New Cooper-Cystein Complexes: Synthesis, Toxicity and Antioxidant Analysis for Soybean Oil

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ABSTRACT: *Four compounds derived from cysteine were synthetized and their chemical structures were confirmed by spectroscopic analyses. All the compounds were evaluated for antioxidant activity and toxicity compared to a commercial antioxidant. The antioxidant capacity was measured based on the DPPH assay and the peroxide value. The (S)-4-methylbenzyl cysteine (mebz-cys,* ***3a****) and (S)-4-chlorobenzyl cysteine (clbz-cys,* ***3b****) not present antioxidant activity in DPPH assay, whereas the complexes [Cu(mebz-cys)]Cl_2 (****4a****) and [Cu(clbz-cys)]Cl_2 (****4b****) confirmed their potential. All the compounds obtained satisfactory results regarding the evaluation of the antioxidative potential through the oxidative stability of the soybean oil. The toxicity was analyzed based on the Hemolysis test. The compounds* ***3a****,* ***3b****,* ***4a*** *and* ***4b*** *showed H_{50} values of 467.3, 470.0, 127,0 and 120.0 µg/mL, respectively. The BHT compound, on the other hand, presented H_{50} at the concentration of 58.5 µg/mL.The synthetized compounds showed a good antioxidant activity and presented lower toxicity.*

KEYWORDS -*Antioxidant activity, Copper complexes, Cystein derivatives, soylbean oil, Toxicity analyses*

I. Introduction

The vegetable oils are of great importance to the human diet because they are rich in a variety of nutrients such as liposolubles vitamins and essential fatty acids. Derivatives of fatty acids act in the control of physiological factors such as blood pressure and cholesterol levels; they also are precursors of prostaglandins, leukotrienes and thromboxanes. During the heating process, the oils are continuously exposed to high temperature and oxygen, causing a great diversity of chemical reactions such as hydrolysis, oxidation and polymerization of the triacylglycerols, leading to the formation of free radicals and various substances harmful to the health of the human beings [1].

Lipid oxidation is responsible for the development of unpleasant flavors and odors, making food unfit for consumption, as well as causing other changes that will affect not only nutritional quality, due to the degradation of fat-soluble vitamins and essential fatty acids, but also the food safety and health through the formation of potentially toxic compounds [2,3]. The use of antioxidants is one of the simplest ways to reduce or minimize these effects. However, some studies have shown that the consumption of synthetic antioxidants used in food has a toxic and carcinogenic effect on the human body [4]. Therefore, there is a great interest in the use of antioxidants derived from natural sources because they are, in general, more secure since they are found in vegetables and fruits.

Antioxidants reduce the risk of diseases by decreasing the concentration of free radicals. Garlic and its products contain powerful antioxidant constituents, as sulfur and phenolic compounds. Garlic also has several other beneficial effects, acting on the immune and cardiovascular system, besides possessing antimicrobial, antibacterial, antifungal and anticarcinogenic properties [5].

Garlic contains high levels of phosphorus, potassium, sulfur compounds and zinc, moderate levels of selenium, vitamin A and C. Some investigations suggest that garlic presents several biological and medicinal functions mainly due to the presence of high amount of organosulfur

compounds. Its primary constituents are *S*-alkenyl-L-cysteine sulfoxide (Fig. 1), such as Alliin (sulfoxide 2-propenyl cysteine), Isoalliin (sulfoxide 1-propenyl cysteine) and Methiin (sulfoxide methyl cysteine) [6].

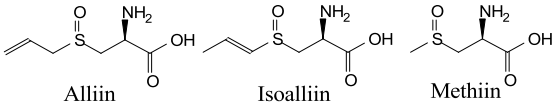


Figure 1. Primary constituents of garlic (*S*-alkenyl-L-cysteine-sulfoxide).

The study of metal complexes has been increased in research areas due to the fact that the transition metals have biological activity, including antiparasitic and antitumor action. In some cases, the activity of the ligand is strengthened when complexed with metals that have some biological activity, such as platinum, tin, gold, palladium, antimony, silver, mercury, gallium, zinc, nickel and copper [7,8,9]. The aminoacids are known to exert a human antioxidant effect. The cysteine is considered one of the most important aminoacids, the knowledge of its chemical and physical properties is necessary to understand its participation in biochemical processes [10]. Due to the formation of disulfide bonds between their thiol groups, cysteines increase molecular stability and resistance to proteolysis, playing a key role in maintaining the tertiary structure of proteins. Cysteine can be found in large quantities in foods such as amaranth, dairy products, broccoli, peppers, meat, fish, soy, garlic, onion, whole grains. Studies of metal complexes with cysteine report, besides antioxidant activity, antibacterial effects [11,12], antifungals [11] and even antidepressants [13].

Copper is an essential metal found in a wide variety of plants, organisms and microorganisms and plays important roles in the biological system. It is the third transition metal or metal-trace most abundant in humans after iron and zinc. It is distributed practically throughout the organism, but in different concentrations, which indicates its various functional roles [14].

This work aims to investigate the antioxidant potential and toxicity of copper complexes with cysteine derivatives ligands containing aromatic groups, as well as to compare their activities against the commercial antioxidant Butylated Hydroxytoluene (BHT).

II. Materials and methods

2.1. Material and techniques

Most of the reagents,2,2-diphenyl-1-picrylhydrazyl (DPPH), cysteine, 4-chlorobenzyl chloride, 4-methylbenzyl chloride, hydrochloric acid, copper chloride dihidrate, Butylated Hydroxytoluene (BHT)and acetic acid were purchasedfrom Sigma-Aldrich Co. (Steinheim, Germany).Sheep red blood cells wereobteined form Kalifarma (São Mateus, Brazil).

Elemental analyses using a Perkin–Elmer 2400 CNH Elemental Analyzer was performed at the *Central Analítica* of Universidade de São Paulo. IR spectra were recorded in AgilentCary 630 FTIR instrument, in the range 4000–400 cm^{-1}, while UV/Vis spectra were recorded on a double beam Q898UVDB equipment from Quimis of our Institution.The ^{1}H and ^{13}C NMR experiments were performed on aNMR spectrometer (Varian VNMRS 11.75 Tesla) at 500 and 125 MHz, respectively, using DMSO-d_6 as solvent at LaboratórioMultiusuário de RMN of Universidade Federal Fluminense.

2.2. Synthesis of compounds

The synthesis of two new complexes were developed from previously known methodologies. The ligands (*S*)-4-methylbenzyl-cysteine (mebz-cys) (**3a**) and (*S*)-4-chlorobenzyl-cysteine (clbz-cys) (**3b**) were initially synthesized; and then the ligands were complexed with copper (II) chloride dihydrate.

2.2.1. Synthesis of ligands

The (*S*)-4-methylbenzyl-cysteine (**3a**) and (*S*)-4-chlorobenzyl-cysteine (**3b**) compounds were synthesized according to the method of Stoll and Seebeck[15] (Fig 2).

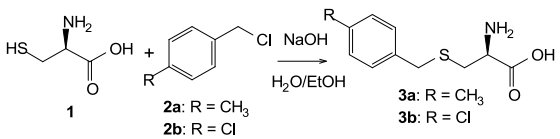


Figure 2. Synthesis of ligands.

In a round bottom flask (250 mL) contained cysteine (0.01 mol) was added an aqueous solution of NaOH (75 mL, 2M) and ethanol (60 mL). The mixture was stirred until complete dissolution. Then the appropriate benzylchloride (0.01 mol) was added and the mixture was stirred for 12 hours, after this time the pH was adjusted to 2 with hydrochloric acid, the product was

concentrated under vacuum and extracted with hot ethanol. The ethanolic extract was concentrated, dissolved in water (25 mL) and the pH of the solution was adjusted to 5. The product was washed with water, ethanol and then dried under vacuum.

2.2.2. Complexes synthesis

In a round bottom flask (100 mL) equipped with a condenser was added the appropriate benzyl cysteine (0.01 mol) dissolved in methanol (50 mL). The mixture was stirred and heated at reflux, until dissolution. Then copper (II) chloride dihydrate (0.01 mol) was added. The precipitate obtained was filtered and dried under vacuum.

2.3. Evaluation of the antioxidant activity

Two tests were carried out to evaluate the antioxidant activity: spectrophotometric analysis using DPPH and peroxide value.

2.3.1. Spectrophotometric analysis (DPPH)

The DPPH radical scavenging activity was examined by using the described method [16].The spectrophotometric analysis was performed with all the compounds synthesized and with the commercial antioxidant BHT. A solution of each compound (1 mg) in 50 mL of DMSO was prepared. The absorbance of the prepared sample was then measured spectrophotometrically at 517 nm. The absorption of the 517 nm band, referring to the DPPH, were compared for each solution.For the comparative calculation, the absorbance of the sample was measured after 15 minutes of the reaction. The ligands were monitored at a concentration range of 6.3 x 10^{-5} to 1.6 x 10^{-4} mol/L, while the complexes and the BHT were monitored in a range of 7.77 x 10^{-5} to 2.95 x 10^{-4} mol/L. The percentage reduction in absorbance of compounds was calculated according to the following formula (1):

$$\%Reduction\ in\ absorbance = \frac{A_{DPPH} - (A_{DPPH+COMPOUND} + A_{COMPOUND})}{A_{DPPH}} \quad (1)$$

Where: A_{DPPH} is absorbance of DPPH solution;
$A_{DPPH+COMPOUND}$ is absorbance of solution containing DPPH and compound;
$A_{COMPOUND}$ is absorbance of compound solution.

2.3.2. Peroxide value

The peroxide content was determined using the method proposed by AOCS [17]. The assay was performed for 12 days at 3 days intervals. In a 250 mL Erlenmeyer flask was added 2.5 g of soybean oil without commercial antioxidants (donated by Cargill Agricultural S/A), 15 mL the acetic acid-chloroform solution (ratio 3:2) and stirred until the sample was dissolved. Then, 0.25 mL of saturated potassium iodide solution was added and allowed to stand out from the light for one minute, after that 5 mL of distilled water was added. Then titration was performed with a 0.1 eq/L sodium thiosulphate solution until the yellow color disappeared. Then, 0.5 mL of 1% starch solution was added and the titration continued until the blue color disappeared. At the same time, a blank test was carried out. The peroxide value was calculated according to Equation (2).

$$IP\left(\frac{meqO_2}{kg}\right) = (A - B) \times (N) \times f \times \frac{1000}{m} \quad (2)$$

Where: A is volume, in ml, of the sodium thiosulphate solution spent on titration of the sample;
B is volume, in mL, of the sodium thiosulfate solution spent on the titration of the blank;
N is normal concentration of sodium thiosulphate solution (eq/L);
f is factor of sodium thiosulphate solution;
m is mass, in grams, of the oil sample.

2.4. Hemolysis test

For this test a stock solution of 0.02 g/mL of compounds was prepared and different volumes were added to the phosphate buffer saline (PBS) to generate eight different concentrations (31.25; 62.5; 125; 250; 500; 1000; 1500 and 2000 µg/mL). The hemolysis test was performed by spectrophotometer method [18], with adaptations. For this method was use sheep red blood cells obtained from Kalifarma® company. A volume of 25 µL of erythrocytes suspension was added to each tube containing 975 µL of eight different concentrations of samples and incubated for 30 min. Having completed this exposure time, samples were centrifuged (FANEM®)at 3000 rpm for 5 min. The free hemoglobin in the supernatant was measured in UV-Vis spectrophotometer at 540 nm (model UV5100) against the white (PBS). Distilled water was used as a positive control and PBS as negative control. The results were expressed as a percentage of the hemolysis present in treated cells (samples) compared with control cells (cells treated with distilled water) in order to calculate the effective

concentration causing 50% hemolysis (H_{50}) and dose-response curves was built.

2.5. Statistical analysis

All tests were conducted in triplicate. For the hemolysis test all results were analyzed statistically by one-way analysis of variance(ANOVA). It was considered to indicate statistical significanceat $p<0.05$ level.

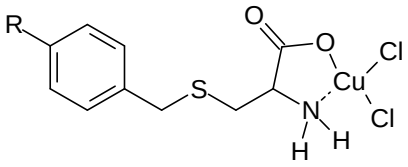
III. Results and discussion

3.1. Characterization of compounds

The (*S*)-4-methylbenzyl-cysteine (mebz-cys) (**3a**) and (*S*)-4-chlorobenzyl-cysteine (clbz-cys) (**3b**) were obtained as a white solid (Yield **3a**: 77% and **3b**: 83%). The structures of the synthesized ligands were established by IR, ^{1}H and ^{13}C NMR. For (*S*)-4-methylbenzyl-cysteine (mebz-cys) (**3a**), IRυ_{max}: 3110, 2979, 2915, 1600, 1568, 1490 cm^{-1}.^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.27 (3H, s, -CH_3), 2.63 (1H, dd, J = 13, 6 Hz, -SCH_2-), 2.73 (1H, dd, J = 13, 6 Hz, -SCH_2-), 3.73 (3H, m, $ArCH_2S$-, NH_2CH-), 7.15 (4H, m, Ar-H). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 20.4 (-CH_3), 33.3 (-SCH_2-), 35.6 ($ArCH_2S$-), 60.1 (NH_2CH-), 128.4 (2C, Ar-H), 128.5 (2C, Ar-H), 135.5 (1C, Ar), 135.8 (1C, Ar), 172.7 (C=O). For (*S*)-4-chlorobenzyl-cysteine (clbz-cys) (**3b**), IRυ_{max}: 3160, 2979, 1600, 1561, 1491, 1090 cm^{-1}.^{1}H NMR (500 MHz, DMSO-D_6): δ = 2.80 (1H, dd, J = 14, 7 Hz, -SCH_2-), 2.91 (1H, dd, J = 14, 5 Hz, -SCH_2-), 3.42 (1H, m, NH_2CH-), 3.77 (1H, d, J = 13 Hz, $ArCH_2S$-), 3.81 (1H, d, J = 13 Hz, $ArCH_2S$-), 7.37 (4H, m, Ar-H). ^{13}C NMR (125 MHz, DMSO-D_6): δ = 31.6 (-SCH_2-), 34.2 ($ArCH_2S$-), 52.4 (NH_2CH-), 128.1 (2C, Ar-H), 130.6 (2C, Ar-H), 131.1 (1C, Ar), 137.1 (1C, Ar), 168.9 (C=O).

The complexes (*S*)-4-methylbenzyl-cysteine copper (II) chloride ([Cu(mebz-cys)]Cl_2) (**4a**) and (*S*)-4-chlorobenzyl-cysteine copper(II) chloride ([Cu(clbz-cys)]Cl_2) (**4b**) were obtained as a green solid (Yield **4a**: 91% and **4b**: 93%).The IR analyses for [Cu(mebz-cys)]Cl_2 (**4a**), υ_{max}: 3510, 3430, 3255, 1618, 1140 cm^{-1} and for [Cu(clbz-cys)]Cl_2 (**4b**)υ_{max}: 3510, 3428, 3255, 1618, 1090 cm^{-1}. The elemental analysis of carbon, hydrogen and nytrogen confirmed the composition of the compounds: [Cu(mebz-cys)]Cl_2 (**4a**) ($C_{11}H_{14}Cl_2CuNO_2S$) calcd. 36.83 %C; 3.93 %H, 3.90 %N; found 36.90 %C, 4.35 %H, 3.74 %N. [Cu(clbz-cys)]Cl_2 (**4b**) ($C_{10}H_{11}Cl_3CuNO_2S$) calcd. 31.68 %C; 2.92 %H, 3.69 %N; found 32.18 %C, 3.58 %H, 3.48 %N.

The IR spectrum of amino acids exhibits significant features in υNH_3 and $\upsilon C{=}O$ regions. The amino acids exist as zwitterions in solution and in solid state. The IR spectra of amino acids exhibited significant features in υNH_3 and $\upsilon COO–$ regions [19]. In the ligands (**3a** and **3b**), the bands in 3160 cm^{-1} – 2800 cm^{-1} region were ascribed to N-H symmetric and asymmetric stretching vibrations. The intense bands in 1600 cm^{-1} – 1490 cm^{-1} region were ascribed to for the carboxylate group. Changes were observed in the IR bands of NH_2 and carboxylate group. The main differences in the spectra of the complexes (**4a** and **4b**) are the shifts of the bands to higher frequencies (3510 cm^{-1} – 3235 cm^{-1} region) and the decrease in intensity, probably owing to interaction of the amino acid NH_2 group with the metal ion. The bands assigned to vibrations of the carboxylate group are shifted, which also indicates the involvement of this group in the metal-ligand bond formation. The new band at 1140 cm^{-1} was assigned for the Cu–O coordination[20]. Thus, we can say that the metal complexes shift in Cu–O and Cu–NH_2 bands as well as the widening of the bands were clearly revealing the formation of metal complexes with a ligand according to the proposed structure (Fig. 3)



4a: R = CH_3
4b: R = Cl

Figure 3. Proposed complex structure.

3.2. Evaluation of antioxidant activity

3.2.1. Spectrophotometric analysis (DPPH)

The DPPH assay was performed to evaluate the efficacy of each complex in capturing free radicals. Therefore, compounds with low IC_{50} values are better antioxidants when compared to those with high IC_{50} values that are considered pro-oxidants. Both complexes and DPPH absorb in the 517 nm wavelength region in which the analysis is performed. The stable DPPH radical is reduced due to the antioxidant causing the change from violet to yellow coloration proportional to the concentration of the reducing substance in the sample.

The ligands did not present a significant decrease in the absorbance against DPPH. With the absorbance data obtained and the concentrations of the complexes and the BHT it was possible to plot the graphs, which provide the IC_{50} (Fig. 4).

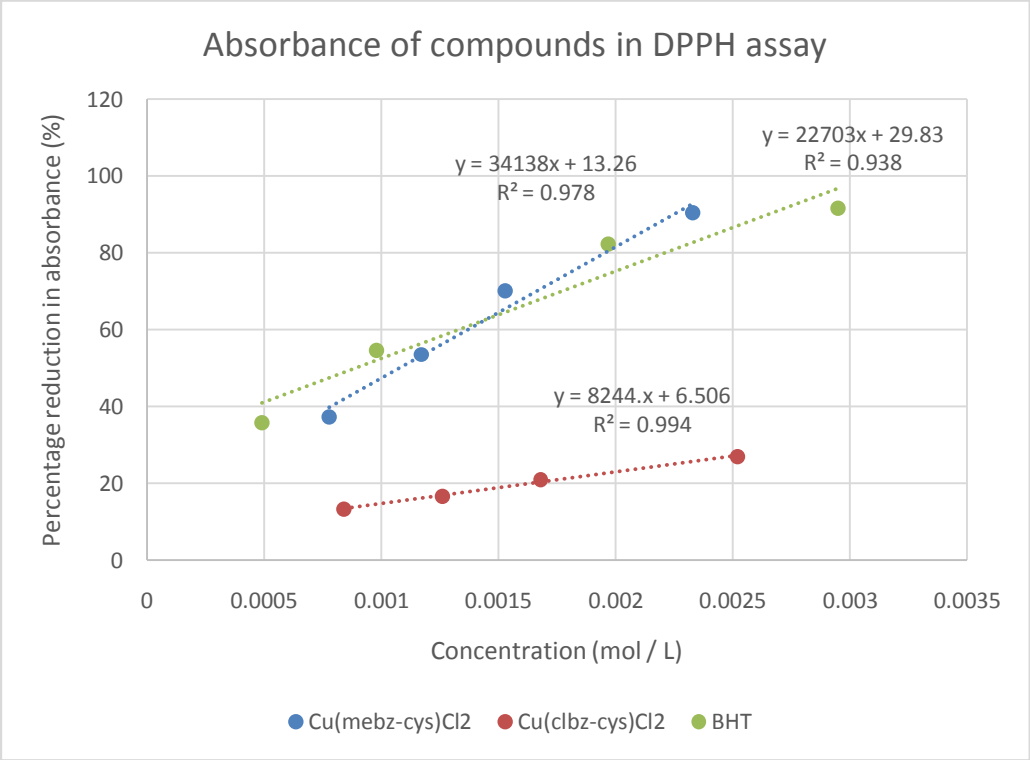


Figure 4. Graph showing the experimental data obtained to calculate the IC_{50} of the compounds.

With the equations of the trend lines of the graphs, it was possible to calculate the IC_{50} value of the compounds. The antioxidant potential of all complexes was confirmed, [Cu(mebz-cys)]Cl_2(**4a**) and [Cu(clbz-cys)]Cl_2 (**4b**) caused IC_{50} at concentrations 1.08 x 10^{-4} and 5.03 x 10^{-4} g/mL, respectively. The BHT compound, on the other hand, presented IC_{50} at the concentration of 0.89 x 10^{-4} g/mL. No complex presented better antioxidant potential than commercial antioxidant, BHT.

3.2.2. Peroxide value

The values of the peroxide value (PV) are shown in Table 1. The test was carried out with soybean oil without commercial antioxidants donated by Cargill Agrícola S/A. The level of oxidation of refined soybean oil is considered low when the IP is between 1.0 and 5.0 meq O_2/kg of oil, moderate level with an IP between 5.0 and 10.0 meq O_2/kg of oil and, with high oxidation with an IP higher than 10,0 meq O_2/kg of oil [21]. Initially, the peroxide value was equivalent to 1.0 meq O_2/kg, being this oil classified as little oxidized.

Analyzing the peroxide value (Table 1), it was verified the increase of this index with the passage of time, as expected. On the 3rd day of heating, pure soybean oil had the highest index (1.70 meq O_2/kg) followed by soybean oil with addition of 1000 ppm mebz-cys (1.64 meq O_2/kg) and soybean oil with addition of 1000 ppm [Cu(clbz-cys)]Cl_2 (**4b**) (1.64 meq O_2/kg). The lowest values observed were those of soybean oil with addition of 2000 ppm of BHT (1.41 meq O_2/kg) and that of soybean oil with addition of 2000 ppm of [Cu(mebz-cys)]Cl_2 (**4a**) (1.47 meq O_2/kg). On the 6th and 9th days of heating, the same profile of the previous analysis was observed, the pure oil presented a higher peroxide value while the oils with the garlic derived compounds had significantly lower values. On the 12th day of heating, soybean oil samples with addition of 2000 ppm [Cu(clbz-cys)]Cl_2 (**4b**) and soybean oil with addition of 2000 ppm [Cu(mebz-cys)]Cl_2 (**4a**)showed the lowest values compared to the other compounds, however they were not lower than the soybean oil with addition of 2000 ppm of BHT.

Table 1.Peroxide value (meq O_2/kg) in soybean oil.

Samples	Heating time at 60 ° C (days)				
	0	**3**	**6**	**9**	**12**
Puresoybeanoil	1.04 ± 0.00	1.70 ± 0.00	2.22 ± 0.00	6.44 ± 0.03	9.44 ± 0.02
Soybean + mebz-cys 1000ppm	1.04 ± 0.00	1.64 ± 0.06	2.10 ± 0.05	6.09 ± 0.03	9.10 ± 0.02
Soybean + mebz-cys 2000ppm	1.04 ± 0.00	1.58 ± 0.13	2.05 ± 0.00	5.74 ± 0.04	8.87± 0.02
Soybean + clbz-cys 1000ppm	1.04 ± 0.00	1.58 ± 0.06	2.16 ± 0.05	6.32 ± 0.03	9.33 ± 0.00
Soybean + clbz-cys 2000ppm	1.04 ± 0.00	1.53 ± 0.12	1.93 ± 0.11	5.98 ± 0.03	9.10 ± 0.02
Soybean + [Cu(mebz-cys)]Cl_2 1000ppm	1.04 ± 0.00	1.58 ± 0.13	2.05 ± 0.00	5.63 ± 0.04	8.75 ± 0.02
Soybean + [Cu(mebz-cys)]Cl_2 2000ppm	1.04 ± 0.00	1.47 ± 0.14	1.82 ± 0.11	5.05 ± 0.04	8.17 ± 0.07
Soybean + [Cu(clbz-cys)]Cl_2 1000ppm	1.04 ± 0.00	1.64 ± 0.06	2.10 ± 0.05	5.74 ± 0.04	8.87 ± 0.05
Soybean + [Cu(clbz-cys)]Cl_2 2000ppm	1.04 ± 0.00	1.58 ± 0.06	1.87 ± 0.09	5.28± 0.04	8.40 ± 0.05
Soybean + BHT 1000ppm	1.04 ± 0.00	1.53 ± 0.12	1.93 ± 0.05	5.51 ± 0.00	8.63 ± 0.00
Soybean + BHT 2000ppm	1.04 ± 0.00	1.41 ± 0.07	1.70 ± 0.00	4.70 ± 0.04	7.82 ± 0.03

All the compounds obtained satisfactory results with the peroxide value, but the complexes (**4a** and **4b**) were superior in relation to the ligands (**3a** and **3b**) antioxidant activity.The peroxide value test was employed to monitor the formation of primary oxidation products in an aqueous/corn oil system(40/60 w/w) supplemented with either Cu–alginate beads or free Cu^{2+} in the aqueous phase (0–5 mM) [22]. It was observed that the binding of pro-oxidant minerals, such as copper, in alginate beads can reduce the levels of oxidation in water/oil mixtures.

3.3. Hemolysis test

The compounds caused lysis in the erythrocytes in a concentration-dependent manner and the results are shown in figure 5. The (S)-4-methylbenzyl cysteine (**3a**) and (S)-4-chlorobenzyl cysteine (**3b**)caused H_{50} at concentrations 467.3 and 470.0 μg/mL, respectively. The [Cu(mebz-cys)]Cl_2 (**4a**) and [Cu(clbz-cys)]Cl_2 (**4b**) resulted in H_{50} at concentrations of 127.0 and 120.0 μg/mL, respectively. The BHT compound, on the other hand, presented H_{50} at the concentration of 58.5 μg/mL. Thus, the ligands and copper complexes have a lower hemolytic capacity than the commercial antioxidant BHT, these results being statistically significant ($p < 0.05$) (Fig. 5).

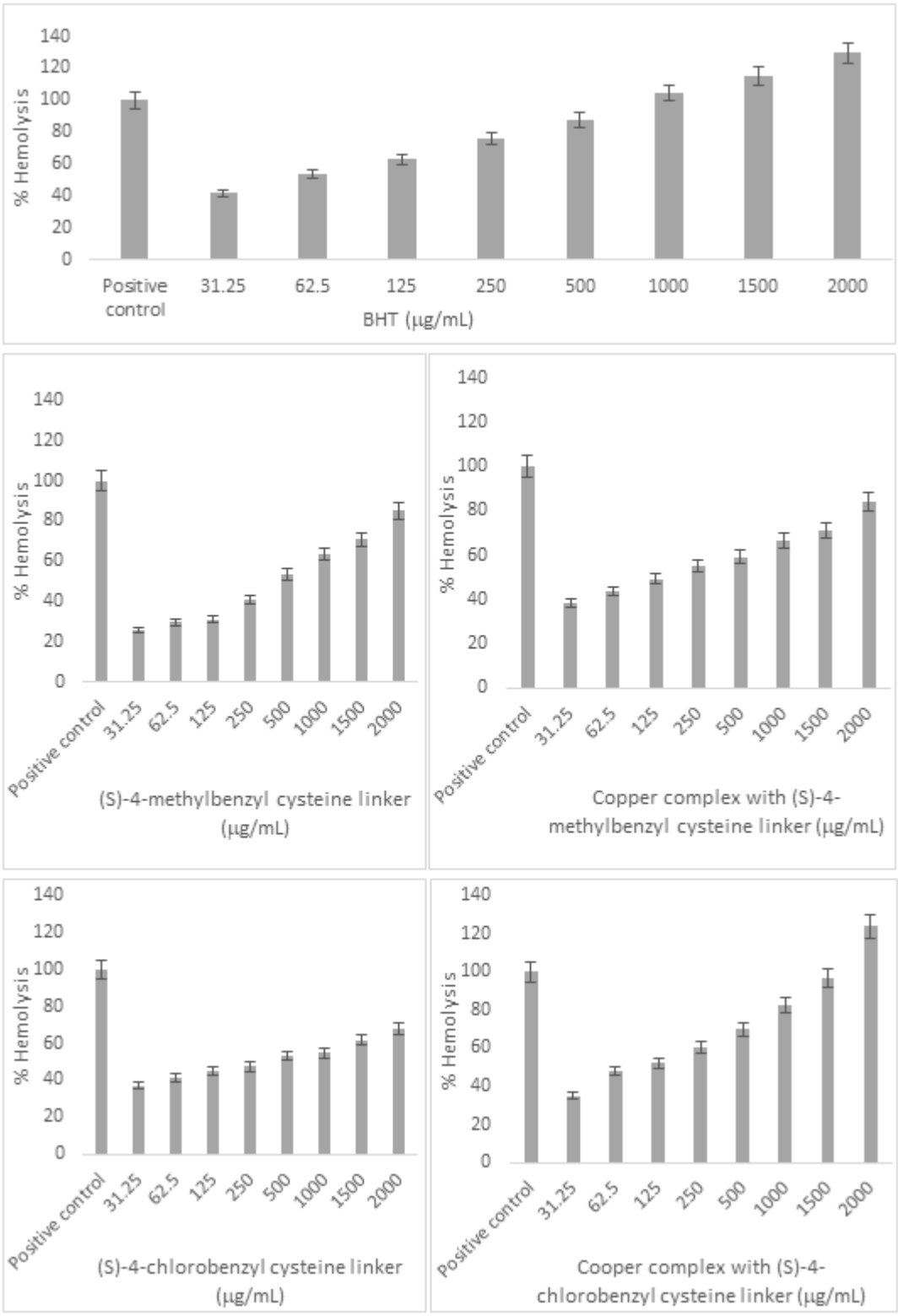


Figure 5.Hemolysis Test. Concentration–response effect of complexes derived from the garlic on hemolysis of erythrocytes. Cells were treated with eight concentrations of compounds (31.25; 62.5; 125; 250; 500; 1000; 1500 and 2000 µg/mL) in sextuplicate. The media of absorbance of each concentration was compared with the control positive group (cells exposed to with distilled) and for this 100% hemolysis was considered. Results represent the mean + standard deviation (SD) of sextuplicate from three independent experiments.

Hemolysis test evaluates the phenomena of cell membrane lysis resulting from the action of the test substance [23] and the evaluation of the mechanical stability of the erythrocyte membrane is a good indicator of damage caused by various compounds such as cytotoxicity screening [24]. Thus, our results suggest that the metallic compounds synthesized by our research group are antioxidants less toxic than the commercial product available on the market.

IV. CONCLUSION

The compounds derived from cysteine and their copper complexes were synthesized. The (*S*)-4-methylbenzyl cysteine (**3a**) and (*S*)-4-chlorobenzyl cysteine (**3b**) obtained 77% and 83% average yield respectively, while the [Cu(mebz-cys)]Cl_2 (**4a**) and [Cu(clbz-cys)]Cl_2 (**4b**) yield 91% and 93%, respectively. These compounds were characterized by spectroscopic techniques such as NMR, IR and elemental analysis, contributing to the elucidation of the structures. The analyzes strongly suggest that the copper is coordinated to the oxygen of the carboxylate group and NH_2. Its antioxidant activities were tested using DPPH and peroxide value. The compounds **3a** and **3b**not present antioxidant activity in DPPH assay, whereas the complexes confirmed their potential, and the [Cu(mebz-cys)]Cl_2 (**4a**) showed a better result compared to [Cu(clbz-cys)]Cl_2 (**4b**). The IC_{50} values were 5.03 x 10^{-5}, 1.08 x 10^{-5} and 0.89 x 10^{-5} g/mL for the [Cu(clbz-cys)]Cl_2 (**4b**), [Cu(mebz-cys)]Cl_2 (**4a**) and BHT, respectively. All the compounds obtained satisfactory results regarding the evaluation of the antioxidative potential through the oxidative stability of the soybean oil, but the complexes are superior than the ligands, further reducing the peroxide value of the samples. No complex presented better antioxidant potential than commercial antioxidant, BHT. However, the synthetized compounds were less toxic than the commercial antioxidant. The H_{50} values were 467.3 and 470.0 µg/mL for ligands **3a** and **3b**, respectively, while the copper complexes presented values of 127.0 and 120 µg/mL for complexes **4a** and **4b**, respectively. The H_{50} value of BHT was 58.5 µg/mL, indicating an elevated toxicity even at lower concentration. The synthetized compounds showed a good antioxidant activity and presented lower toxicity. For the use of these novel antioxidant agents as food additives, further studies about their effectiveness on real foods should be conducted.

Acknowledgements

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