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Net Effects of Cashew Nuts in *Saccharomyces cerevisiae* front to Damage Induced by Hydrogen Peroxide

ORIGINAL

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Abstract

Cellular injury associated with oxidative stress has been related in the etiology and progression of many diseases, including neurodegenerative diseases and cancer. Shell of cashew nut releases the alkylphenols oil, known as cashew nut shell liquid (CNSL), which can be characterized according to the method of production such as technical (tCNSL) and in natural (iCNSL) types. This study aimed to characterize the chemical constituents in both liquids by gas chromatography coupled to mass spectrometry (GC-MS), and to evaluate the net effect of them in proficient (SODWT) and deficient (Sod1Δ, Sod2Δ, Cat1Δ, Sod1ΔSod2Δ, Sod1ΔCat1Δ) *Saccharomyces cerevisiae* oxidative damaged induced by hydrogen peroxide (H_2O_2 (10 mM); stressor) at concentrations of 17.37, 34.7 and 69.5 µg/ml in pre-, co- and post-treatment. Both iCNSL and tCNSL produced no oxidizing effect to the tested yeast strains, otherwise they protected against damage induced by stressor (STR). However, tCNSL was found more prominent than the iCNSL in restorative activity. In conclusion, CNSLs (iCNSL/tCNSL) exhibited excellent protective, antioxidant and repair capabilities compared to the damage induced by STR in *S. cerevisiae* cells, thus may be a suggestion for its biotechnological production especially for pharmaceutical account.

Keywords
Oxidative Stress; Cashew Nut Shell; Liquid; *Saccharomyces Cerevisiae.*

Introduction

During normal metabolism, oxygen consumed by our breathing and some immune functions mediated by our cells produce reactive oxygen

species (ROS). They are known as oxidants and pro-oxidants [1, 2]. The imbalance between ROS and antioxidants can cause oxidative stress, which can induce peroxidation of cell membranes, oxidation to essential macromolecules such as – proteins and DNA, inhibition to the electron transport chain in mitochondria [3]. Molecules that inhibit or prevent the oxidation of other molecules are termed as antioxidants. They can prevent the breaks in double strands of DNA, atherosclerosis and some cancers [4].

Due to the presence and detrimental effects of free radical (RL) and ROS in human cells, a search of antioxidants in natural sources especially in plant origins has been taken into vast interest. The RL and ROS are responsible for various diseases such as stroke, heart disease, cancer [5], and other acute hepatic insufficiency and chronic hepatic diseases [6]. Many studies have associated new strategies for disease treatment as neurodegenerative using antioxidants [7-9].

Medicinal plants are known as a source of natural products that may have therapeutic potentials. Many of their secondary metabolites have been associated with various biological activities such as -antimutagenic, antioxidant [4, 10, 11], antimicrobial and anticarcinogenic [12].

Currently, there is a growing interest in natural substances with antioxidant properties and they are useful for human health in food consumptions as well as protective substances that may be specific to pharmaceutical accounts. It is well known that plants possessing antioxidant and pharmacological properties are related to the presence of phenolic compounds, particularly phenolic acids and flavonoids with potential scavengers of free radicals [4].

Non-isoprenoid phenolic lipids are found in large quantities in the Anacardiaceae family, more specifically in *Anacardium occidentale*, a widely distributed species in Brazil and known as cashew tree [13]. Phenols can be used to obtain compounds of interest for the chemical and pharmaceutical industries [14].

The fruit of the cashew tree, popularly known as cashew nuts, is an achene long and variable width, smooth leathery shell, mesocarp honeycomb, filled with a dark, caustic, flammable liquid, called Net peel the cashew nut (CNSL). The CNSL may be classified according to the methodology of obtaining, in: CNSL in nature (iCNSL) and technical CNSL (tCNSL) [15]. According to Oliveira et al. [16], the iCNSL comprises anacardic acid (62.9%); cardol (23.98%) and cardanol (6.99%), while tCNSL, extracted at 80-200° C comprises of cardanol by 52-60%, cardol and polymeric materials by 10% and 30%, respectively [17].

CNSL has been already reported in some literature as: natural anti-inflammatory [18], antioxidant [19], antifungal [20], larvicidal [16], antitumor [21, 22], anticholinesterase [23], non-mutagenic in mice [24] lipoxygenase [25] and xanthine oxidase inhibitory [19, 26] activities.

However, the evaluation of antioxidant capacity using laboratory animals is generally a difficult as it requires a large number of animals to ensure statistically significant results. Therefore, tests with microorganisms are easy, fast and can be used a large number of cells with the same genetic characteristics [27].

The eukaryote, yeast (*S. cerevisiae*) is widely used in the biological tests for screening and predicting the ability of various antioxidant compounds. In addition, all aerobes have a variety of antioxidant defenses, including superoxide dismutase (Sod) and catalase (Cat) [28] as their metabolism is similar to that of the higher eukaryotes, with own mechanisms metabolic activation (P450) and detoxification, which is not present in bacteria [29].

Thus, the study aimed to evaluate the oxidant activities, protective, antioxidant and repairing capabilities of iCNSL and tCNSL in *S. cerevisiae*, proficient and deficient strains damage induced by H_2O_2 in pre-, co- and post-treatments.

Materials and Methods

Obtaining the CNSL and choice of doses

Shells of cashew nuts were collected in Teresina, Brazil and were stored in a cooler in liquid nitrogen for 5 minutes. Then, they were crushed and subjected to extraction with n-hexane in a Sohxlet apparatus for 16 hours. After evaporation of the solvent, there was obtained iCNSL. tCNSL was kindly provided by Europa Chestnut Industry Ltda group, located in Altos, Piauí, Brazil. The nuts were immersed in a warm bath (195 °C) for 3 h (according to the company's instructions). Finally, tCNSL was filtered and stored at room temperature [30]. In this study we select lower doses as there is a previous talk for an antioxidant activity of tCNSL at 100-500 mg/ml in *S. cerevisiae*.

Derivatization and characterization of the chemical constituents in CNSLs by gas chromatography-mass spectrometry (GC-MS)

Briefly, in a 5.0 ml flask 5.0 mg CNSL was added which was previously purred by 0.2 ml of distilled pyridine and 100 ul of N-bis-trimethylsilyl tri fluoracetamide (BSTFA). The system was subjected to heating at 60 °C for about 60 min under magnetic stirring. The reaction product was analyzed by GC-MS under the conditions described below.

The analysis of samples was performed in a Shimadzu GC-17A equipment coupled to a mass spectrometer QP5050A equipped with a capillary column DB-5HT (95% dimetilpolisiloxilano and 5% phenyl, 30 meters length, 0.25 mm inner diameter, 0.1 mm in film thickness); carrier gas: helium 1.0 ml/min; inlet pressure of 107.8 kPa column; Column flow = 1.7 ml/min; detector temperature 300 ° C; line speed = 47.3 cm/s; total flow of 24 ml/min; carrier flow 24 ml/min; injector temperature 260 °C; Column temperature 60 °C (0.5 min) with a heating rate of 6 ° C/min to 260 °C (5 min), then increased at a rate of 12 ° C/min up to 300 °C (10 min).

The analysis with the mass detector was held in scan mode, analysis time 52.21 min; the acquisition of mass spectra was made in the range of 47-600 Daltons electron impact with ionization energy of 70 eV (voltage 1.5 kV, the quadrupole analyzer) and ion source at 200 °C.

Saccharomyces cerevisiae strains

The test strains of *S. cerevisiae* used in this study are shown in **Table 1**.

Culture media

Cells growth was done in complete liquid medium (YEL) containing 1% yeast extract, 2% bactopepto-

Table 1. Proficient and dificient strains of *S. cerevisiae* used in this study.

Strains	Genotype	Lack of the enzymatic defense	Origin
EG103 *(Sodwt)*	MATα leu2-3,112 trp1-289 ura3-52 GAL+	None	Edith Gralla, L Angeles[1]
EG118 *(Sod1Δ)*	Sod1:URA3 all other markers as EG103	Superoxide dismutase cytosolic Cu-Zn	
EG110 *(Sod2Δ)*	Sod2:TRP1 all other markers as EG103	Superoxide dismutase mitochondrial	
EG133 *(Sod1ΔSod2Δ)*	Sod1:URA3 Sod2:TRP1 double mutant all other markers as EG103	All superoxide dismutases	
EG223 *(Cat1Δ)*	EG103, except Cat1: TRP1	Cytosolic catalase	
EG *(Sod1ΔCat1Δ)*	EG103, except Sod1: URA3 and Cat1: TRP1	Cu Zn-superoxide dismutase and cytosolic catalase	

Strains were kindly provided by the research group in Genetic Toxicology at the Federal University of Rio Grande do Sul (UFRGS).

ne and 2% glucose; as YEPD solid medium, which was prepared by adding to the YEL medium composition 2% bacto-agar.

Oxidant capacity assessment, antioxidant and repair capacity assessment for iCNSL and tCNSL in *S. cerevisiae* **strains**

Briefly, iCNSL and tCNSL as 17.37, 34.75 and 69.50 µg/ml were tested in this in vivo assay. The strains were inoculated in YEL at 28 °C in an orbital stirrer until they reached the stationary growth phase according to Rosa et al. [31]. Suspended cells were seeded from the center to the edge of a sterile, solidified YEPD media containing petridish in a continuous cycle, for both sides of the plate in the center containing a sterile filter paper disk.

Ascorbic acid (vitamin C' AA) (50 mM) was used as positive control. For the evaluation of oxidantive effect, the plates were seeded with 100 ul of iCNSL and tCNSL to see if or not inhibited the growth of seeded yeasts. In the pretreatment, a 5 ul of iCNSL and tCNSL (specified concentrations) were added to the disk and let them incubation for four hours at 35±1 °C then followed by the addition of STR (H_2O_2). To know the antioxidative defense, a 5 ul of test samples along with 5 µl of STR (10 mM) was added subsequently. In the repair capability test, 5 µl of STR (10 mM) addition followed by addition of 5 µl of samples after the incubation (35±1 °C for 4 h) period STR. After a 72 h of incubation in an incubator at 35±1 °C inhibition zones were measured in milimeters (mm) taking full growth 0 mm to no growth 40 mm in lenghts. All assays were performed in duplicate.

Statistical analysis

The results were statistically analyzed using the program GraphPad Prism 6.0, with two-way analysis of variance (ANOVA) followed by Tukey multiple comparison test, comparing the test treatments with the negative control (saline 0.9%) and STR (H_2O_2) used. Data presented as mean±standard deviation (SD); level of significance at $p < 0.05$; $p < 0.01$ and $p < 0.001$.

Results

Chromatographic analysis of cashew nut liquids

Figures 1 and **2** show the chromatographic profiles (total ion chromatogram) of iCNSL and tCNSL, respectively. The assignment of the peaks of the identified compounds as well as the retention time and the relative percentage of the height of the compounds are shown in **Tables 2** (iCNSL) and **3** (tCNSL). Integration was performed manually. The larger silylated derivatives found in iCNSL were cardanol monounsaturated (10.76%), unsaturated (double) (12.80%), saturated (2.69%), followed by tri unsaturated cardol (6.71%), unsaturated (double) (11.56%) and methyl saturated cardol (4.36%). In the presence of monounsaturated anacardic acids (3.59%), unsaturated (double) (12.66%) and saturated (8.38%) **(Table 2)**.

For the tCNSL, derivatives silylated results showed to presence of monounsaturated cardanol (27.99%), unsaturated (double) (15.35%) and satu-

Figure 1: Total ion chromatogram (TIC) for iCNSL analyzed by GC-MS.

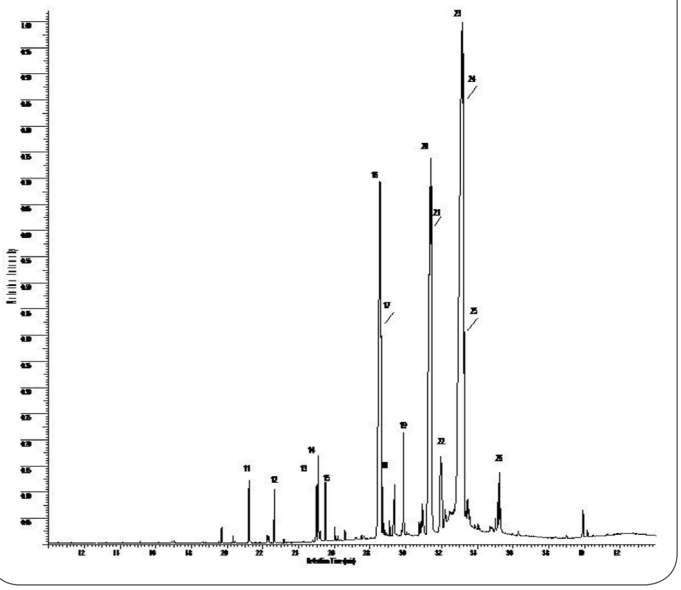

Figure 2: Total ion chromatogram (TIC) for tCNSL analyzed by GC-MS.

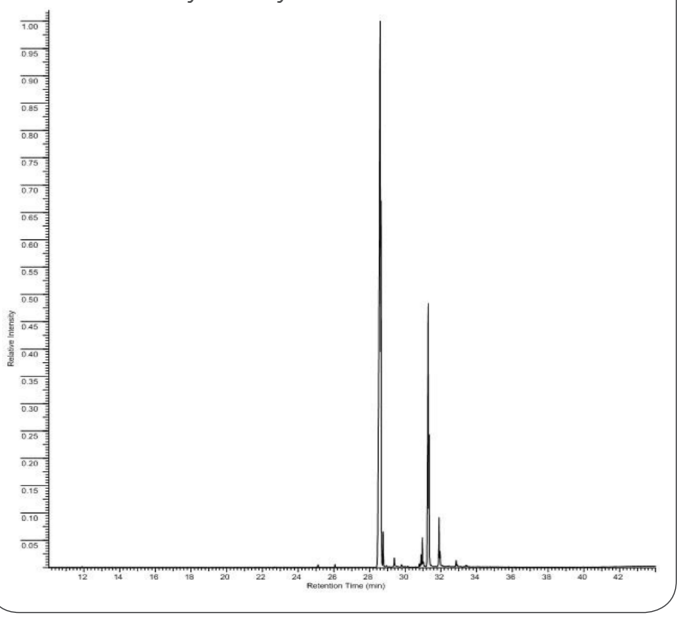

Table 2. Main components in the iCNSL, identified the silylated derivative.

Peak	Retention time (min)	Compounds	Relative abundance (%)
11	21.217	Debutila phthalate	3.77
12	22.633	Hexadecanoic acid	3.21
13	25.000	9.12-octadecadienoic acid (Z, Z) or linoleic acid	3.32
14	25.100	TRANS-9-octadecenoic acid	4.57
15	25.492	Octadecanoic ccid	3.42
16	28.542	Monounsaturated cardanol	10.76
17	28.600	Cardanol unsaturated (double)	12.80
18	28.717	Cardanol saturated	2.69
19	29.867	Not Identified	4.73
20	31.392	Tri unsaturated vitro	6.71
21	31.442	Unsaturated (double) vitro	11.56
22	31.950	Saturated methylcardol	4.36
23	33.133	Anacardic acid monounsaturated	3.59
24	33.200	Anacardic unsaturated(double) acid	12.66
25	33.242	Anacardic acid saturated	8.38
26	35.233	N.I.	3.47

N.I.: Not identified

rated (6.63%), a total of 49.97%, followed by cardols double (27.5%), monounsaturated (14.39%) with 41.89%. however, the important constituent, anacardic acid appeared in very low relative abundance, it is not possible integration **(Table 3)**.

Table 3. Main components in tCNSL, identified as silylated derivative.

Peak	Retention time (min))	Compounds	Relative abundance (%)
1	28.529	Monounsaturated cardanol	27.99
2	28.717	Cardanol unsaturated	15.35
3	28.797	Cardanol saturated	6.63
4	29.365	Dioctila phthalate	1.52
5	30.854	N.I.	1.33
6	30.927	Benzoic acid, 2-(12-heptadecenil)-6-methoxy-, methyl ester	2.16
7	31.313	Cardol unsaturated (double)	27.50
8	31.353	Cardol mono unsaturated	14.39
9	31.865	N.I.	3.13
10	-	Anacardic acid	Trace amount

N.I.: Not identified

Due to the high concentration of injected sample there was a broadening of the peak of the mixture of anacardic acids, thus hindering their integration. This may have influenced by their relative abundance, which would explain its lowest level in relation to previous literature evidences.

Assessment of the effects of iCNSL and tCNSL as the oxidizing action in S. cerevisiae

In *S. cerevisiae* assay, the iCNSL and tCNSL showed no oxidative effects to the strains Sodwt, SodΔ1, Sod2Δ, Sod1Δ/Sod2Δ, Cat1Δ, Sod1Δ/Cat1Δ at all concentrations tested as compared to the NC.

Protective effects of iCNSL and tCNSL by oxidative damage induced with hydrogen peroxide in *S. cerevisiae*

Table 4 showing the protective effcts by the iCNSL and tCNSL tells that tCNSL provide more protective effects to the *S. cerevisiae* strains than the iCNSL.

Antioxidant effects of iCNSL and tCNSL by STR induced oxidative damage to the *S. Cerevisiae*

Both iCNSL and tCNSL exhibited antioxidative defense in oxidative damage indiced by STR. However, tCNSL was more strongly protects the *S. cerevisiae* strains than the iCNSL, where the protection of single mutant Cat1Δ and double mutant Sod1ΔCat1Δ were found to be more prominent **(Table 5)**.

Repair capability of iCNSL and tCNSL by STR induced oxidative damage to the *S. Cerevisiae*

The iCNSL exhibited a decreased repair capacity at the highest concentration (69.50 µg/ml) compared to the tCNSL. Moreover, tCNSL at the dose 17.37 µg/ml effectively repaired the oxidative damage induced by the STR (10 mM) than the tCNSL other treated doses and iCNSL in comparision to the standard, AA **(Table 6)**.

Table 4. Protective effects of iCNSL and tCNSL in comparison to controls in *S. cerevisiae*.

Pre-treatment	NC	STR	AA	tCNSL			iCNSL		
				17.37 µg/mL	34.75 µg/mL	69. 50 µg /mL	17.37 µg/mL	34.75 µg/mL	69.50 µg/mL
Sodwt	5.50±2.08	16.75±1.25***a	7.00±1.41***b	9.5±1.00***b	8.00±2.44***b	8.50±1.00***b	9.70±0.57***b	8.50±0.55***b	7.75±2.06***b
Sod 1Δ	17.75± 5.62	28.00±7.25***a	11.00±0.81***b	10.50±1.29***b	9.75± 0.50***b	10.25±4.11***b	10.75±2.87***b	10.00±0.81***b	10.00 ± 1.63***b
Sod 2Δ	12.25±2.06	25.25±6.18***a	12.75±0.95***b	9.25±0.95***b	8.00±2.70***b	8.00±2.82***b	12.75±1.50***b	10.00±0.81***b	9.75±0.50***b
Sod 1ΔSod2Δ	11.75±2.3	30.25±2.36***a	10.75±1.50***b	7.25±1.50***b	8.00±2.30***b	6.00±2.00**a***b	10.75±0.95***b	9.75±2.75***b	10.00±0.81***b
Cat 1Δ	10.25±1.25	19.00±1.15***a	5.75±1.70***b	3.50±0.57***a***b	5.75±1.70**a ***b	3.00±1.41***a ***b	10.25±1.70***b	10.75±0.95***b	11.25±0.95***b
Sod 1ΔCat 1Δ	12.00±2.44	17.00±1.82***a	2.75±1.70***b	2.50±0.57***a ***b	4.25±0.95***a ***b	5.25±1.50***a ***b	11.00±1.15***b	11.75±0.95**b	11.55±2.36**b

Values are mean±SD (mm); NC: negative control (0.9% NaCl solution); STR: stressor (H2O2); AA: ascorbic acid (vitamin C); tCNSL: technical cashew nut shell liquid; iCNSL: cashew nut shell liquid natural; *** ($p < 0.001$), ** ($p < 0.01$) and * ($p < 0.05$). "a" compared to the NC, "b" compared to the STR; two-way analysis of variance (ANOVA) followed by Tukey multiple comparison test

Table 5. Antioxidant activity of iCNSL and tCNSL in comparison to controls in *S. cerevisiae*.

Co-treatment	NC	STR	AA	tCNSL			iCNSL		
				17.37 µg/mL	34.75 µg/mL	69. 50 µg /mL	17.37 µg/mL	34.75 µg/mL	69.50 µg/mL
Sodwt	11.25±2.98	0.25±0.50***a	8.00±1.41***b	8.75±2.50***b	7.25±2.25***b	11.50±2.38**b	10.50±4.20**b	11.00±2.94**b	9.25±1.50***b
Sod 1Δ	10.25±1.70	15.75±2.98***a	6.75±1.70***b	11.00±1.15	11.00±7.70	11.50±6.60	2.50±1.73**a***b	3.25±2.36**a***b	1.50±1.95***a***b
Sod 2Δ	15.75±2.21	1 7.00±2.70***a	7.00±2.16**b	10.50±2.38	9.25±5.31*b	8.75±3.31*b	8.25±2.36*a*b	8.95±2.36*a*b	7.00±0.81***b
Sod 1ΔSod2Δ	11.75±5.37	2 5.25±3.50***a	6.75±1.70***b	7.25±2.63**a***b	9.15±5.37*a***b	9.75±4.11*a***b	7.75±3.59**a***b	5.00±1.41***a***b	9.75±0.95**a***b
Cat 1Δ	12.25±1.25	1 6.25±2.21***a	6.50±2.08***b	11.00±3.16	4.50±2.38***a***b	4.50±1.29***a***b	10.25±1.30**b	9.25±0.95**b	8.00±2.44*a***b
Sod 1ΔCat 1Δ	13.25±2.36	1 5.50±0.57***a	6.75±1.7 ***b	9.25±1.50*b	4.50±0.57***a***b	7.25±2.63*a***b	9.25±0.06**b	7.25±3.94***b	7.50±2.38*a***b

Values are mean±SD (mm); NC: negative control (0.9% NaCl solution); STR: stressor (H2O2); AA: ascorbic acid (vitamin C); tCNSL: technical cashew nut shell liquid; iCNSL: cashew nut shell liquid natural; *** ($p < 0.001$), ** ($p < 0.01$) and * ($p < 0.05$). "a" compared to the NC, "b" compared to the STR; two-way analysis of variance (ANOVA) followed by Tukey multiple comparison test.

Table 6. Repairs activity of iCNSL and tCNSL in comparison to controls in *S. cerevisiae*.

Post-treatment	NC	STR	AA	tCNSL			iCNSL		
				17.37 µg/mL	34.75 µg/mL	69. 50 µg /mL	17.37 µg/mL	34.75 µg/mL	69.50 µg/mL
Sodwt	9.50±1.29	20.00±3.55***a	7.75±2.06***b	8.75±1.89***b	9.00±1.41***b	10.25±0.50***b	10.25±0.50***b	10.75±0.95***b	7.75±1.70***b
Sod 1Δ	8.25±1.70	15.75±2.98***a	5.75±2.06***b	8.00±0.81***b	7.75±1.78***b	9.00±1.15***b	11.50±0.57*b	11.00±1.41**b	13.75±0.95
Sod 2Δ	8.25±2.75	16.00±1.15***a	5.75±2.06***b	6.00±0.81***b	7.75±1.70***b	8.75±0.95***b	9.50±0.35**b	11.50±1.41***b	14.00±0.75
Sod 1ΔSod2Δ	11.00±1.82	22.50±2.08***a	8.50±1.29***b	6.00±1.41**a***b	7.50±1.29***b	8.75±1.50***b	12.00±1.15***b	10.50±0.57***b	13.00±2.16***b
Cat 1Δ	8.75±1.70	16.50±1.73***a	5.50±2.08***b	9.75±1.25***b	9.50±1.29***b	8.00±1.63***b	10.75±1.50***b	13.25±1.50	13.00±0.10
Sod 1ΔCat 1Δ	7.00±0.81	14.00±3.16***a	5.25±2.21***b	9.75±0.50	10.75±0.95	11.25±0.95	10.75±3.40	11.75±1.50	16.25±2.06

Values are mean±SD (mm); NC: negative control (0.9% NaCl solution); STR: stressor (H2O2); AA: ascorbic acid (vitamin C); tCNSL: technical cashew nut shell liquid; iCNSL: cashew nut shell liquid natural; *** ($p < 0.001$), ** ($p < 0.01$) and * ($p < 0.05$). "a" compared to the NC, "b" compared to the STR; two-way analysis of variance (ANOVA) followed by Tukey multiple comparison test

Discussion

Oxidative stress has been associated with overexpression of ROS that can interact with DNA, proteins and lipids. This association may result in mutations [32]. The DNA damage is known as a path responsible for mutagenesis and carcinogenesis [33]. The phytochemicals present in plants, has called attention for its therapeutic properties in the treatment of oxidative stress and related disorders due to mutation [34]. The consumption of these chemopreventive substances reduces the incidence of cancer by their antioxidative, antimutagenic and antiproliferative capabilities [35].

In GC-MS analysis, tCNSL presented a mixture of cardanols by 49.97% in its composition, while iCNSL by 26.25% cardanols and 24.63% anacardic acid as major components (**Table 2** and **3**). The chemical composition found in this study was different from the earlier reported in literatures [16,19]. There may be effects due to extraction processes, climate conditions and the origin of the nuts. The lack of anacardic acid in tCNSL is due to its thermal instability, as they it may easily decarboxylated by heat induced during extraction process. Oxidant is a process of electron addition to the substrate in which by-products are highly unstable thus makes a chain reaction [36, 37]. The cytotoxic model in *S. Cerevisiae* is a simple, rapid and highly sensitive one for the assessment of varieties of biologicals, natural and synthetic chemicals [38].

In our present study, both iCNSL and tCNSL showed no oxidative effect in all test yeast strains. It is evident that phenolic compounds are substances with high antioxidant power, which can attack the RL formation during oxidation processes [39]. Therefore these substances are efficient in non-enzymatic guards against oxidative stress and may act by preventing metal ions responsible for beginning oxidation, or inhibit the oxidative enzymes [40]. The anacardic acid extracted and purified from iCNSL is an antioxidant that can inhibit the generation of superoxide radicals ($O2^{\bullet -}$) by sequestering the hydroxyl radical ($^{\bullet}OH$) [26].

According to Correia et al. [13] the plant family, Anacardiaceae is rich with phenolic lipids and which are good sources for antioxidative and chemopreventive substances as they can inhibit diseases related to oxidative stress, ROS mediated injuries to cells [41], cardiovascular, neurodegenerative and cancers [7, 9].

It is also evident that CNSL derivatives (cardanol, cardol, cardanol, and tert-butylated all hydrogenated) may inhibit oxidation to a variety of organic substances. Otherwise, each test organism has peculiarities regarding the evaluation of toxic products with cytotoxic and antioxidants capabilities; although there is a good correlations of test eukaryotes to the mammals [42, 43]. Thus the anacardic acid, cardanol and cardol may have cytotoxic activity also.

The protective effects of iCNSL and tCNSL (Table 4) can be explained by the presence of phenolic compounds, especially the presence of anacardic acid (70%) in iCNSL and cardanol in iCNSL and tCNSL as major components. These results corroborate the literature, in which the anacardic acid and cardanol are reported as for their antioxidant activities [16].

Hoever, Morais et al. [44] observed that the anacardic acid provides gastric protection through antioxidant mechanisms. *In vitro* antioxidant studies carried out in DPPH and ABTS scavenging tests tells that cardanol ia the most active compound, followed by anacardic acid and cardol [16]. Similarly, Andrade et al. [19] found that 100 ug/ml of iCNSL can reduce the level of DPPH radicals by 88.9% and can effectively eliminate hydroxyl radicals in the xanthine oxidase assay. It is otherwise well known that most plants having phenolic and polyphenolic compounds may have great antioxidant potential and thus be exploited in the preparation of food and pharmaceutical products [45, 46]. They exert their activity by protecting mainly

because of their redox properties acting as reducing agents, hydrogen donors and singlet oxygen scavenger. Thus, the prophylactic benefits can be attributed to the antibacterial, anticancer, anti-inflammatory, antiviral, anti-allergic and immuno stimulatory activities [45].

In addition to In vitro studies [16, 19] a recent in vivo study with the doses of 100-500 mg/ml of tCNSL was found evident to have protective effect against oxidative stress in yeasts [19]. Our studies performed with much lower doses than the earlier reported studies shows prominent antioxidant capacity in *S. cerevisiae* **(Table 5)**.

The principle of antioxidant activity is based on the availability of electrons to neutralize the RL. This antioxidant activity may be related to the number and nature of hydroxylation of the aromatic ring in the chain of the compound in question. It is assumed that the capacity to act as a hydrogen and oxidation inhibitor is improved by increasing the number of hydroxyl groups in the phenolic ring [47]. Thus, the antioxidant properties of both liquids **(Table 5)** may be related to the chemical composition of the tested compounds, as in the presence of anacardic acid and cardanol in iCNSL tCNSL as main components [16, 19, 26].

Chandrasekara and Shahidi [48] suggested antioxidant effect of cashew in relation to temperature. They found that the roasted chestnuts to 130 ° C for 33 minutes had higher antioxidant capacity by eliminating DPPH, hydroxyl as well as oxygen radicals.

These findings may be were associated with the presence of flavonoids such as (+) - catechin, (-) - epicatechin and epigallocatechin. Cashew is also evident to have these components [49] along with phenolics [50], flavonoids [48, 49], tannins, terpenoids, alkaloids [50], β-carotene, lutein and α-tocopherol [49].

The enzymatic antioxidant defenses include the activity of superoxide dismutase (Sod), catalase (Cat), glutathione peroxidase (GPx), glutathione reductase (GR) and Glutathione S-transferase(GST). To minimize the toxic effects of RL formed, it is necessary an appropriate balance of antioxidant enzymes as mentioned above [51]. Starcevic et al. [52] demonstrated that the use of H_2O_2 induces significant damage to the DNA and the emergence damage is concentration dependent. Sensitivity to the damage caused by H_2O_2 decreased with the increasing rate of change. The decreased sensitivity can be attributed to increased expression of enzymes antioxidants Cat and Sod1 and Sod2 expressed in these cells.

The cashew nut shells are rich in phenolic compounds with antioxidant activity and can be used as a natural source of antioxidants to be used in food consumptions, and especially for protection and pharmacological treatment of various diseases [53].

The anacardic acid and cardanol isolated from LCCI showed excellent antioxidant activity associated with the side chain carbon 15 by inhibiting pro-oxidative enzymes. There are also reports that the cardol present in highest percentage in tCNSL, can be used as an antioxidant agent [54]. From the above discussion it may be told that there may be synergistic and/or additive effects of phenolic compounds present in the iCNSL and tCNSL [26].

It should be emphasized that the effects of tCNSL and iCNSL in mutated strains catalase, including Cat1Δ and Sod1ΔCat1Δ, indicate that these natural products may also be able to dismutate H_2O_2, forming water and molecular oxygen as proposed by Fridovich [55].

Rodrigues et al. [54] examined the antioxidant activity of CNSL derived from the thermal oxidation films of cis-1,4-poly isoprene. All materials showed an increased in the induction time and reduction at constant speed. The authors attributed the introduction present in the CNSL and cardanol to their antioxidant activity, suggesting a better antioxidant activity for the phenolic compounds in the CNSL complex mixture of what they isolated by arranging the order: CNSL>> cardanol = hydrogenated carda-

nol and alkylated> hydrogenated cardanol, which may also explain synergistic and/or additive effects of tCNSL.

Thus, the iCNSL and tCNSL may probably have to catalase activity, like the mechanisms reported for vitamin C (AA); which, in our experiments, showed an excellent antioxidant activities (Table 5). There are reports that the hydroxyl radicals released by H_2O_2 can be hijacked by the chemical components of the products tested, such as the cardol, cardanol and anacardic acid [56].

Both iCNSL and tCNSL may also decrease the toxicity of H_2O_2 due to the phenolic compounds present in both liquids, thus the possible antioxidant activities may be due to their metal chelating and/or free radical scavenging as described by Khokhar et al. [57]. However, Kamath and Rajini [53] tested the antioxidant capacity of the ethanol extract of A. occidentale nuts showing the association capacity of phenolic compounds with transition metal inhibiting chemical reactions that release free radicals.

The excellent results of antioxidants of iCNSL and tCNSL in *S. cerevisiae* are the indicator of both liquids to have the action against deleterious effects of free radicals produced by the H_2O_2 **(Table 4**, **5** and **6)**, indicating inhibition of oxidative stress by natural products. Thus, these products can be used in pharmaceutical formulations to protect neurodegenerative diseases, including cognitive deficits that occur during normal aging brain, Alzheimer's disease and Parkinson's disease. Otherwise, oxidative stress results from ROS, is associated with the induction of breaks in double and single stranded DNA. Those events are considered most important for the emergence of degenerative human diseases such as cancer and aging. The ROS generated during physiologicals procdess, pathophysiological conditions, cell metabolism, cell proliferation, and so on may be kept in homeostatic accounts [52].

However, inappropriate repair of oxidative damage to the DNA may link to an increased risk of cancer in men. The most critical level of damage would be chromosomal breaks, because they reflect other factors beyond repair material, including kinetic antioxidant protection of the cell cycle. ROS are so intense that the oxidative damage is unavoidable and repair of genetic material can be lethal disabling mutations [58]. Small changes in the DNA base, including oxidation and alkylation, are principally repaired by means known as excision repair bases (BER). A lesion-specific glycosylase remove base, resulting a spot at the site of apurinic pyrimidine (AP). This site is filled by DNA polymerase with the aid of a ligase [59].

According to Stepanenko et al. [60], resorcinols/cardol can repair DNA damage. The later one is present in both iCNSL and tCNSL, thus the damage repair activity may linked with.

In a recent work of our research group found excellent antimutagenic, protection and improvement activities as observed by inhibition of micronucleus (MN) and chromosomal aberrations (CAs) induced by copper sulfate for both CNSLs, except for the highest concentration of iCNSL (69.5 mg/mL) by means of *Allium cepa* [30].

There are also reports that the cashew juice and cajuína (clarified cashew juice) from *A. occidentale* had repair effects in *S. typhimurium* due to the presence of phenolic compounds in both juices, possibly by stimulating repair or reversal of damage [61]. Phenolic compounds, like the flavonoids may be not only involved in antioxidative defense but also with DNA repair in human cells [62].

Damage to the genome spontaneously or induced by chemical and/or physical agent as well as for errors in replication leading to mutations results in inherited diseases and aging. The damage in bases can be generated ROS, which can also generate DNA breaks and $O2^{\bullet -}$, $^{\bullet}OH$ and H_2O_2. ROS can also oxidize RNA, lipids, proteins and nucleotides. The first line of defense against superoxide and RL are the enzyme superoxide sidmutase, and catalases that inactivate the toxicity of H_2O_2. A second line

defense is the DNA base incorporation, which is prevented by hydroxylase/oxidases (dNTPs), such as the 8-oxodGTP. The third line defense corresponds to damage repair mechanisms, base excision (BER), coupled to transcription repair, global genome repair, as well as the repair by homologous and non-homologous recombination. Moreover, these mechanisms are also integrated into the regulation of the cell cycle, transcription and replication [63].

Ascorbic acid, used in this study, has activity in DNA repair modulation, depending on the subject, nutritional status and plasma concentration of vitamin [64]. In vitro studies showed that this vitamin is involved in the expression of gene repair that could protect, prevent and reduce oxidative damage, interacting cytoplasmic or extracellular fluid, and directly interact with the RL, thus preventing oxidative damage [65].

Epidemiological and molecular studies show that AA plays an important role on genetic damage from DNA adducts, tapes breaks and oxidative damage. These effects were observed in the blood plasma of patients exposed to mutagens and supplementation with this vitamin (50 mM/lit), the decreased in chromosomal aberrations and micronuclei, as well as the frequency of genomic translocations. However, the impact of supplementation depends on the dose-response for each individual as well as the level of exposure to xenobiotic (air pollution, ionizing radiation, exposure to hydrocarbons) and oxidative stress [66].

Conclusion

Both CNSLs (iCNSL and tCNSL) produce no oxidative damage, otherwise effectively protects the tested proficient and mutant *Saccharomyces cerevisiae*. In addition, antioxidative defense and damage repair capabilities ensuring the earlier evidences on cashew nuts. Thus the compounds in cashew nuts may be consided as pharmaceutical and food consumptions as they may be an alternate economical source of natural antioxidants, with promising pharmacological actions for neurodegenerative diseases and cancer.

Ethical statement

A protocol number (0440051000-07) was deposited and approved by the Research Ethics Committee the National Council of Scientific and Technological Development (CNPq) under CAAE, Brazil.

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Conflict of Interests

The authors declare no conflict of interests.

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