPPH-HPLC analysis revealed that demethoxycurcumin and curcumin reacted strongly with DPPH. Combined results suggest that these flours can be used to balance the effects of oxidative stress, when introduced in the human and animal diets and aggregate value to the local production of these products. This work provides relevant information and reinforces, once more, the interest for more research on the mechanisms of action and signaling pathways that are involved in antioxidant and anti-inflammatory properties of *Curcuma longa*.

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