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FOOD TECHNOLOGY

Extraction of bioactive compounds of lemongrass, antioxidant activity and evaluation of antimicrobial activity in fresh chicken sausage

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ABSTRACT: *The aim of this work was to determine the best extraction condition of bioactive compounds from lemongrass (Cymbopogon citratus), using the conventional method and ultrasonic assisted extraction, varying the temperature, in order to evaluate the antioxidant activity and the antimicrobial activity of the extract with higher antioxidant power in fresh chicken sausages during the storage period. The extracts were obtained by the conventional method (solvent extraction) and by ultrasound assisted extraction, varying the temperature (20°C, 40°C and 60°C). Phenolic compounds, total flavonoids and antioxidant activity were measured by the DPPH, FRAP, ORAC methods. Conventional extraction and ultrasound methods influenced the phenolic and total flavonoid content at all tested temperatures. Conventional and ultrasonic methods did not influence the IC_{50} at temperatures of 40°C and 60°C. The antioxidant activity by the DPPH method and by the FRAP method was superior in the conventional method at the temperature of 60°C, however by the ORAC method the best results were in the extraction by ultrasound. The results demonstrate that the conventional extraction at 60°C was better to obtain extracts of lemongrass with greater amount of bioactive compounds. The antimicrobial capacity evaluated in sausage of fresh chicken showed that in the concentration of 1.0% of the extract protected the product as the growth of mesophilic aerobes and against the growth of psychotrophic bacteria. Lemongrass can be considered as a natural alternative to obtain extracts rich in bioactive compounds, with antioxidant activity and high antimicrobial capacity.*
Key words: *Cymbopogon citratus, natural antioxidants, ultrasound, chicken sausage.*

Extração de compostos bioativos de capim-limão, atividade antioxidante e avaliação da atividade antimicrobiana em linguiça frescal de frango

RESUMO: *O objetivo deste trabalho foi determinar a melhor condição de extração de compostos bioativos do capim-limão (Cymbopogon citratus), usando o método convencional e extração assistida por ultrassom, em diferentes temperaturas, a fim de avaliar a atividade antioxidante e a atividade antimicrobiana do extrato com maior poder antioxidante em linguiças de frango frescal durante o período de armazenamento. Os extratos foram obtidos pelo método convencional (extração com solvente) e por extração assistida por ultrassom, variando a temperatura (20°C, 40°C e 60°C). Foram medidos os compostos fenólicos, flavonoides totais e atividade antioxidante pelos métodos DPPH, FRAP, ORAC. Os métodos de extração convencional e ultrassom influenciaram no teor de fenólicos e flavonoides totais em todas as temperaturas testadas. Os métodos convencional e ultrassom não influenciaram no IC_{50} nas temperaturas de 40°C e 60°C. A atividade antioxidante pelo método DPPH e pelo método FRAP foi superior no método convencional na temperatura de 60°C, entretanto pelo método ORAC os melhores resultados foram na extração por ultrassom. Os resultados demonstram que a extração convencional a 60°C foi melhor para obter extratos de capim-limão com maior quantidade de compostos bioativos. A capacidade antimicrobiana avaliada em linguiça frescal mostrou que na concentração de 1,0% do extrato protegeu o produto quanto o crescimento de aeróbios mesófilos e contra o crescimento de bactérias psicotróficas. O capim-limão pode ser considerado uma alternativa natural para obtenção de extratos ricos em compostos bioativos, com atividade antioxidante e elevada capacidade antimicrobiana.*
Palavras-chaves: *Cymbopogon citratus, antioxidantes naturais, ultrassom, linguiça de frango.*

INTRODUCTION

Lemongrass (*Cymbopogon citratus*), which belongs to the Poaceae family, is an aromatic plant cultivated for the commercial production of essential oil, which usually has the citral monoterpenes (mixture of geranial and neral isomers) and myrcene as main constituents (PRINS et al., 2008).

Many plant species have antimicrobial and antioxidant properties, among them, the medicinal plants stand out as they can perform an inhibitory action on the growth of undesirable microorganisms and reduce the oxidative processes, which makes

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them useful and a healthier alternative for the food industry, compared to chemical preservatives (JAYASENA & JO, 2014).

Meat products can deteriorate rapidly due to oxidative processes and microbial growth during the processing and storage steps. Therefore, delaying and preventing these processes are determining factors in the final quality of the product (KRISHNAN et al., 2014).

In order to ensure the safety of these products, increase their shelf life, control lipid oxidation and microbial growth, the industry uses chemical preservatives (MOREIRA et al., 2005). However, research associates these preservatives with mutagenic and carcinogenic effects. Given this, there has been a broad interest in finding phytochemicals from natural sources that could replace synthetic antioxidants. Compounds such as phenolics and flavonoids are widely found in plant products. These polyphenols are a large group of natural compounds, which have broad biological activity with applicability in several areas due to their effects as antioxidants, antitumor, antiviral, anti-inflammatory, antibiotic and allelopathic. (TANASE et al., 2015).

The extractive methods influence the rates of obtaining the bioactive compounds present in the plant matrices. Over the last decade, a number of new extraction techniques have been introduced and investigated, and the choice should be based on economic viability and suitability for each particular situation. Among these, ultrasonic extraction is a promising extractive technique because it promotes the exhaustive extraction of active plant principles with relatively small energy expenditure, time savings and greater safety in the process (VIZZOTTO & EREIRA, 2011).

Considering the above, the aim of this work was to determine the best extraction condition of bioactive compounds from lemongrass (*Cymbopogon citratus*), using the conventional method and ultrasonic assisted extraction, varying the temperature, in order to evaluate the antioxidant activity and the antimicrobial activity in chicken sausages during the storage period.

MATERIALS AND METHODS

The leaves of lemongrass were harvested in January in the rural area of Santa Maria (RS). The lemongrass was oven dried at 45°C (±5) for 48 hours, ground in a Willy-type knife mill and passed through a 20-mesh sieve. The material was stored in a freezer in polyethylene bags at -18°C until the end of the analysis.

Hydroalcoholic extracts were prepared from 5g of milled lemongrass added with 50ml of cereal alcohol 70%, 1:10 (w/v) ratio. The extraction time was set at 20 minutes in accordance with PIOVESAN et al. (2017). Conventional and ultrasound-assisted extraction was performed according to BOEIRA et al. (2018). For the extraction by the conventional method, an ultra-thermostatized bath (Marconi, model MA-184, São Paulo, Brazil) was used with constant shaker agitation (Marconi MA-039, São Paulo, Brazil). Ultrasonic bath (QUIMIS®, model Q335D, São Paulo, Brazil) was used for the ultrasonic extraction method operating at 40kHz frequency. Extracts were subsequently centrifuged at 202g for 10 minutes and filtered; the volume was adjusted to 50mL and they were then packed in amber bottles and stored in a freezer (-18°C) until analysis.

The determination of total phenolic compounds was performed by the Folin-Ciocalteu method described by ROESLER (2007). The content of total phenolic compounds was expressed in milligrams of gallic acid/g of dry lemongrass (mg GAE g^{-1}).

The total flavonoid content was determined by the method proposed by ZHISHEN, MENGCHENG & JANMING (1999). The total flavonoid content was expressed in mg quercetin equivalent/g of dry lemongrass (mg EQ g^{-1}).

The ferric reducing antioxidant power (FRAP) activity was determined according to the methodology described by BENZIE & STRAIN (1996). The calculation was performed by a calibration curve using TEAC (trolox equivalent antioxidant capacity). The results are expressed in µmol equivalents of trolox/g dry lemongrass (µmol TEAC g^{-1}).

DPPH

The determination of the antioxidant activity by the DPPH (2,2-diphenyl-1-picryl-hydrazyl) method was done according to BRAND-WILLIAMS, CUVELIER & BERSET (1995). The results were expressed as percentage (%) of inhibition of DPPH radical oxidation, according to equation (1):

$$\%DPHH_{radicalinhibition} = [(A_0 - A_s) \div A_0] \times 100 \quad (1)$$

Where A0 is control absorbance, As is the sample absorbance. For the calculation of the IC 50, the equation of the line obtained from the absorbance values (AA%) of the increasing concentrations of the samples was used, substituting the value of Y for 50, obtaining the value of X as the sample concentration with capacity to reduce 50% of DPPH. The IC_{50} value will be determined by the equation

of the line plotted through the results containing the concentration values (mg/mL) used on the X axis and the percentages of protection found on the Y axis. A standard Trolox curve in *µmol* versus % inhibition will also be developed, where the result will be expressed in *µmol* equivalents of TEAC/g of dry lemongrass (µmol TEAC g^{-1}).

The oxygen radical absorbance capacity (ORAC) was analysed as proposed by DÁVALOS, GÓMEZ-CORDOVÉS & BARTOLOMÉ (2004). The standard curve was prepared with Trolox solution (0 to 96mM), and the results were expressed in µmol Trolox equivalents per gram of lemongrass (µmol Trolox/g).

The process of the chicken sausage was carried out in a meat pilot plant (Rural Sciences Centre, UFSM) and the recommendations described by TERRA (1998) were used, following the quantities of ingredients described in the Legislation (BRASIL, 2000) according to table 1. The elaboration of chicken sausages started with the separation of meat and skin (boneless chicken breast and thigh) using sterilized knives. Then, the grinding of the chicken meat and skin (Jamar PJ22 Grinder, Jamar Ltda, São Paulo, Brazil) was performed on a disc with 12mm diameter, and were taken to the mixer (Jamar MJI 35, Jamar Ltda, São Paulo, Brazil) for the addition of the other ingredients until the formation of the alloy.

In order to provide a better comparison of the results, the following was elaborated: a standard formulation (T1), added with synthetic antioxidant, which is the same used in commercial sausages (Sodium erythorbate); a control formulation (T2), with no addition of antioxidants; addition of 0.5% and 1.0% lemongrass extract (T3 and T4), respectively (Table 1). The addition of predefined aliquots of hydroalcoholic extracts was added manually. Three replicates were performed for each treatment (N=12). For storage, the chicken sausages were packed in polystyrene trays, packed with film paper, identified and stored in a refrigerator at 4°C.

Microbiological analyses were performed to verify the stability of the sausage. Total aerobic and total psychrotrophic analyses were followed up every seven days, until the forty-two days of storage at 4°C were completed. Subsequently, portions of 25g of chicken sausage were homogenized with 225mL of peptone water and the dilutions (up to 10^{-5}) in 0.1% peptone water were used for microbiological analyses. Total aerobic mesophilic microorganisms were counted using standard agar culture medium in plates by depth (37°C/48h) and the counting of psychrotrophic microorganisms was done using the standard agar culture medium with inoculation in surface plates (7°C/7 to 10 days) (BRASIL, 2003).

The extracts and the analyses were conducted in triplicate. The experiment was repeated

Table 1 - Formulation used for fresh chicken sausages elaboration.

Ingredients (%)	T1	T2	T3	T4
Chicken patches	88	88	88	87.53
Chicken skin	5.43	5.53	5.03	5.0
Water/ice	3.0	3.0	3.0	3.0
Salt	234	2.34	2.34	2.34
Quick cure	0.23	0.23	0.23	0.23
Ground garlic	0.09	0.09	0.09	0.09
Ground black pepper	0.09	0.09	0.09	0.09
Glutamate	0.04	0.04	0.04	0.04
Color fastener	0.23	0.23	0.23	0.23
Seasoning for chicken sausage*	0.45	0.45	0.45	0.45
Sodium erythorbate	0.1	-	-	-
Lemongrass extract	-	-	0.5	1.0
Total	----------100%----------			

T1- Added synthetic antioxidant (Sodium Erythorbate).
T2 - No addition of antioxidant.
T3 - With addition of 0.5% lemongrass extract.
T4 - With addition of 1.0% lemongrass extract.
*Amount recommended by the supplier - Bremil Food industry LTDA, Arroio do Meio, Rio Grande do Sul - Brazil.

three times. The results were submitted to analysis of variance (ANOVA) and the means compared to each other through the Tukey test, at 5% significance, through the statistical software Statistica® 7.0.

RESULTS AND DISCUSSION

The total phenolic and total flavonoids content of lemongrass extracts are presented in table 2. The extract obtained by the conventional method at 20°C presented higher phenolic content when compared to the other temperatures tested in this method. Studies by Mokrani & Madani (2016) corroborate that the increase in temperature from 25°C to 70°C reduced the content of phenolic compounds of peach (*Prunus persica* L.) fruit. Temperature above certain values can promote concomitant degradation of phenolic compounds that have been previously mobilized or even the decomposition of remaining phenolic residues in the plant matrix.

For the ultrasonic extraction method, the increase in temperature positively influenced the total phenolic content. TABARAKI et al. (2012) reported that heating plant extracts at temperatures between 52°C and 67°C can soften the cell wall tissue and hydrolyze the phenolic compounds (phenol-protein or polysaccharide-phenol), thereby increasing their solubility. When comparing the two methods of extraction, it is possible to observe that at 40 and 60°C, the extracts obtained did not show a significant difference in phenolic content, that is, the extraction method did not influence the results at these tested temperatures.

Table 2 - Total phenolics, total flavonoids, inhibitory capacity (IC_{50}), iron reduction capacity (FRAP), oxygen radical absorption capacity (ORAC) and antioxidant activity (DPPH) of extracts obtained by different extraction methods at different temperatures.

Temperature (°C)	Conventional method	Ultrasound method
----------Total phenolics (mgGAE/g)----------		
20°C	$141.42^{Aa} \pm 1.71$	$111.73^{Bb} \pm 2.94$
40°C	$121.80^{Cb} \pm 3.92$	$114.22^{Bb} \pm 0.24$
60°C	$133.84^{Ba} \pm 2.29$	$135.81^{Aa} \pm 4.58$
----------Total flavonoids (mgQE/g)----------		
20°C	$13.99^{Aa} \pm 1.52$	$10.33^{Ab} \pm 1.16$
40°C	$13.53^{Aa} \pm 1.40$	$11.46^{Ab} \pm 0.64$
60°C	$13.42^{Aa} \pm 0.27$	$12.09^{Ab} \pm 0.48$
----------IC_{50} (mg/mL)----------		
20°C	$0.89^{Ab} \pm 0.68$	$1.46^{Aa} \pm 0.20$
40°C	$0.71^{Aa} \pm 0.11$	$0.63^{Ba} \pm 0.08$
60°C	$0.45^{Ba} \pm 0.01$	$0.52^{Ba} \pm 0.10$
----------FRAP (µmol TEAC/g)----------		
20°C	$8.20^{Ba} \pm 0.64$	$3.37^{Ab} \pm 0.84$
40°C	$8.10^{Ba} \pm 0.52$	$5.40^{Ab} \pm 1.36$
60°C	$9.70^{Aa} \pm 0.69$	$4.80^{Ab} \pm 0.83$
----------ORAC (µmol Trolox/g)----------		
20°C	$326.35^{Ca} \pm 4.31$	$283.65^{Cb} \pm 3.32$
40°C	$364.00^{Aa} \pm 1.20$	$355.60^{Bb} \pm 3.11$
60°C	$353.95^{Bb} \pm 5.40$	$405.85^{Aa} \pm 4.50$
----------DPPH (µmol TEAC/g)----------		
20°C	$40.37^{Ca} \pm 3.08$	$25.69^{Cb} \pm 4.61$
40°C	$64.12^{Ba} \pm 2.43$	$39.59^{Bb} \pm 7.73$
60°C	$89.47^{Aa} \pm 1.66$	$80.05^{Ab} \pm 1.45$

Results are expressed as Mean±SD (n=9).
GAE=Gallic acid equivalent; QE: Quercetin equivalent.
TEAC=trolox equivalent antioxidant capacity.
^{A,B}Equivalent capital letters in the same column did not present significant differences by Tukey's test (P>0.05) in the same extraction method between the different tested temperatures. ^{a,b}Equal lowercase letters on the same line do not show significant difference between the same temperatures for the different extraction methods.

In addition, by comparing the methods in relation to the flavonoids, the extract obtained by the conventional method proved to be better at all temperatures. Research has shown that a number of beneficial effects on health can be attributed to the phenolic compounds present in fruits, vegetables and medicinal herbs, such as antioxidant, anti-inflammatory, antimicrobial and anticarcinogenic activity. In addition, the consumption of flavonoid-containing substances is associated with a lower mortality risk due to cardiovascular diseases, and even in small amounts present may be beneficial to human health (MCCULLOUGH et al., 2012; SOMPARN et al., 2018).

Different techniques are used to determine in vitro antioxidant activity in order to allow the selection of pure substances and matrices with this property. In the present study, the antioxidant activities of lemongrass extracts were evaluated by the following methods: DPPH, FRAP and ORAC (Table 2). The antioxidant capacity of extracts can be influenced by many factors, such as extraction methods and/or methods used for quantification, so it is necessary to perform different evaluations to determine the various mechanisms of action that can develop. Most of the analyzes carried out use oxidative processes, which involve the addition of an initiator to accelerate the process, such as temperature, agitation, oxygen availability, transition metal or even light exposure (ANTOLOVICH et al., 2002).

The IC_{50} is a parameter used to determine the antioxidant potential of plants. It demonstrates the amount of plant extract that is needed to capture the DPPH radical by 50%. The higher the IC_{50}, greater the amount of substance required to perform antioxidant activity, thus, a low IC_{50} means the plant has a great antioxidant power (NEGRELLE & GOMES, 2007). Table 2 shows that in the extraction by the conventional method at 60°C the IC_{50} of the extract was lower consequently, the temperature rise increased the antioxidant activity, in the same way the FRAP method the conventional extraction was influenced by the temperature increasing the activity with the temperature increase, however by the ORAC method the temperature favoring the conventional extraction was 40°C.

The method of extraction assisted by ultrasound positively influenced the antioxidant activity by the ORAC in the temperature of 60°C differing statistically from the others. The antioxidant activity by DPPH in the conventional method was higher at the temperature of 60°C, coinciding with the lower IC_{50}, as well as in the ultrasound method the increase in temperature influenced positively. According to SHIRSATH et al. (2012), one of the parameters that are noteworthy in the extraction by ultrasound is the frequency of the device, since according to the author, lower frequencies of about 20kHz are more effective in materials of plant origin. This is the case of lemongrass, due to the effects promoted by cavitation during extraction, since the bubbles generated in the system can implode more easily than the bubbles generated at high frequency, thus facilitating the release of the compound (ESCLAPEZ et al., 2011). In this research, the frequency of the device used was 40kHz, which may justify the fact that the conventional method obtained better antioxidant activity by the DPPH and FRAP methodologies.

In general, the conventional extraction was better to obtain extracts of lemongrass, rich in bioactive compounds with high antioxidant activity. The results also show that the temperature of 60°C presented higher antioxidant activity.

In order to verify the antimicrobial activity of the lemongrass extracts during the storage period of the chicken sausages, the psychotrophic and aerobes mesophilic microorganisms were counted (Figure 1). According to TERRA (1998), the count of up to $10^6 CFU.g^{-1}$ is considered an acceptable range of microbial contamination in food, which also indicates the food sanitary quality.

In the results obtained from the counts of psychotrophic microorganisms (Figure 1), the addition of the lemongrass extract at 1.0% concentration (T4) maintained the product stable until the end of the storage when compared to the other treatments, in a total of 42 days. Psychotrophic microorganisms are not inhibited by the refrigeration effect and, consequently, they reduce the shelf life of fresh products (FRANCO & LANDGRAF 1999).

Furthermore, it is observed that for the counting of aerobes mesophilic, in the period from 0 to 14 days of storage, in all treatments were less than 10^6 CFU g^{-1} (Figure 1). The addition of the extract in the fresh sausage and chicken, in proportions 0.5% and 1.0%, provided the microbiological stability up to the 21st day of storage, emphasizing that the 1.0% concentration protected the product until the 42st day against the growth of psychrotrophs. VIEDMA et al. (2017), when evaluating the antimicrobial activity of raw Stevia extracts (*Stevia rebaudiana Bert*.) at concentrations above 0.5% in salmon paste, observed that for the total of mesophiles there was protection efficiency only until the fourteenth day. Therefore,

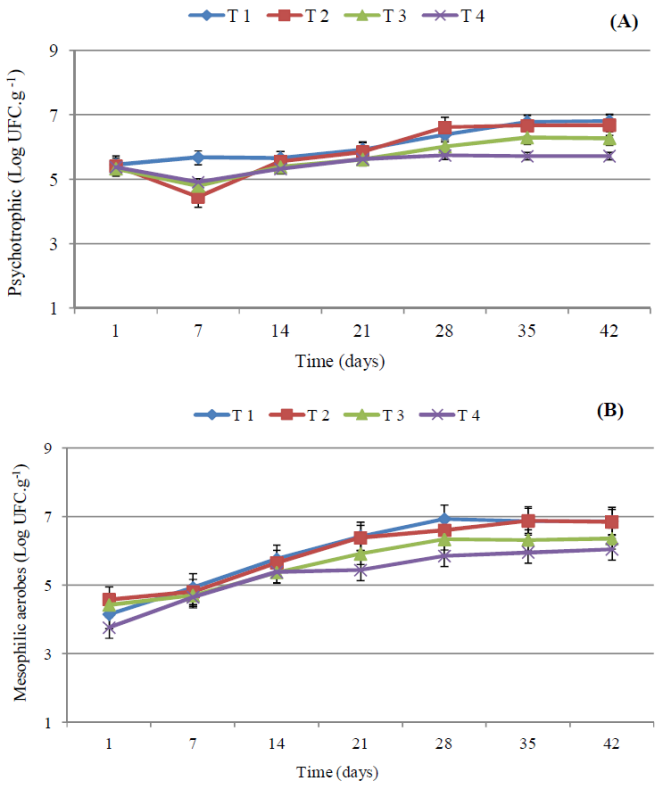

Figure 1 - Total psychrotrophic (A) and aerobic mesophilic (B) microorganisms from fresh chicken sausages treated with different antioxidants during the storage period at 4°C. T1- Added synthetic antioxidant (Sodium Erythorbate); T2 - No addition of antioxidant; T3 - With addition of 0,5% of lemongrass extract; T4 - With addition of 1,0% lemongrass extract.

it is noteworthy that the addition of the lemongrass extract (1.0%) in the present study was efficient in keeping fresh chicken sausage stable until the 21st day of storage in relation to total mesophiles.

CONCLUSION

Conventional extraction was better to obtain extracts of lemongrass, rich in bioactive compounds with high antioxidant activity. The results also show that the temperature of 60°C presented higher antioxidant activity. The evaluation of antimicrobial capacity in fresh sausage showed that the concentration of 1.0% extract maintained the product stable until the 21st day of storage for the growth of mesophilic aerobes and protected the product during the 42 days of storage against the psychotrophic bacteria. Thus, lemongrass can be considered a promising natural source of extracts that are rich in antioxidant and antimicrobial compounds in order to replace synthetic antioxidants in the food industry, due to its low cost and high availability.

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DECLARATION OF CONFLICTING INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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