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Solid-state fermentation as an efficient strategy for the biotransformation of lentils: enhancing their antioxidant and antidiabetic potentials

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Abstract

Background: Fermentation is a classic industrial process that can be applied as an efficient strategy to increase the release of bioactive compounds with antioxidant and antidiabetic activities.

Methods: This work reported the effects of solid-state fermentation (SSF) performed using strains of *Aspergillus oryzae* and *Aspergillus niger* on the antioxidant (DPPH, ABTS and FRAP) and in vitro antidiabetic (inhibition of α-amylase and α-glucosidase activities) potential of lentils.

Results: The results showed that the profiles of the biological activities of the extracts obtained from the fermented samples varied greatly with respect to both the microorganism involved and the fermentation time. The extracts obtained from the fermented lentils by *A. oryzae* after 72 h and by *A. niger* after 48 h using the FRAP assay showed the most remarkable changes in the antioxidant activity, increasing by 107 and 81%, respectively, compared to the nonfermented lentils. The lentil extracts produced by fermentation with *A. niger* after 48 h were able to inhibit the α-glucosidase activity by up to 90%, while a maximal inhibition of amylase (~ 75%) was achieved by the lentil extract obtained after 24 h of fermentation with *A. oryzae*. The content of the total phenolic compounds (TPCs) and the identification of them in lentil extracts correlated well with the improvement of the biological activities.

Conclusion: These results suggested that SSF was feasible to obtain extracts of fermented lentils with improved antioxidant and antidiabetic properties. Additionally, these results indicated that the proper choice of microorganism is crucial to direct the process for the production of compounds with specific biological activities.

Keywords: Lentils, Solid-state fermentation, Antioxidant compounds, Antidiabetic potential

Introduction

The association between legume consumption and its positive effect on chronic diseases such as obesity, cardiovascular disease and type 2 diabetes has attracted great interest from food professionals (Vaz-Patto et al. 2015). Type 2 diabetes mellitus is a metabolic disorder characterized by a resistance to insulin action, which interferes with the proper control of blood glucose levels. An efficient way to control postprandial hyperglycemia is to decrease the absorption of glucose in the small intestine, which can be accomplished by inhibiting the amylolytic enzymes; amylolytic enzymes are responsible for the hydrolysis of starches and oligosaccharides during digestion, and their inhibition results in the release of glucose (Oseguera-Toledo et al. 2015).

Hyperglycemia can also induce the production of free radicals, which damage various tissue types and are related to the onset of cancer and premature aging (Oseguera-Toledo et al. 2015). The negative effects promoted by the action of free radicals can be minimized by the presence of compounds with antioxidant activities. Antioxidants are considered important nutraceuticals

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that have several health benefits and are defined as any substance that significantly slows or inhibits the oxidation of a substrate (Ibrahim et al. 2014).

Lentils (*Lens culinaris* L.) are an important pulse crop worldwide and have notable health benefits; lentils are grown in over 70 countries and are consumed in more than 100 countries (Campos-Vega et al. 2010; Bautista-Expósito et al. 2018a). Compared to other grains, lentils are emerging as a major pulse crop because lentils are an excellent fit in existing crop rotations, and as a result, a fivefold increase in the global production of lentils has occurred during the last five decades (Ekanayake et al. 2015; Joshi et al. 2017). This legume is a dietary source of carbohydrates (40–50%), protein (20–30%), fiber, essential minerals and other micronutrients that are important to human nutrition (Bautista-Expósito et al. 2018a; Siva et al. 2018). Moreover, lentil grains are also a source of bioactive molecules, such as phenolic compounds and precursor proteins of bioactive peptides with antioxidant and antidiabetic properties (Mirali et al. 2016; García-Mora et al. 2017).

Fermentation is a classic industrial process applied to enhance the shelf-life and nutritional and organoleptic properties of food substrates (Frias et al. 2005; Kaprasob et al. 2017). Additionally, bioactive compounds may be produced via secondary metabolic pathways or released from the substrate by enzymes produced by microorganisms during fermentation (Dey et al. 2016; Dulf et al. 2016; Handa et al. 2019). This current application has been explored as an efficient strategy to enhance the synthesis of microbial metabolites with biological properties using several substrates to achieve diverse functional benefits, such as antioxidant, antidiabetic and antihypertensive activities (Fujita et al. 2017; Bier et al. 2019; Handa et al. 2019). Proteins and phenolic compounds are compounds that can be targeted for enhancing functional health benefits by novel microbial biotransformation (Kaprasob et al. 2017; Toldrá et al. 2018).

The strains of *Aspergillus oryzae* LBA01 and *Aspergillus niger* LBA02, which were used in this work, were reportedly able to simultaneously produce several enzymes, such as cellulase, α-amylase, β-glucosidase and protease, during solid-state fermentation (de Castro and Sato 2013; de Castro et al. 2015; Ohara et al. 2018). Thus, the hypothesis of this study is that the solid-state fermentation of lentil flour using strains of *Aspergillus*, which are known to produce a range of enzymes that can act specifically and effectively to hydrolyze plant cell wall matrices, increases the biological activity of fermented lentils by releasing insoluble phenolic compounds and generating bioactive peptides.

Additionally, to our knowledge, all the works reported in the literature have studied the biotransformation of lentils using bacterial strains, and this study is the first to apply strains of *Aspergillus* to obtain compounds with antioxidant and antidiabetic potentials.

Materials and methods

Reagents

The reagents Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid), sodium nitrite, aluminum chloride, sodium hydroxide, vanillin, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), hydrated iron chloride and the enzymes α-amylase (Termamyl® 2×) from *Bacillus licheniformis* and α-glucosidase (EC 3.2.1.2) from *Saccharomyces cerevisiae* were purchased from Sigma Aldrich (Darmstadt, Germany). All the other reagents used in the experiments were of analytical grade.

Preparation of lentil flour for use as a substrate in solid-state fermentation

Fresh lentils were purchased from a local market (Campinas, Sao Paulo, Brazil) and used to prepare the lentil flour that was used for the fermentation process. The lentil grains were crushed in a blender, frozen and freeze-dried. The powder product obtained after the freeze-drying process was stored under refrigeration for use as a substrate in solid-state fermentation.

Microorganisms

The microorganisms used in this study were *Aspergillus oryzae* LBA01 and *Aspergillus niger* LBA02, which were obtained from the culture collection of the Laboratory of Food Biochemistry, School of Food Engineering, University of Campinas, Brazil.

Solid-state fermentation (SSF) of the lentil flour

Lentil flour was used as a substrate for the biotransformation by *A. oryzae* LBA01 and *A. niger* LBA02, which was achieved by SSF using 250-mL Erlenmeyer flasks. The substrate was prepared to obtain a final amount of 20 g of media with an initial moisture adjusted to 50%, The media were sterilized at 121 °C for 15 min and incubated under the following conditions: the incubation temperature was 30 °C, and an inoculum of 10^7 spores g^{-1} was obtained. The fermentation process was performed for 24, 48, 72 and 96 h. A crude extract containing compounds with antioxidant and antidiabetic potentials was obtained by the addition of 100-mL of distilled water, and this extract was homogenized and equilibrated for 1 h. Then, the solution was filtered through a filter membrane (Whatman® qualitative filter paper, Grade 1) to obtain an extract free of any solid material.

Determination of the antioxidant properties

Determination of the ABTS radical cation scavenging activity

An ABTS assay (Al-Duais et al. 2009) was performed using a Multiskan™ GO Drop Plate microplate reader (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation solution (generated by oxidation with potassium persulfate) was prepared in a 75-mM potassium phosphate-buffered saline solution (PBS) (pH = 7.4). At the time of the analysis, the ABTS radical cation working solution (potassium persulfate (140 mM, 0.088 mL) and ABTS (7 mM, 10 mL) in PBS) was prepared by diluting the stock solution in PBS to achieve an absorbance value of 0.70 ± 0.02 (734 nm).

Aliquots of 20 µL of each lentil extract (30 mg mL^{-1}) were added to 220 µL of the ABTS radical cation solution, and the absorbance was read at 734 nm after 6 min. A control assay was made with distilled water in place of the samples. A standard curve was prepared using different concentrations of Trolox (2.5–200 µM), and the results were expressed as µmol of Trolox equivalents per g of sample (µmol TE g^{-1}).

Determination of the DPPH radical scavenging activity

The DPPH radical scavenging activity was determined using the method described by Al-Duais et al. (2009). Briefly, 134 µL of a 150-µM DPPH radical solution, which was freshly made in ethanol, was added to 66 µL of appropriately diluted extracts (30 mg mL^{-1}) or standards. After 45 min of incubation at room temperature in the dark, the absorbance was measured at 517 nm using the Multiskan™ GO Drop Plate microplate reader (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Ethanol was used as the blank. A standard curve was prepared with different concentrations of Trolox (20–140 µM), and the results were expressed as µmol TE g^{-1}.

Measurement of the ferric reducing/antioxidant power (FRAP) assay

A FRAP assay was performed according to Benzie and Strain (1996) with some modifications proposed by Wiriyaphan et al. (2012). The FRAP reagent was prepared by mixing 2.5 mL of a 10-mM TPTZ solution in 40-mM HCl, 25 mL of a 0.3-M acetate buffer (pH = 3.6), and 2.5 mL of a 20-mM $FeCl_3 \cdot 6H_2O$ solution. Aliquots of 100 µL of the lentil extracts (15 mg mL^{-1}) and the blank or Trolox (5–250 µM) were mixed with 1 mL of the fresh FRAP reagent. Then, the reaction mixture was incubated at 37 °C for 15 min in a water bath. The absorbance of each sample was monitored at 593 nm. The results were expressed as µmol TE g^{-1}.

In vitro antidiabetic properties: inhibition of the α-amylase and α-glucosidase activities

The inhibition of both enzyme activities was accessed according to a method described by Apostolidis et al. (2007). To evaluate the α-amylase inhibition, 0.5 mL of a starch solution (1% w v^{-1}), 0.5 mL of an enzymatic solution and the lentil extracts (10 mg mL^{-1}) that were previously prepared in phosphate buffer (50 mM, pH = 7.0) were incubated for 30 min at 70 °C. A control assay was performed, in which distilled water was added to the reaction mixture instead of the sample. After that, the 3,5-dinitrosalicylic acid (DNS) method (Miller 1959) was used to analyze the content of the reducing sugar by performing spectrophotometry at 540 nm.

The α-glucosidase inhibition was determined using a Multiskan™ GO Drop Plate microplate reader (Thermo Fisher Scientific, Waltham, Massachusetts, USA). In a 96-well microplate, 50 µL of sample (10 mg mL^{-1}) in the phosphate buffer (50 mM, pH = 7.0), 50 µL of the substrate 4-nitrophenyl α-D-glucopyranoside (5 mM) and 100 µL of the enzymatic solution (0.1 U mL^{-1}) were added. The final mixture was maintained at 37 °C for 10 min, and data were collected after every 1 min to evaluate the enzymatic kinetics. A control assay was performed using distilled water instead of the sample. The spectrophotometry analyses were performed using a wavelength of 405 nm.

The inhibition of the α-amylase and α-glucosidase activities was calculated according to Eq. 1:

$$\text{Enzyme inhibition (\%)} = \left[\frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of control})} \right] \times 100 \quad (1)$$

Phenolic compounds in the fermented lentils

Total phenolic content (TPC)

The total phenolic content was estimated according to the method of de Camargo et al. (2012), who modified a method originally developed by Hillis and Swain (1959). The Folin and Ciocalteau's phenol reagent (0.5 mL) was mixed with 0.5 mL of extracts in a tube along with 4 mL of distilled water. After 3 min of incubation, 1 mL of a saturated sodium carbonate solution (0.3 g mL^{-1}) was added to each tube. The reaction mixtures were allowed to stand for 2 h at room temperature in the dark. The absorbance was read at 760 nm. The total amount of phenolic compounds was expressed as mg of gallic acid equivalents (GAE) per gram dry weight of defatted sample (mg GAE g^{-1}).

Identification of the phenolic compounds by UHPLC–ESI–MS/MS

The phenolic compounds were identified using an ACQUITY Ultra Performance LC™ system (Waters, Milford, MA, USA) that was linked simultaneously to a Micromass Quattro *micro*™ API benchtop tandem quadrupole mass spectrometer (Waters MS Technologies, Manchester, UK) according to the method described by Xavier et al. (2017). The multiple reaction monitoring (MRM) mode as well as the *m/z* transitions of the precursor and product ions was employed to identify and confirm the presence of phenolic compounds in the samples. Chlorogenic acid, protocatechuic acid, myricetin, caffeic acid, vanillic acid, gallic acid, 3,4-di-hydroxybenzoic acid, quercetin, sinapic acid, ferulic acid, rutin and coumaric acid were used as the standards for the identification of the phenolic compounds.

Calculations and statistics

The results were statistically analyzed according to the Tukey test using the software Minitab® 18 from Minitab Inc. (USA). The values were expressed as the arithmetic mean and were considered significantly different when the p value was ≤ 0.05.

Results and discussion

Lentil grains are an excellent source of nutrients, such as carbohydrates, proteins, essential minerals and micronutrients, which makes lentils a suitable substrate for microbial growth during fermentative processes. The microorganisms *A. oryzae* LBA01 and *A. niger* LBA02 were used in this study and grew satisfactorily in the culture medium that was formulated with lentil flour, as evidenced by Figs. 1 and 2.

The production of ingredients with enhanced antioxidant activities is one of the challenges of the fermentation of legumes (Limón et al. 2015). The antioxidant activities of the lentil extracts obtained from nonfermented samples and samples fermented by *A. oryzae* LBA01 and *A. niger* LBA02 with respect to the fermentation time are presented in Table 1. In general, the antioxidant properties of the lentil extracts obtained after fermentation with *A. niger* were markedly better than those of the lentil extracts fermented with *A. oryzae*. The DPPH radical scavenging that occurred in the fermented lentils by *A. oryzae* ranged from 5.06 (96 h of fermentation) to 6.81 μmoL TE g^{-1} (24 h of fermentation), whereas this activity in the lentils fermented by *A. niger* ranged from 4.41 (96 h of fermentation) to 6.97 μmoL TE g^{-1} (48 h of fermentation). The extracts from the lentils fermented by *A. niger* possessed the highest ABTS radical scavenging activity after 96 h of fermentation, reaching 11.56 μmoL TE g^{-1}, while the samples obtained after 72 h of fermentation with *A. oryzae* presented with the highest antioxidant activity, as measured by the FRAP assay, in which 15.41 μmoL TE g^{-1} was detected. The highest percentage increases of the fermented samples compared to the nonfermented samples were detected for the antioxidant activity, which was evaluated by the FRAP assay. Variations of approximately 107 and 81% were observed for the extracts of the lentils fermented by *A. oryzae* and *A. niger*, respectively (Table 1).

The inhibition of α-amylase and α-glucosidase of the lentil extracts obtained from the samples fermented by *A. oryzae* LBA01 and *A. niger* LBA02 and the nonfermented samples is shown in Tables 2 and 3, respectively. The capacity of α-the glucosidase inhibition by the extracts obtained from the lentils fermented by *A. niger* was higher than that obtained after fermentation with *A. oryzae* (Table 2). Compared to the extracts produced from the nonfermented lentils, all the samples obtained from the fermentation process with *A. oryzae* showed a decreased capacity to inhibit α-glucosidase, whereas the samples obtained by the 48- and 72-h fermentation process with *A. niger* exhibited significantly higher capacities to inhibit α-glucosidase. On the other hand, the extracts from the lentils fermented by *A. oryzae* achieved the best α-amylase inhibition regardless of the fermentation time (Table 3). The lentil extracts obtained after 48 h of fermentation with *A. niger* inhibited the α-glucosidase activity by approximately 90% (Table 2). Meanwhile, the samples fermented for 24 and 48 h by *A. oryzae* inhibited the α-amylase activity by approximately 71–75%, but the extract obtained from the nonfermented lentils should be emphasized because it has an equivalent ($p \leq 0.05$) capacity to inhibit this enzyme (Table 3).

The microorganisms used in the fermentative processes of this study are known to be excellent producers of hydrolytic enzymes, such as proteases, amylases, cellulases and lipases (de Castro and Sato 2013; Ohara et al. 2018). Therefore, it is possible to infer that the main effects of fermentation on the biological properties of legumes are related to the release of conjugated phenolic compounds into cell walls by hydrolysis, in which the phenolic compounds become soluble with the subsequent increase in the content of these compounds in aqueous extracts. To monitor the variation of the content of these compounds, TPC values were determined throughout the fermentation process. The results showed that there was an increase in the content of phenolic compounds for all the fermented lentil extracts compared to the nonfermented samples (Fig. 3). A linear profile of the TPC increase during the fermentation kinetics was observed for all the lentil extracts produced by both microorganisms. The highest TPC values were detected

in the lentil extracts produced after fermentation with *A. oryzae*, and a TPC value of 4.27 mg GAE g^{-1} was reached after 96 h of fermentation (Fig. 3). The increase in the TPC values in the lentil extracts obtained after fermentation corroborated the positive changes observed for the antioxidant and antidiabetic properties.

Due to the positive effects of the fermentation time on the phenolic compounds, antioxidant activity and antidiabetic properties, UPLC–MS/MS analysis was performed on the extracts obtained at 96 h of fermentation to identify changes in the phenolic compound profiles during this process (Table 4). Phenolic compounds that are present in the nonfermented lentil samples were not detected because their concentrations could possibly be below the detection limit of the equipment. On the other hand, the lentil extract fermented with *A. niger* contained four phenolic compounds: quercetin, which is a flavonoid, ferulic acid, 3,4-dihydroxybenzoic acid and vanillic acid. These last two compounds were also present in the lentil extract fermented with *A. oryzae* (Table 4).

The change in the profile of these compounds resulted in the enhanced biological properties of the fermented legumes (Agil et al. 2013; Torino et al. 2013; Simsek et al. 2014). In addition, the differences in the antioxidant and antidiabetic properties of the lentil extracts obtained after fermentation with different strains (*A. oryzae* and *A. niger*) could be associated with qualitative and quantitative variations in the enzyme activities.

The antioxidant and antihypertensive properties of the liquid- and solid-state fermented lentils were reported by Torino et al. (2013). Lentil extracts were obtained after liquid fermentation with *Lactobacillus plantarum*, which was used as the inoculum, while solid-state fermentation was performed using a strain of *Bacillus subtilis*. A comparative analysis showed that the lentil extracts obtained after liquid fermentation contained more free amino groups, a higher GABA content, and better antioxidant and angiotensin I-converting enzyme inhibitory activities than the extract produced after solid-state fermentation. These authors suggested that complex polyphenols can be hydrolyzed to other simpler and more biologically active compounds during fermentation. In addition, bioactive peptides can be produced during fermentation by microbial proteases, which improves the biological properties of the fermented products. Therefore, it was possible to establish a positive correlation with the microorganisms used in our study. Strains of *Aspergillus niger* and *Aspergillus oryzae* have a long history of use in the industry due to their high proteolytic activity. More specifically, the strains *A. oryzae* LBA01 and *A. niger*

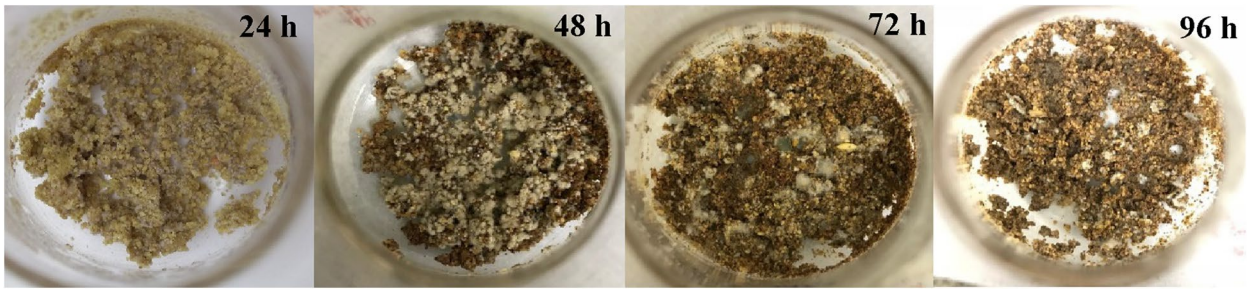

Fig. 1 SSF after 24, 48, 72 and 96 h using lentil flour as the substrate and *A. oryzae* LBA01 as the inoculum to obtain extracts with antioxidant and antidiabetic properties

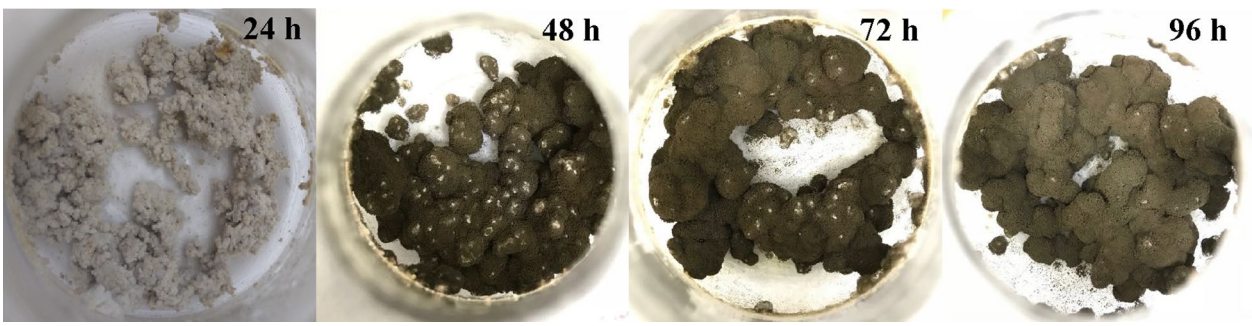

Fig. 2 SSF after 24, 48, 72 and 96 h using lentil flour as the substrate and *A. niger* LBA02 as the inoculum to obtain extracts with antioxidant and antidiabetic properties

Table 1 Antioxidant properties of the lentil extracts obtained after 0 (nonfermented), 24, 48, 72 and 96 h of solid-state fermentation with *A. oryzae* LBA01 and *A. niger* LBA02

Microorganism	Fermentation time (h)	Antioxidant activity (μmoL TE g^{-1})[1]					
		DPPH	Variation (%)[2]	ABTS	Variation (%)[2]	FRAP	Variation (%)[2]
Aspergillus oryzae	0	5.82^{b}	0.00	9.60^{a}	0.00	7.46^{c}	0.00
	24	6.81^{a}	17.06	7.48^{b}	−22.08	6.57^{c}	−11.97
	48	5.78^{bc}	−0.64	5.35^{d}	−44.27	9.56^{b}	28.13
	72	5.73^{c}	−1.49	5.96^{c}	−37.92	15.41^{a}	106.60
	96	5.06^{d}	−13.08	7.53^{b}	−21.56	10.20^{b}	36.68
Aspergillus niger	0	5.82^{c}	0.00	9.78^{b}	0.00	7.46^{c}	0.00
	24	6.46^{b}	11.07	8.92^{c}	−8.76	5.31^{d}	−28.80
	48	6.97^{a}	19.75	10.02^{b}	2.44	13.49^{a}	80.86
	72	6.51^{b}	11.77	9.99^{b}	2.11	9.46^{b}	26.83
	96	4.41^{d}	−24.30	11.56^{a}	18.24	3.00^{e}	−59.72

[1] Results are expressed as mean ($n = 3$) and those with different letters are significantly different in the same column ($p \leq 0.05$) for each microorganism

[2] The variation (%) was calculated as a function of the values of μmol TE g^{-1} obtained for the fermented samples in relation to their respective 0 (nonfermented sample)

Table 2 Antidiabetic properties in terms of the α-glucosidase inhibition (%) of the lentil extracts obtained after 0 (nonfermented sample), 24, 48, 72 and 96 h of solid-state fermentation with *A. oryzae* LBA01 and *A. niger* LBA02

Time of reaction (min)	α-glucosidase inhibition (%)								
	Nonfermented	24 h fermentation		48 h fermentation		72 h fermentation		96 h fermentation	
		A. oryzae	*A. niger*	*A. oryzae*	*A. niger*	*A. oryzae*	*A. niger*	*A. oryzae*	*A. niger*
1	43^{a}	9^{a}	21^{a}	21^{a}	91^{a}	34^{a}	66^{a}	32^{a}	33^{a}
2	33^{b}	2^{b}	15^{b}	13^{b}	90^{a}	25^{b}	62^{a}	23^{b}	27^{b}
3	26^{c}	-3^{c}	12c	6^{c}	90^{a}	18^{c}	$58^{a, b}$	$17^{b, c}$	$21^{b, c}$
4	20^{c}	-7^{c}	$9^{c, d}$	2^{d}	90^{a}	13^{d}	$55^{a, b}$	12^{c}	17^{c}
5	16^{c}	-9^{c}	8^{d}	-1^{d}	90^{a}	9^{e}	53^{b}	9^{c}	14^{c}
6	13^{d}	-11^{c}	7^{d}	-3^{d}	89^{a}	6^{f}	51^{b}	6^{c}	$12^{c, d}$
7	11^{d}	-12^{c}	6^{d}	-5^{d}	89^{a}	$4^{f, g}$	50^{b}	4^{c}	11^{d}
8	9^{d}	-14^{c}	6^{d}	-6^{d}	89^{a}	3^{g}	49^{b}	2^{c}	10^{d}
9	8^{d}	-14^{c}	6^{d}	-7^{d}	88^{a}	2^{g}	48^{b}	1^{c}	9^{d}
10	6^{e}	-15^{c}	6^{d}	-8^{d}	88^{a}	1^{g}	47^{b}	0.3^{c}	8^{d}

Results are presented as the mean ($n = 3$) and those with different letters are significantly different on the same column with $p \leq 0.05$

LBA02 used in our study were reported as good producers of proteases (de Castro and Sato 2013; de Castro et al. 2015), which is a characteristic that is intrinsically linked to the generation of peptides with antidiabetic and antioxidant potentials.

The fermentation of vegetable juices containing germinated seeds and sprouts of lentils and cowpea and the effects on their antidiabetic properties were studied by Simsek et al. (2014). An analysis was performed comparing the nonfermented samples to the fermented samples, and the results showed that there were no significant differences between the α-glucosidase inhibitory activities of the samples. However, the fermentation process negatively affected the α-amylase inhibitory capacity, in which the nonfermented sample had an IC_{50} of 41 μM, while the fermented sample had an IC_{50} of 149 μM, which was similar to the results we observed for the lentil extracts obtained after fermentation with *A. niger*. These authors further propose the importance of the inhibition of the α-glucosidase and α-amylase activities as an effective strategy for the management of type 2 diabetes.

Limón et al. (2015) reported the effects of SSF and liquid state fermentation (LSF) on the content of bioactive compounds in water-soluble extracts of kidney beans.

Table 3 Antidiabetic properties in terms of the α-amylase inhibition (%) of the lentil extracts obtained after 0 (nonfermented sample), 24, 48, 72 and 96 h of solid-state fermentation with *A. oryzae* LBA01 and *A. niger* LBA02

Fermentation time (h)	*A. oryzae*		*A. niger*	
	Enzyme activity (U/mL)[1]	α-amylase inhibition (%)[2]	Enzyme activity (U/mL)[1]	α-amylase inhibition (%)[2]
Control	13,600[a]	–	13,600[a]	–
0	3900[c]	71[a]	3700[d]	73[a]
24	3400[c]	75[a]	10,700[c]	21[b, c]
48	3700[c]	73[a]	11,400[b]	16[d]
72	10,200[b]	25[b]	11,000[b, c]	19[c, d]
96	10,900[b]	20[b]	11,700[b]	14[d]

[1] Results are expressed as mean ($n = 3$) and those with different letters are significantly different in the same column ($p \leq 0.05$)

[2] α-*amylase* inhibition was calculated as a function of the values of enzyme activity obtained for the enzyme activity in the presence of samples (fermented or nonfermented) of sample in relation to the values of enzyme activity in the absence of samples (control)

For this study, a strain of *Bacillus subtilis* was used for the SSF, while LSF was carried out by natural fermentation using a strain of *Lactobacillus plantarum*. The results obtained in their study showed that the antioxidant properties of the extracts obtained from samples fermented under SSF significantly increased. The authors suggested that the phenolic compounds largely contribute to the antioxidant activity of the extracts in terms of the release of these compounds to simpler forms. Additionally, it was highlighted that the antioxidant activity of samples obtained from kidney beans fermented by *Bacillus subtilis* under SSF cannot be attributed to the formation of bioactive peptides since low degrees of protein hydrolysis were observed during the process (Limón et al. 2015).

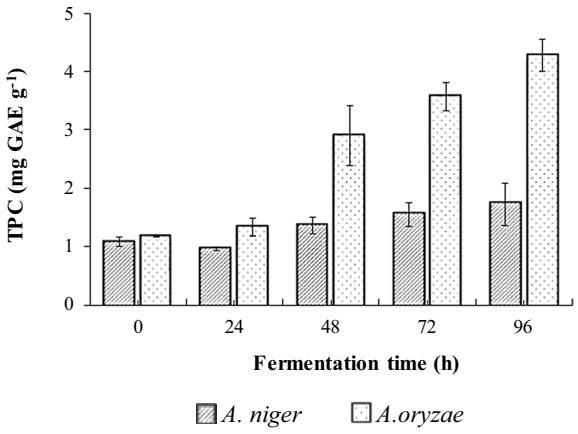

Fig. 3 TPC variation during lentil fermentation using *A. oryzae* LBA01 and *A. niger* LBA02 after 0 (nonfermented), 24, 48, 72 and 96 h

Gunenc et al. (2017) investigated the effects of submerged fermentation using kefir as the microbial culture on the phenolic contents and antioxidant activity of germinated and nongerminated lentils. The results showed that germination and fermentation significantly increased the total phenolic content as well as the antioxidant properties of the lentil samples in comparison to the nongerminated grains. Phenolic compounds such as catechin, protocatechuic acid, rutin, pyrogallol, syringic acid and ρ-coumaric acid were identified in the fermented lentils. The release of bound phenolic compounds from macromolecules such as complex polysaccharides, proteins and lipids in lentils during kefir fermentation was reportedly the main cause of the improvements to the antioxidant activity of the fermented products.

Bautista-Expósito et al. (2018b) reported that alkaline fermentation with *Lactobacillus plantarum* affected the phenolic compound and peptide compositions of lentils. According to their results, the strain *L. plantarum* CECT 748 was able to release peptides from lentil proteins at alkaline pH values, which was attributed to the action of intracellular peptidases produced by the microorganism. After fermentation, higher concentrations of p-hydroxybenzoic and vanillic acids, isorhamnetin glucuronide and kaempferol di-rutinoside were detected in the lentil extracts. The authors described that in addition to proteases, *L. plantarum* also produces enzymes responsible for releasing bound phenolic compounds into plant cell wall matrices, and these enzymes include carbohydrolases, β-glucosidases and a wide range of esterases. Thus, this finding agrees with the reported ability of *A. niger* LBA02 by solid-state fermentation to simultaneously produce several enzymes, such as cellulase, α-amylase, β-glucosidase and protease (de Castro et al. 2015; Ohara et al. 2018), which can act specifically and effectively to hydrolyze plant cell wall matrices, releasing insoluble phenolic compounds.

Table 4 Phenolic compounds identified in extracts obtained from the fermented lentil samples by UHPLC–ESI–MS/MS

Compound	Retention time (min)	$[M–H]^-$ (*m/z*)	Confirmation transition (*m/z*)	Extracts
3,4-dihydroxybenzoic acid	3.4	153	80.9/108.9	N96; O96
Ferulic acid	4.4	192.9	134/178	N96
Vanillic acid	1.5	166.9	122.9/152	N96; O96
Quercetin	7.6	301	151/179.1	N96

O96, Lentil extract obtained after fermentation with *A. oryzae* at 96 h; N96, Lentil extract obtained after fermentation with *A. niger* at 96 h

Different bioactivities of solid-state fermented lupin, quinoa and wheat using strains of *Lactobacillus* spp. were reported by Ayyash et al. (2019). The antioxidant properties of the fermented samples were measured by the DPPH and ABTS methods, in which significant improvements ($p \leq 0.05$) in these properties were detected after all the samples were fermented. Solid-state fermentation also positively influenced the α-glucosidase inhibition of all the fermented seeds, ranging from 18 to 60%. The changes in the bioactivities were attributed to the nature of the proteolytic products released from the fermented samples to the phenolic compounds.

Additionally, some studies have reported the production of phenolic compounds with biological potentials by *Aspergillus* under solid-state fermentation (Aguilar et al. 2008; Dey and Kuhad 2014; Sheih et al. 2014). The extracellular phenolic compounds produced by different filamentous fungi showed that these compounds not only are extracted from plant materials during fermentation processes but may also be produced as secondary metabolites of these microorganisms. However, the metabolic pathways involved in this phenomenon are not completely understood (Dey et al. 2016).

Conclusion

The current study documented the changes that occurred in the antioxidant and antidiabetic properties of lentil extracts obtained after fermentation using strains of *A. oryzae* and *A. niger*. The antioxidant activities of the lentil extract produced after *A. oryzae* fermentation for 72 h remarkably improved and ranged from 7.46 (nonfermented) to 15.41 μmoL TE g^{-1}, which represented a 107% increase in the activity evaluated by the FRAP method. Interestingly, the α-glucosidase inhibition by the lentil extracts obtained after 48 and 72 h of fermentation using *A. niger* was better than that of the nonfermented samples, and these samples inhibited the α-glucosidase activity by up to 90%. Meanwhile, the samples produced after fermentation with *A. oryzae* had drastically reduced abilities to inhibit α-glucosidase. Finally, it was possible to conclude that SSF can be applied as an efficient strategy to modify the bioactive profile of lentils.

Abbreviations

SSF: solid-state fermentation; LSF: liquid-state fermentation; *A. oryzae*: *Aspergillus oryzae*; *A. niger*: *Aspergillus niger*; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid; TPTZ: 2,4,6-tris(2-pyridyl)-s-triazine; PBS: phosphate-buffered saline; TE: Trolox equivalents; HCl: hydrochloric acid; DNS: 3,5-dinitrosalicylic acid.

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Authors' contributions

Conceived and designed the experiments: RJSC. Performed the experiments: AEAM, GBR and LCS. Analyzed the data: AEAM, LCS, GBR and RJSC. Wrote the paper: AEAM, LCS and RJSC. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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