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Assessment of continuous and intermittent drying on antixidant activity of corn grains

Avaliação da secagem contínua e intermitente sobre a atividade antioxidante de grãos de milho

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Grain drying is essential for storage to preserve material quality. However, drying can affect the composition of agricultural material, as exposure to high temperatures for long periods can reduce the quality of the grain, in terms of antioxidant activity and germination power. The objective of this work was to assess the continuous and intermittent drying, in order to maximize the preservation of corn grains antioxidant potential. Experiments were conducted at 40, 55, and 70 °C with tempering periods of 5 and 10 minutes. Solvents used were methanol/water 70/30 (v/v), methanol/water 95/5 (v/v), ethanol/water 70/30 (v/v), and ethanol/water 95/5 (v/v) and extraction times of 1, 4, and 24 hours. Antioxidant activity analysis was carried by DPPH and ABTS radicals scavenging and the best results of antioxidant activity for *in natura* grains were obtained with methanol/water 70/30 (v/v) and 4 h. Intermittent drying of 5 and 10 minutes' periods at 40 °C led to least reduction of antioxidant activity percentage compared to *in natura* corn results. This study demonstrated that intermittent drying conducted at low temperatures is an effective alternative to preserve bioactive compounds of corn.
Keywords: drying methods, extraction, bioactive compounds.

A secagem do grão é essencial para o armazenamento no intuito de preservar a qualidade do material. No entanto, a secagem pode afetar a composição do material agrícola, pois a exposição a altas temperaturas durante longos períodos pode diminuir a qualidade do grão, em termos de atividade antioxidante e poder germinativo. O objetivo deste trabalho foi avaliar a secagem contínua e intermitente, a fim de maximizar a preservação do potencial antioxidante dos grãos de milho. Os experimentos foram conduzidos a 40, 55 e 70 °C com períodos de intermitência de 5 e 10 minutos. Os solventes utilizados foram metanol/água 70/30 (v/v), metanol/água 95/5 (v/v), etanol/água 70/30 (v/v), e etanol/água 95/5 (v/v) e tempos de extração de 1, 4 e 24 horas. A análise da atividade antioxidante foi realizada por sequestro de radicais DPPH e ABTS e os melhores resultados da atividade antioxidante para grãos *in natura* foram obtidos com metanol/água 70/30 (v/v) e 4 h. A secagem intermitente com períodos de intermitência de 5 e 10 minutos a 40 °C levou à menor redução da porcentagem de atividade antioxidante em comparação com os resultados do milho *in natura*. Este estudo demonstrou que a secagem intermitente realizada em baixas temperaturas é uma alternativa eficaz para preservar os compostos bioativos do milho.
Palavras-chave: métodos de secagem, extração, compostos bioativos.

1. INTRODUCTION

The agroindustry is one of the most important sectors in the world economy, mainly in relation to grain production, and corn, wheat, rice, barley, and sorghum are some of the principal products in this grain production [1].

Corn (*Zea mays* L.; Poaceae family) is a cereal consumed worldwide, and it contains high nutritional power, with high content of starch, protein, vitamins, and minerals [2-6]. It is also composed of phytochemicals with bioactivity as carotenoids (lutein, zeaxanthin, and β-cryptoxanthin), tocopherols, phytic acid, anthocyanins (cyanidin-3-glycoside, pelargonidin-3-glycoside, and peonidine-3-glycoside), flavonoids (hirsutrin, 3'-methoxyhirsutrin), phenolic compounds, as ferulic acid, *p*-coumaric acid, *o*-coumaric acid, protocatechuic acid, vanylic acid, 2,4,6-trihydroxybenzoic acid, and *p*-hydroxycinnamic acid [2-8].

As this grain is produced on large scale due to its demand in animal and human feed, it needs to be stored. Drying is an essential process for this storage to preserve grain quality. Corn grains are recommended to be stored at a moisture content from 12.5 to 14% on a dry basis (d.b.), and it is harvested in a range between 25 and 28% (d.b.) of moisture content [9].

The types of drying most used are continuous and intermittent, in which occurs mass and heat transfer between the grain and the hot air simultaneously. During drying process, an amount of heat is applied to the material, which provides the grain and its inner water heating, leading to the liquid evaporation. As the energy demand is high during this process and there are problems in grain quality under continuous drying, such as crack, loss of vigour, decrease in germination power and antioxidant activity and fissure, intermittent drying is an alternative applied that aims to minimize these problems [10].

During intermittent drying process, at first there is injection of hot air followed by tempering period. This procedure is repeated until the ideal moisture content of storage is achieved. The exposure of hot air enables the evaporation of water at the surface of the grain, and during the tempering periods water diffuses from the middle to the surface. Intermittent drying operation is also beneficial to thermosensitive materials, such as grains, including soybean, corn, coffee, rice, vegetables, fruits, and herbs, due to the fact that the time in which the material is in contact with hot air is reduced [11].

Furthermore, intermittent drying is also beneficial when drying is controlled by internal mass transfer due to water diffusion inside the grain. It is commonly observed during drying that the internal moisture distribution is not uniform. However, tempering periods between hot air injections leads to greater stabilization of moisture content distribution inside the grain. In general, materials in which there is a predominance of internal mass transfer are agricultural products. Intermittent drying of corn grains provided higher physiological quality grains and greater storage potential, in relation to continuous operation [12].

Optimization of intermittent drying related to inlet air temperature and tempering periods leads to reduction of energy expenditure, increase of dried grain quality retention and preservation of the bioactive components of the final product [10].

Among bioactive compounds present in corn, there are compounds that present antioxidant potential. Antioxidants are substances found in several foods, such as grains, green leaves, grains, and fruits, which are characterized by the ability of eliminating free radicals present in human body, chelating activity of transition metals, and singlet oxygen deactivators, preventing the development of diseases [13].

Compounds with antioxidant potential are related with biological activities, such as anticarcinogenic, anti-inflammatory, and prevent diseases like respiratory, neurodegenerative, diabetes, and obesity [14]. Currently, several methodologies have been developed in order to analyse antioxidants. Choice of solvent for extraction of compounds with antioxidant properties is an essential factor. Water in combination with other organic solvents contributes to creation of a moderately polar medium, which promotes the extraction of polyphenols [15].

Extraction time is also important, as it can influence the extraction efficiency of antioxidant compounds. If extraction time is not sufficient, equilibrium concentration is not reached, and if it is too high, a reduction of the compounds can occur due to oxidative stress and decomposition [16].

Studies evidenced that by operating with intermittent drying it was possible to increase the conservation of bioactive compounds in natural products, when compared to conventional operation. Qu et al. (2016) [17] demonstrated that nuts submitted to intermittent drying reported higher levels of flavonoids in comparison to conventional drying.

In this context, the objective of this work was to assess corn seed drying under continuous and intermittent operation, aiming to obtain the best operational conditions to maximize the preservation of grain quality in terms of antioxidant activity. It was also studied the extraction time and four hydroalcoholic solvent systems to optimize the antioxidant compounds extraction from the grains.

2. MATERIALS AND MÉTHODS

2.1 Drying process

Drying of corn grains (NS 90 RR2/PRO/PRO2 hybrid) was carried out in oven drying (Nova Ética) at temperatures of 40 °C, 55 °C, and 70 °C. 100.00 g of grains were placed in thin layer trays. Experiments were carried out in duplicate with random sequence, in continuous and intermittent operation, with tempering periods of 5 and 10 minutes. The total drying time considered for all experiments was 90 minutes. Grains samples were left at 105 ± 2 °C during 24 hours in the oven to determine an average of moisture content over time.

2.2 Preparation of extracts

Four hydroalcoholic solvent systems were assessed to determine the best solvent and extraction time for *in natura* grains that presented the highest levels of extract antioxidant activity. Extractions were conducted with solvents methanol/water (MeOH/H_2O) 70/30 (v/v), methanol/water (MeOH/H_2O) 95/5 (v/v), ethanol/water (EtOH/H_2O) 70/30 (v/v), and ethanol/water (EtOH/H_2O) 95/5 (v/v) and extraction times of 1, 4, and 24 hours.

Grains samples were grinded and 1.0000 g of the sample was diluted in 100.0 mL of each hydroalcoholic solvent system. Extraction was carried out in a shaker (Marconi, model MA-420) with stirring of 180 rpm at room temperature. Extracts were filtered with an analytical funnel and qualitative filter paper (80 g m^{-2}) to a 100.0 mL volumetric flask, solution volume was adjusted, and extracts were stored under refrigeration in amber glass to further analysis. All extractions were done in duplicate at random sequence.

After determining the solvent and extraction time that provided the best results to antioxidant activity, extracts were prepared with dehydrated corn grains submitted to intermittent and conventional drying.

2.3 Determination of antioxidant activity by the DPPH free radical scavenging method

It was prepared a 0.1192 mM solution of DPPH (2,2-Diphenyl-1-picrylhydrazyl) in methanol (MeOH) and a control by adding 1.0 mL of MeOH and 2.0 mL of DPPH ($A_{control}$) solutions. A blank was prepared for each sample, using 1.0 mL of extract and 2.0 mL of solvent (A_{blank}). Samples were prepared with 1.0 mL of extract and 2.0 mL of DPPH (A_{sample}). Samples were analysed in triplicate with random sequence, according to Brand-Williams et al. (1995) [18] with modifications. Absorbance of samples were obtained on spectrophotometer (CARY 60 UV-VIS) at 517 nm after 30 minutes. Percentage of antioxidant activity (%AA) was calculated as expressed in Equation 1. BHT (butylhydroxy-toluene) and ascorbic acid at a concentration of 0.1 g L^{-1} were used as standard.

$$\%\, AA\ DPPH = \frac{A_{control} - (A_{sample} - A_{blank})}{A_{control}} . 100 \quad (1)$$

2.4 Determination of antioxidant activity by the ABTS free radical cation scavenging method

ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) solution was prepared with 192 mg of ABTS diluted in 50 mL of distilled water and a potassium persulfate solution was prepared with 378.4 mg of salt diluted in 10 mL of distilled water. Solutions were stored in amber glasses under refrigeration. $ABTS^{\cdot+}$ radical cation solution was prepared with 5 mL of ABTS solution and 88 µL of potassium persulfate solution. This solution was stored for 16 hours in amber glasses at room temperature. 1.0 mL of $ABTS^{\cdot+}$ radical cation solution was diluted in approximately 70 mL of solvent, until reaching an absorbance of 0.900 ± 0.050 at 734 nm. Solutions were prepared and used for analysis at the same day. During absorbance measures, it

was prepared a control with 30 µL of EtOH or MeOH and 3 mL of $ABTS^{\cdot+}$ solution ($A_{control}$), a blank using 30 µL of extract and 3 mL of solvent EtOH or MeOH (A_{blank}) and a sample with 30 µL of extracts and 3 mL of $ABTS^{\cdot+}$ solution (A_{sample}) [19]. Absorbance readings were obtained on a spectrophotometer (CARY 60 UV-VIS) at 734 nm after 6 minutes. Percentage of antioxidant activity (%AA) was calculated as expressed in Equation 2. BHT (butylhydroxy-toluene) and ascorbic acid at a concentration of 0.1 g L^{-1} were used as standard.

$$\%\ AA\ ABTS^{*+} = \frac{A_{control} - (A_{sample} - A_{blank})}{A_{control}} . 100 \quad (2)$$

2.5 Statistical analysis

The results presented were obtained through the mean of the repetitions ± standard deviation and were statistically analysed by the Tukey test ($p < 0.05$), with multiple comparisons. Statistical analyses were performed using the Statistica® 8.0 (StatSoft, Inc.) software.

3. RESULTS AND DISCUSSION

3.1 Effect of solvents on extractions

Results of antioxidant activity by DPPH radical scavenging method for corn samples *in natura* (Table 1) demonstrated that the best conditions were observed for solvent MeOH/H_2O 70:30 (v/v) with extractions times of 1 h and 4 h. It was verified that shorter extraction times favor the antioxidant activity of the corn seed because it is a reaction that quickly reaches equilibrium concentration, and at high reaction time there can be decomposition of sample's antioxidant compounds. Regarding the methanol/water extract in comparison with ethanol/water, for DPPH free radical analyses, the methanolic extract favors in comparison to the ethanolic extract.

Table 1: Results of percentages of antioxidant activity (% AA) of corn extracts in natura.

Time	Solvent (v/v)	%AA DPPH	%AA ABTS
1h	MeOH/H_2O 95:5	50.58±1.59^{d}	12.96±0.39^{c}
	MeOH/H_2O 70:30	79.17±1.44^{a}	11.47±0.13^{d}
	EtOH/H_2O 95:5	27.21±194^{f}	11.34±0.32^{d,e}
	EtOH/H_2O 70:30	56.98±0.60^{c}	1.90±0.28^{g}
4h	MeOH/H_2O 95:5	70.55±0.55^{b}	11.32±0.12d,e
	MeOH/H_2O 70:30	77.69±0.87^{a}	14.72±0.42^{a,b}
	EtOH/H_2O 95:5	32.66±0.67^{e}	8.34±0.15^{f}
	EtOH/H_2O 70:30	71.78±0.83^{b}	10.11±0.24^{e}
24h	MeOH/H_2O 95:5	53.06±0.53^{c,d}	13.50±0.22^{b,c}
	MeOH/H_2O 70:30	68.54±0.80^{b}	15.04±0.24^{a}
	EtOH/H_2O 95:5	53.52±1.42^{c,d}	13.45±0.23^{c}
	EtOH/H_2O 70:30	70.97±1.11^{b}	15.82±0.20^{a}
Standard			
BHT	-	99.45	98.24
Ascorbic Acid	-	99.68	97.73

Results expressed as mean (n = 6). Equal letters in the same column indicate that there are no significant differences ($p < 0.05$) by the Tukey test.

It was found that shorter extraction times provided better values of antioxidant activity by the DPPH method, demonstrating that the extraction kinetics of bioactive compounds was fast. With increasing extraction time, decomposition of the sample's antioxidant compounds may occur. This same behavior was observed for the extraction of phenolic compounds for dry rice grains [20].

For the ABTS method, the best extraction conditions were obtained by MeOH/H_2O 70:30 (v/v) solvents with 4 h and 24 h of extraction and by EtOH/H_2O 70:30 (v/v) with 24 h. It was also verified no significant difference between these values. Results observed by the ABTS method were different from those of the DPPH in relation to the extraction time. For the ABTS method, longer times provided extracts with greater antioxidant potential. These results are in agreement with Spigno et al. (2007) [16], who verified that longer extraction times generally lead to greater amounts of extracted antioxidant compounds, resulting in better results by the ABTS method.

Based on these results, extractions using MeOH/H_2O 70:30 (v/v) solvent and 4 h of extraction time was carried out to assess the antioxidant potential by free radical scavenging DPPH and ABTS methods for corn dried grains. The results indicate that type of solvents and time extraction influence antioxidant activity potential for corn grains. Several studies found in the literature report the influence of the type of solvent extractor in natural products on the antioxidant activity of the extracts [21-23], which is in agreement with the present work.

3.2 Effect of drying on corn grains antioxidant activity

After extraction conditions were optimized, extracts were prepared with corn grains submitted to continuous and intermittent drying, and their antioxidant potentials were assessed to verify the influence of drying operation on antioxidant activity preservation.

Extracts were prepared with corn grains dried under continuous and intermittent operation, and their antioxidant activities were assessed by DPPH and ABTS tests. The percentage of antioxidant activity results are listed in Table 2.

Table 2: Results of percentages of antioxidant activity (% AA) of corn extracts (MeOH/H2O 70:30; 4 h) subjected to drying.

Drying	Temperature (ºC)	%AA DPPH	%AA ABTS
Continuous	40	55.14 ± 0.74^{c}	8.82 ± 0.14^{b}
	55	$52.94\pm0.55^{c,d}$	$5.21\pm0.13^{d,e}$
	70	51.13 ± 0.64^{d}	4.68 ± 0.15^{e}
Intermittent 5 min	40	69.52 ± 0.64^{a}	$8.98\pm0.14^{a,b}$
	55	71.38 ± 0.55^{a}	6.38 ± 0.12^{c}
	70	46.06 ± 0.89^{e}	4.03 ± 0.11^{f}
Intermittent 10 min	40	68.40 ± 0.78^{a}	9.39 ± 0.09^{a}
	55	58.98 ± 0.55^{b}	5.53 ± 0.08^{d}
	70	$51.91\pm1.26^{c,d}$	5.39 ± 0.10^{d}

Results expressed as mean (n=6). Equal letters in the same column indicate that there are no significant differences ($p < 0.05$) by the Tukey test.

The results of percentage of antioxidant activity of the DPPH radical demonstrate that the intermittent dryings of 5 minutes at 40 °C and 55 °C, and 10 minutes at 40 °C were the ones that presented the best responses, not presenting significant differences between their average values ($p < 0.05$). For the ABTS method, the best results were obtained with the extracts from intermittent drying of 5 and 10 minutes at 40°C, not presenting significant differences between their average values ($p < 0.05$). These results demonstrate that intermittent drying at lower temperatures is an efficient alternative for maintaining corn antioxidant compounds. A

considerable decrease in antioxidant activities was observed when the drying temperature was 70 °C for both methods studied, which can be attributed to the decomposition of antioxidant compounds in corn.

It was also observed that for all drying tests (Table 2) there was a decrease in antioxidant activity when compared to MeOH/H_2O 70/30 and 4h extract from *in natura* grains (Table 1). By the DPPH method, the decrease in antioxidant activity was 10.52%, 8.12%, and 11.96% for intermittent drying of 5 minutes at 40 °C and 55 °C, and 10 minutes at 55 °C, respectively. The greatest reduction in activity was 40.71% for intermittent drying of 5 minutes at 70 °C. These results are in agreement with the observation by Keser et al. (2020) [24], who report a decrease in antioxidant activity due to exposure of grains to temperatures higher than the room temperature.

Xu et al. (2010) [6] demonstrated that phenolic content of corn grains was positively correlated to DPPH radical scavenging, suggesting their decomposition with exposure to temperatures higher than 55 °C.

Figure 1 depicts kinetic curves related the percentage of DPPH scavenging over time at room temperature. It can be verified, according to the classification of Sánchez-Moreno et al. (1999) [25], that reactions obtained from drying with intermittence of 5 minutes at 40 °C and 55 °C and intermittence of 10 minutes at 55 °C extracts can be considered as fast kinetics (consumption of 50% of free radical is lower than 5 minutes). Reactions obtained from intermittent drying of 5 minutes at 70 °C extracts were described by slow kinetics (consumption of 50% of free radical is higher than 30 minutes). The other extracts reactions were described by intermediate kinetics (consumption of 50% of free radical is between 5 and 30 minutes). These results indicate that reaction kinetics related to DPPH scavenging by extracts components are influenced by air drying temperature. This may also indicate decomposition of antioxidant compounds with temperature increase.

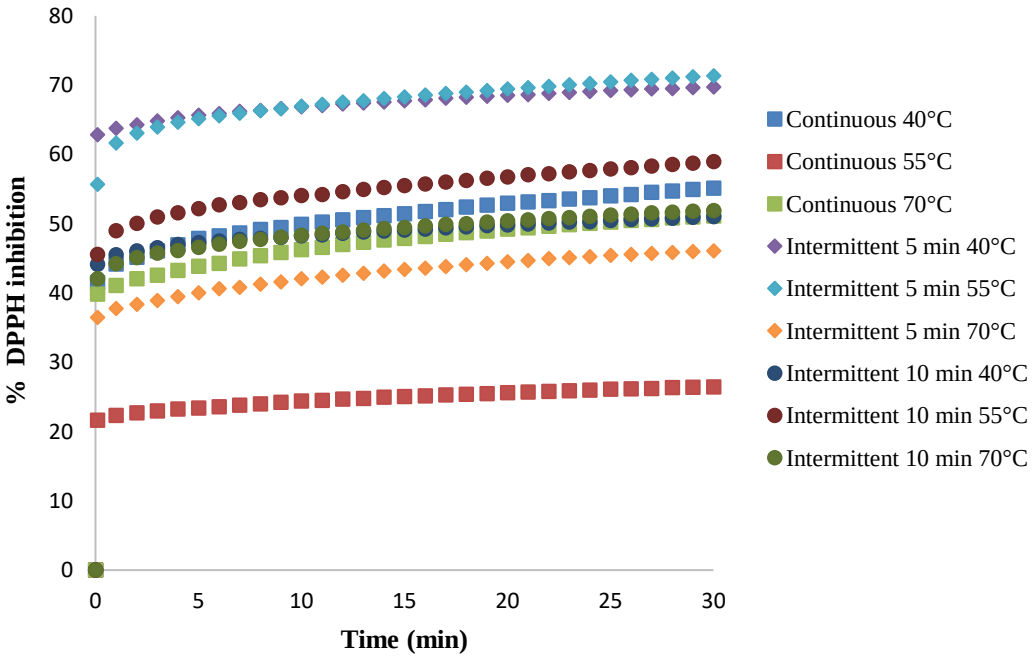

Figure 1: Kinetic curves in relation to the percentage of DPPH radical scavenging.

It was also possible to observe a decrease in antioxidant potential of extracts prepared with dehydrated corn grains (Table 2) in comparison to corn *in natura* (Table 1) for the ABTS method. There were also lower radical inhibition values for drying carried out at 70 °C in comparison to drying at 40 °C and 55 °C results, which demonstrate that bioactive substances composition of grains was influenced by air temperature.

The lowest reductions in antioxidant activity for the ABTS method in comparison to *in natura* extracts prepared with MeOH/H_2O 70/30 during 4 h were observed for samples extracts submitted to intermittent drying of 5 and 10 minutes at 40 ºC. Reduction percentage averages were 38.99% and 36.21%, respectively. Extracts prepared from grains submitted to conventional drying at 70 °C and intermittent drying of 5 min at 70 °C were the ones that presented the greatest decrease in antioxidant potential (68.21% and 72.62%, respectively).

Kinetic curves of the ABTS inhibition percentage over time at room temperature are depicted in Figure 2. It is possible to observer a slight increase in inhibition percentages over time. Analogously to DPPH kinetic curves, it was also verified that reaction kinetics related to ABTS inhibition by extracts components are influenced by air drying temperature, which may also be related to decomposition of antioxidant compounds with temperature increase.

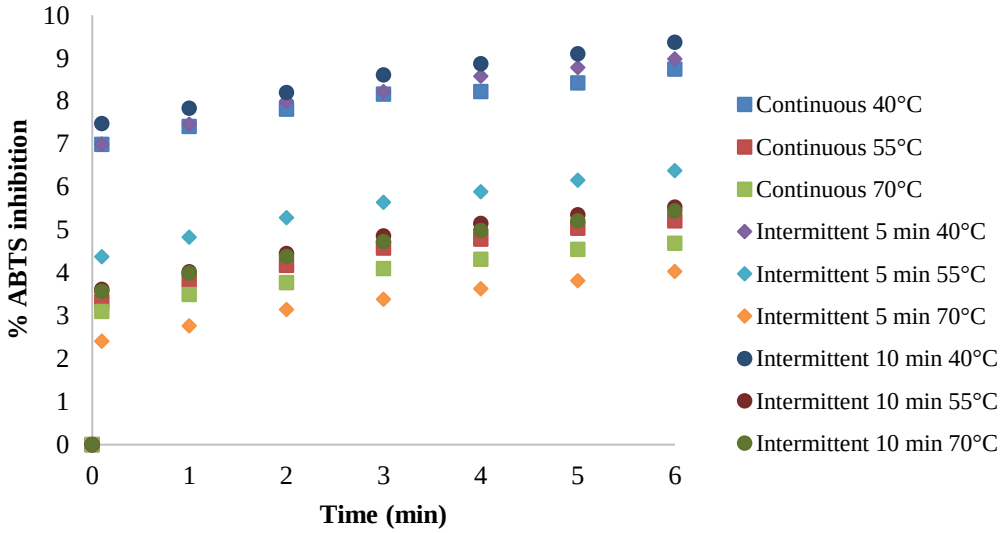

Figure 2: Kinetic curves in relation to the percentage of ABTS radical scavenging.

Based on these results obtained by DPPH and ABTS methods, intermittent drying at 40 °C and 5 and 10 minutes of tempering time provided the lowest reductions in antioxidant potential in comparison to *in natura* grains extracts results.

It can be analyzed that for all method assessed, high temperatures lead to a significant decrease in antioxidant potential of corn seed extracts, even when submitted to intermittent drying. However, antioxidant potential of seed extracts submitted to intermittent drying were higher in comparison to continuous drying results in general. Manach et al. (2004) [26] suggested that a higher temperature during drying may accelerate the oxidation of phenolic compounds and decrease their specific activity. And the phenolic compounds of different agricultural products degrade at different rates during drying, due to the physical and physical-chemical conditions of the individual phenolic compounds of each product and their interactions with other components in the food matrix. Zilic et al. (2012) [8] demonstrated that a higher content of phenolic compounds in corn grain contributes to its greater antioxidant activity.

Intermittent drying at lower temperatures leads to a decrease in contact time with hot air, which promotes antioxidant activity preservation, being considered beneficial its use for corn grains drying. However, sudden variation in air temperature can affect this potential as it was observed for extracts antioxidant potentials from grains submitted to intermittent drying at 70 °C.

Optimization studies are indicated to determine optimum temperatures and tempering times to maximize material quality preservation submitted to drying process and to avoid thermal degradation of phytochemicals.

4. CONCLUSION

Based on results, it was observed that corn grains submitted to intermittent drying, mainly at low temperatures, presented lower decrease in antioxidant potential when compared to conventional drying. It was also observed that air temperature increase provides a reduction in antioxidant activity of dried grains. There are indications that sudden variations in air temperature may interfere negatively in bioactive components of corn grains as it was observed in intermittent drying at 70 °C. In this context, it would be interesting to conduct optimization studies to determine the best drying air temperature modulation to maximize antioxidant activity preservation of grains.

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