Nutritional and antioxidant changes in lentil and quinoa induced by fungal solid-state fermentation with Pleurotus ostreatus

Janaina Sánchez-García
Universitat Politecnica de Valencia
Andrea Asensio-Grau
Universitat Politecnica de Valencia
Jorge García-Hernández (jorgarhe@btc.upv.es)
Universitat Politecnica de Valencia https://orcid.org/0000-0003-1258-6128
Ana Heredia
Universitat Politecnica de Valencia
Ana Andrés
Universitat Politecnica de Valencia

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Abstract

Solid-state fermentation (SSF) could be considered a suitable bioprocess to produce protein-vegetal ingredients with increased nutritional and functional value. This study aimed at assessing changes in phenols and antinutrients contents, along with biomass and protein production, resulting from the metabolic activity of *Pleurotus Ostreatus,* and edible fungus, in lentil and quinoa during 14 days of solid-state fermentation. The impact of particle size on these parameters was also assessed as the process was conducting in both seeds and flours. According to the obtained results, fungus biomass raised along fermentation and reached 30.0 ± 1.4 mg/ g dry basis and 32 ± 3 mg/ g dry basis in lentil grain and flour, and 52.01 ± 1.08 mg/ g dry basis and 45 ± 2 mg/ g dry basis in quinoa seed and flour after 14 days of SSF. Total protein content also increased about 20-25% along fermentation, excepting in lentil flour. However, soluble protein fraction remained constant. Regarding phytic acid, a positive impact of SSF was found with a progressive decreased, being higher in flours (90% of reduction) than in seeds. With respect to antioxidants properties, autoclaving of the substrates promoted the release of polyphenols together with an antioxidant activity (ABTS, DPPH and FRAP) in all substates. These parameters, however, drastically decreased as along as fermentation progressed. In conclusion, these results provide scientific knowledge for producing a based-lentil or quinoa fermented ingredients with higher protein content and low antinutrients content than their counterparts.

Introduction

The increasing interest in seeking plant protein sources as an alternative to replace animal proteins, is driven by environmental sustainability, low-cost and food security motivations. In this sense, both legumes and pseudocereals are relevant in the agricultural and food security context since they present environmental and economic benefits associated with their ability to fix nitrogen in soils (Khazaei et al. 2019). This allows the replacement of synthetic fertilizers and helps to mitigate the emission of greenhouse gases (Nemecek et al. 2008).

On one hand, legumes are one of the most consumed foods worldwide, being an essential component not only of Mediterranean diet, but also in developing countries (Clemente and Jimenez-Lopez, 2020). Among others, lentils, chickpeas, beans or peas are rich sources of protein and complex carbohydrates such as insoluble fibre, which present a low glycaemic index (Bouchenak and Lamri-Senhadji, 2013; Dhull et al. 2020). They also stand out for their content in bioactive compounds such as B vitamins, minerals such as potassium or magnesium, and polyphenols (Becerra-Tomás et al. 2019; Khazaei et al. 2019). Among them, lentils (*Lens culinaris*) are frequently noted for their protein content, essential micronutrients and antioxidants (Khazaei et al. 2019). The presence of phenolic compounds and precursor proteins of bioactive peptides, known as bioactive molecules, provide them with antioxidant and antidiabetic activity of significant interest (Magro et al. 2019). On the other hand, pseudocereals such as amaranth, buckwheat or quinoa differ from cereals in some morphological properties and distinct chemical composition (high protein and gluten free). Specifically, quinoa (*Chenopodium quinoa Willd.*), original crop from Andean region, has been stated as one of the grains of the 21st century and their

cultivation spread out to European countries, EEUU and Canada (Romano and Ferranti, 2019). It is considered a source of high-quality protein that contains the nine essential amino acids, with a high content of lysine, methionine and cysteine, in comparison with common cereals (Motta et al. 2019). Despite lentils and quinoa high-quality nutritional profile, they also contain antinutrients (phytates, polyphenols such as tannins or gastric protease inhibitors) which hinder their digestibility and absorption of nutrients (Schlemmer et al. 2009; Nkhata et al. 2018; Asensio-Grau et al. 2020). Phytates mainly affect the bioavailability of minerals, as may also happen with tannins (Bouchenak and Lamri-Senhadji, 2013; Khazaei et al. 2019). Tannins, in turn, react with amino acids such as lysine or methionine limiting their bioavailability. On the other hand, protease inhibitors irreversibly alter gastric proteases such as trypsin, leading to a decrease in protein digestion and amino acid absorption (Khazaei et al. 2019).

Thus, cooking methods are known to reduce the negative impact of antinutrients and improve food digestibility (Muzquiz et al. 2012; Shi et al. 2017). Many of these molecules such as protease inhibitors are thermosensitive, while others, such as tannins, saponins or phytates, can be reduced by soaking, germination or even fermentation (Muzquiz et al. 2012). Fermentation is a biological process, which implies the conversion of substrates into new added-value products by the metabolic actions of microorganisms. Thus, the resulting fermented foods present, compared with their non-fermented counterparts, improved nutritional composition and functionality by hydrolysis of complex macromolecules (fats, carbohydrates and proteins) into low molecular weight compounds susceptible to be easier digested, and further bio-absorbed (Şanlier et al. 2017; Gupta et al. 2018). The improved digestibility can be considered especially relevant for some population groups suffering from gastrointestinal disorders such as pancreatic insufficiency or for vegans whom their main protein intake comes from vegetables, for instance.

Among the different fermentation processes, solid state fermentation (SSF) arises as an environmental and economical sustainable alternative to classical liquid state fermentation (submerged method, SmF). SSF is carried out in the absence of free water and the microorganism is in direct contact with gaseous oxygen (Raghavarao et al. 2003). In addition, SSF is a technique that allows fermentation in a great variety of substrates, that can also result very cheap, such as agro-industrial waste. Furthermore, SSF reaches higher final product concentrations, with a conversion rate ranging from 20 to 30% of the substrate (compared to a 5% associated with SmF). Potential benefits of solid-state fermentation have been described in the revaluation of industrial by-products, such as the production of ethanol from lignocellulosic waste (Raghavarao et al. 2003; Gupta et al. 2018). SSF has also entailed an important advance in the production of protein-enriched foods from carbohydrate-rich substrates (Raghavarao et al. 2003). Moreover, the positive effect of SSF on the nutritional profile of legumes such as chickpeas (Xiao et al. 2014), beans (Espinosa-Páez et al. 2017) and lentils, both in grain (Dhull et al. 2020) and flour (Magro et al. 2019) has been reported. However, temperature, humidity, available gases and pH, together with the inoculum selection, are some of the key processing variables to optimize SSF process (Pandey 2003).

In this sense, the employment of different microorganisms has been reported in the SSF of dietary substrates (Couto and Sanromán, 2006). Particularly, mushrooms are considered one of the sources of high nutritional value due to their content of carbohydrates, essential amino acids, fibre, vitamins and minerals (Espinosa-Páez et al., 2017). Their potential medicinal and pharmacological benefits are also emphasized (Atlý et al., 2019). In this regard, the genera *Ganoderma, Lentinula, Trametes, Cordyceps, Hericium* and *Pleurotus* stand out (Atlý et al. 2019). The edible species of the genus *Pleurotus*, catalogued as Generally Recognized As Safe (GRAS), have been pointed out by different authors for their ability to synthesize essential amino acids while developing characteristic organoleptic properties (Espinosa-Páez et al. 2017). The *Pleurotus ostreatus* species is one of the most grown and produced worldwide. It is a mushroom capable of growing on lignocellulosic substrates, which makes it especially suitable for the degradation of substrates like legumes, seeds, grains, etc.

In this context, the aim of this work was to analyse the impact of solid-state fermentation with *P. ostreatus* on protein, phytates and polyphenols contents as well as antioxidant activity in lentil and quinoa substrates.

Materials And Methods

Materials

Lentil (*Lens culinaris*) of “pardine” variety and quinoa (*Chenopodium quinoa Wild*) grains or seeds and flours were acquired from Molendum ingredients S.L. (batch: 19011573). The *Pleurotus ostreatus* strain was obtained from the Spanish Type Culture Collection (CECT) (20311; batch: 18-10-2016) at the Universitat de València (València, Spain). To formulate the culture media, malt extract, glucose, mycopeptone and agar powder were supplied by Scharlab (Barcelona, Spain).

The analytical determinations required the following reagents: sodium hydroxide (NaOH), acetylacetone ($Ce_5H_80_2$), ethanol (CH_3OH), methanol (CH_3CH_2OH), galic acid ($C_7H_6O_5$), trolox ($C_{14}H_{18}O_4$), DPPH reagent ($C_{33}H_{44}N_5O_6$), iron chloride hexahydrate ($FeCl_3$ (III) · $6H_2O$), TPTZ reagent ($C_{18}H_{12}N_6$), acetic acid ($C_2H_4O_2$), ABTS reagent ($C_{18}N_{24}N_6S_4$), Folin-Ciocalteu reagent, thioglycolic acid ($C_2H_4O_2S$), potassium persulfate ($K_2S_2O_8$), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), p-dimethylamine benzaldehyde ($C_9H_{11}NO$) and glucose ($C_6H_{12}O_6$) were acquired from Sigma-Aldrich (St Louis MO, USA). The total starch kit (AA/AMG) was obtained from Megazyme (Ireland). Glucosamine (TCI Chemicals, USA), acetylacetone ($C_5H_8O_2$), sulfuric acid (H_2SO_4), ammonium iron sulphate ($NH_4Fe\ (SO_4)_2 \cdot 12H_2O$), chlorohydric acid (HCl) were acquired from AppliChem Panreac (USA), while sodium phytate from Biosynth Carbosynth (USA) and sodium carbonate (Na_2CO_3) from Scharlab (Barcelona, Spain).

Fungal solid-state fermentation

Starter culture preparation

Pleurotus ostreatus colonies were isolated from the agar plate and cultured in agar petri dishes made with 2% glucose, 2% malt extract, 0.1% mycopeptone and 1.5% agar, and then incubated for 14 days at 28 ºC (Selecta J.P.200207, Germany). The obtained mycelium was inoculated with a loop in the culture broth (2% glucose, 2% malt extract and 0.1% mycopeptone) and incubated again at 28 ºC for 14 days and subsequently was used to prepare the starter culture previously fermentation.

For the preparation of the starter culture, glass petri dishes containing 10 g of lentil or quinoa flour with a 65% of moisture were sterilised (121 ºC, 20 min), inoculated with 1 ml of *Pleurotus ostreatus* in the liquid medium previously prepared and incubated at 28 ºC during 14 days until the lentil or quinoa surface was completely colonized by the mycelium.

Fermentation process

Lentil (grain and flour) and quinoa (seed and flour) were subjected to fungal solid-state fermentation according to (Asensio-Grau et al. 2020) with some modifications. Glass jars (250 ml) containing 35 g of grains or flour were moistened to 65% and sterilised at 121 ºC for 20 min. Then, glass jars were inoculated with one portion of the starter culture previously divided in eight portions. Then, glass jars were incubated at 28 ºC for 14 days. Three glass jars were taken at different fermentation times 0, 2, 4, 6, 8, 10, 12 and 14 days to conduct the corresponding analytical determinations.

Analytical determinations

Substrate composition

Protein, lipid, ash and moisture content were determined by the AOAC methodologies in lentil and quinoa (AOAC 2000). Carbohydrates were estimated subtracting lipid, protein and ash to the total solid content.

Fungus biomass

Glucosamine content was used to estimate the fungus growing considering glucosamine such as a product of the chitin hydrolysis (Scotti et al. 2001). For fungal chitin hydrolysis into N-glucosamine, 100 mg of dried lentil and quinoa samples were incubated with 2.4 ml of 72% sulfuric acid (H_2SO_4) at 25ºC for 24 h. Then, samples were diluted with 55 ml of distilled water and the hydrolysis was carried out by sterilising the sample during 2 h at 121 ºC. The hydrolysed products were neutralised to pH 7 using sodium hydroxide (NaOH) 10M and 0.5M. Afterwards, 1 ml of hydrolysed products was added together with 1 ml of acetylacetone reagent (1 ml of acetylacetone and 50 ml of sodium carbonate 0.5M) in glass tubes and incubated in a boiling water bath for 20 min. After cooling tubes, 6 ml of ethanol and 1 ml of Erhlick reagent (2.67 g p-dimethylamine benzaldehyde and ethanol:HCl solution (1:1 (v:v)) were mixed into a 100 ml volumetric flask) were added into the mixture. Then, samples were incubated at 65º C for 10 min and absorbances were measured at 530 nm using a spectrophotometer (Thermo scientific, Helios Zeta UV/Vis). A calibration line performed with glucosamine (0-0.5 mg/ml) as standard, was used to quantify the fungus biomass. Results were expressed as mg of glucosamine per g dry basis.

Protein content

Protein content was determined by Kjeldhal method according to AOAC methodologies (AOAC 2000). Results were expressed such as g of protein in 100 g dry basis.

TCA soluble protein

Amino acids released during fermentation were estimated as the amount of soluble protein in trichloroacetic acid (TCA) according to Asensio-Grau et al. (2020) and Gallego et al. (2021). Samples (100 mg) were mixed with TCA solution to a final concentration of 12% and incubated at 4 ºC for 15 min. Then, samples were centrifugated (Eppendorf MiniSpin Plus) at 11000 rpm for 10 min. The supernatant was diluted with 50 mM EDTA and 8M UREA buffer (pH 10) and the absorbance was measured by ultraviolet spectrophotometry (Helios Zeta UV/Vis, Thermo Scientific) at 280 nm. A calibration line was used for quantification using tyrosine as standard. Results were expressed such as g of soluble protein in TCA per 100 g of protein.

Phytate content

Phytate was determined according to the method published by Haug & Lantzsch (1983) and adapted from Peng et al. (2010). This method is based on the precipitation of phytic acid using an acidic iron solution. The decrease of the iron in the supernatant is proportional to the amount of phytic acid in samples. Ferric solution (0.2 g of $NH_4Fe\ (SO_4)_2 \cdot 12H_2O$ in 100 ml HCl 2N and the volume was made to 1000 ml with distilled water) and bipyridine solution (1 g 2,2-bipyridine and 1 ml of thioglycolic acid and the volume was made to 100 ml with distilled water) were previously prepared. For the analysis, 50 mg of sample was extracted with 10 ml HCl 2N overnight at 4ºC. Then, samples were vortex and 0.5 ml of the extract was added into a glass tube with cap with 1 ml of ferric solution and were placed in a boiling water bath for 30 min. After cooling samples to the room temperature, 2 ml of bipyridine solution was added and samples were vortex and immediately measured by spectrophotometry at 519 nm (Helios Zeta UV/Vis, Thermo Scientific). For the quantification, a calibration line was made using phytic acid as standard (0-0.15 mg/ml) and results were expressed such as mg of phytic acid/ g of dry sample.

Total polyphenols

Polyphenols were determined in samples with the Folin-Ciocalteu method according to Espinosa-Páez et al. (2017) and Chang et al. (2006). An extraction with methanol 80% during 2 h in agitation (55 rpm, 25 ºC, Intelli-Mixer RM-2) was performed in order to recover the hydrosoluble compounds from samples. Methanol was added to the sample in a proportion 1:20 (w:v). After agitation, samples were centrifugated (20 min, 14 g-force, 20 ºC) and supernatant was used to quantify polyphenols by visible spectrophotometry (Helios Zeta UV/Vis, Thermo Scientific). A gallic acid line was used to quantify total polyphenols (0-200 mg/l) and results were expressed such as mg of gallic acid per g dry basis.

Antioxidant activity

Three different methods (ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), DPPH: 2,2-diphenyl-1-picrylhydrazyl, and FRAP: Ferric reducing antioxidant power) were used for measuring antioxidant activity in fermented samples according to Thaipong et al. (2006) and Espinosa-Páez et al.

(2017). An extraction with methanol 80% was conducted as for antioxidant activity determination. After centrifugation, supernatants were used for quantification using a spectrophotometer (Helios Zeta UV/Vis, Thermo Scientific). In all the methods, a calibration line was required using trolox such as standard (0-200 mg/l). Results were expressed as mg of trolox per g dry basis.

Statistical analysis

Simple factorial analysis of variance (ANOVA) was performed with a confidence interval of 95% ($p < 0.05$) to study possible differences in structure (between grain/seed and flour) and fermentation time (days) using the statistical program Statgraphics Centurion-XV. Fermentation and analyses were performed at triplicate.

Results And Discussion

Lentil and quinoa can be considered good providers of nutrients for microorganisms' growth during fermentative processes. However, any modification (chemical or physical) of the starting substrate could impact the fermentative process even when the same microbial specie is used (Michael et al. 2011; Limón et al. 2015; Espinosa-Páez et al. 2017). Table 1 shows the nutritional composition (in dry basis) of lentil and quinoa, before (grain/seed) and after milling and sieving (flour). As it can be seen, quinoa is richer in lipids, minerals, phytates and phenols than lentils. Regarding protein content, all substrates presented higher than 30 g per 100 g of dry basis, excepting quinoa grain for which was lower. The remotion of some fibrous part of quinoa seeds during milling and after sieving could be responsible of the differences found between seed and flour in terms of protein, as well as in carbohydrates and lipids contents. The antioxidant activity values (mg trolox/ g dry basis) of the substrates based on radical-based scavenging assays 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH)) and on non-radical redox potential-based one (FRAP) are also gathered in Table 1. According to the results, higher values were obtained in ABTS-assay than in DPPH and FRAP ones regardless the substrate. Moreover, lentils exhibited slightly higher capacity to quench the ABTS and DPPH radicals than quinoa, despite of the lower phenolic content of lentils. A positive relationship among total phenolic content and radical-based scavenging assays has been previously reported in vegetal foods (Marathe et al. 2011; Devi et al. 2019). In this study, it seems to be related to the phenolic profile rather than to the total content. Thus, phenolic compounds from lentils exhibited higher antioxidant activity than those from quinoa. Hence, chemical species with hydrogen atom or electron donating ability exert antioxidant properties. In the case of phenols, these capabilities seem to be related to the position and number of hydroxyl groups attached to the aromatic rings. Catechin and proanthocyanidin compounds represent 69% of the identified phenols in Pardina lentils (Aguilera et al. 2010); while phenolic acids together with flavanols comprised 60% of total compounds in white quinoa (Rocchetti et al. 2019).

Table 1
Proximate composition (g/ 100 g dry basis), phytic acid content (mg phytic acid/ g dry basis), total phenolic content (mg gallic acid/ g dry basis), and antioxidant activity by ABTS, DPPH and FRAP assays (mg trolox/ g dry basis) in lentil grain (LG) and flour (LF), and quinoa seed (QS) and flour (QF) before SSF.

	Lentil grain (LG)	Lentil flour (LF)	Quinoa seed (QS)	Quinoa flour (QF)
Protein	31.9 ± 0.5^{A}	32.3 ± 0.3^{A}	25.2 ± 0.2^{A}	31.1 ± 0.3^{B}
Lipids	0.86 ± 0.08^{A}	1.19 ± 0.10^{B}	3.4 ± 0.3^{A}	8.6 ± 0.3^{B}
Ash	2.76 ± 0.09^{B}	2.58 ± 0.05^{A}	3.60 ± 0.01^{A}	4.09 ± 0.02^{B}
Carbohydrates	64.5 ± 0.7^{B}	63.9 ± 0.4^{A}	67.7 ± 0.6^{B}	56.2 ± 0.7^{A}
Moisture	10.15 ± 0.05^{B}	9.35 ± 0.02^{A}	10.34 ± 0.09^{B}	8.97 ± 0.05^{A}
Phytic acid content	4.5 ± 0.4^{A}	4.8 ± 0.4^{A}	15.2 ± 0.9^{A}	19.9 ± 0.2^{B}
Total phenolic content (TPC)	1.28 ± 0.05^{A}	1.19 ± 0.07^{A}	1.57 ± 0.06^{A}	2.00 ± 0.08^{B}
Antioxidant activity (ABTS)	3.8 ± 0.2^{A}	3.5 ± 0.2^{A}	2.4 ± 0.2^{A}	3.1 ± 0.2^{B}
Antioxidant activity (DPPH)	1.26 ± 0.09^{A}	1.10 ± 0.06^{A}	0.82 ± 0.05^{A}	0.94 ± 0.04^{B}
Antioxidant activity (FRAP)	2.09 ± 0.10^{A}	1.8 ± 0.2^{A}	1.8 ± 0.2^{A}	2.3 ± 0.2^{B}

The results represent the mean of three repetitions with their standard deviation. Different capital letters indicate significant differences between grain/seed and flour with a 95% ($p < 0.05$) significance level.

On the other hand, no statistical significative differences were found among samples on their capacity to reduce the ferric 2,4,6-tripyridyl-s-triazine complex $[Fe^{3+}–(TPTZ)_2]^{3+}$ from ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in acidic medium.

The evolution of the fermentation process was followed by estimating the unicellular biomass generation in the medium. Fungal biomass is difficult to assess, since fungal cells do not easily separate from the solid substrate. The measurement of glucosamine (chitin monomer and major constituent of the cell wall in fungi) is an acceptable indicator to the estimation of fungal mycelium development (Scotti et al. 2001). The biotransformation of the substrate by *P. ostreatus* will depend on its ability to grow and secrete certain enzymes (mainly oxidative and hydrolytic) able to metabolize substrates rich in lignocelluloses (Rodrigues Da Luz et al. 2012), which are not directly fermentable. White rot fungi such as *P. ostreatus*, present 2 types of extracellular enzyme systems: a hydrolytic system that produces hydrolases responsible for the degradation of polysaccharides and an extracellular and oxidative lignolytic system that degrades lignin (Ergun and Urek 2017). The growth of *P. ostreatus* (CECT 20311) observed by following the evolution of glucosamine content is not only affected by the amount of

nutrients but also by the morphological characteristics, grain or flour, of the substrate (Fig. 1). The initial section of the curve, between day 0 and 4, corresponds to the latency phase of the fungus. On the fourth day of fermentation an exponential increase in the growth of the mycelium begins to occur without reaching a stationary phase during the observed period. Despite some differences observed in the growing rate between grains and flours, similar values were found after 14 days of incubation although higher biomass production was obtained over quinoa substrates as compared to lentils.

In order to evaluate the impact of biomass growing on the protein of the fermented samples, total protein content as well as the protein fraction soluble in TCA were evaluated at different times of the bioprocess (Table 2). Consistently with the biomass growth over the different substrates, a positive correlation between biomass and protein content was observed; the increase of total protein content was significantly higher in quinoa substrates (25.2% in seed after 14 days and 30.8% in flour after 6 days) than in lentils (25.5% in grain after 4 days and in 33.3% in flour after 14 days). However, no significant changes were observed in terms of soluble protein in TCA remaining constant along the process. The impact of the particle size of the substrate on the protein content is unclear and dependent on of the type of the substrate since the increase of protein was higher in lentil flour than in grain, in quinoa being the opposite. An increase of total protein content has been found in SSF with *P. ostreatus* in other pulses such as kidney beans (13%) and black beans (6%) (*Phaselous vulgaris*) (Espinosa-Páez et al. 2017) or in castellana lentils 18.5%), a different variety to the one used in this study. This protein increment could be explained as during fermentation, carbohydrates serve as an energy source for fungus growth and some of them may have been bioconverted into complex proteins, peptides or even free amino acids (Asensio-Grau et al. 2020). Furthermore, after fermentation with *P. ostreatus* of castellana variety lentils, an increase of soluble protein fraction was observed contributing to a higher digestibility in the resulting flour. Similar results were found by (Mora-Uzeta et al. 2019), in which protein content increased in tepary beans (*Phaselous acutifolius*) (+ 35%) fermented by *Rhizopus oligosporus*.

Table 2

Evolution of total protein (g protein/ 100 g dry basis) and soluble protein in TCA fraction* (g soluble protein/ 100 g protein), with respect to non-inoculated substrate (time 0), in lentil grain (LG) and flour (LF), and quinoa seed (QS) and flour (QF) at different SSF times.

Fermentation time (days)	Total protein content and soluble fraction in TCA			
	Lentil grain (LG)	Lentil flour (LF)	Quinoa seed (QS)	Quinoa flour (QF)
0	24.4 ± 1.5^{abc} (12.7 ± 0.7^{d})	27.5 ± 0.9^{a} (12.75 ± 0.15^{g})	20 ± 2^{a} (14.30 ± 1.15^{c})	25.5 ± 0.7^{a} (18.5 ± 0.5^{c})
2	24.5 ± 0.7^{abc} (12.1 ± 0.7^{cd})	29.0 ± 0.3^{b} (11.0 ± 0.2^{f})	20.4 ± 1.0^{ab} (13.1 ± 0.4^{b})	27.7 ± 0.9^{b} (20.0 ± 1.3^{d})
4	25.5 ± 1.3^{c} (11.3 ± 0.3^{abc})	30.8 ± 0.4^{c} (10.50 ± 0.04^{e})	21.7 ± 0.4^{bc} (11.1 ± 0.4^{a})	28.2 ± 0.6^{b} (16.67 ± 0.15^{b})
6	25.1 ± 0.6^{bc} (11.3 ± 0.5^{abc})	30.3 ± 0.3^{c} (10.34 ± 0.12^{e})	22.5 ± 0.2^{cd} (11.5 ± 0.2^{a})	30.8 ± 0.3^{d} (16.1 ± 0.3^{b})
8	24.7 ± 0.4^{bc} (10.6 ± 0.2^{a})	30.48 ± 0.15^{c} (10.04 ± 0.05^{d})	22.9 ± 0.3^{cd} (12.6 ± 0.4^{b})	29.36 ± 0.10^{c} (14.9 ± 0.2^{a})
10	24.5 ± 0.6^{abc} (11.8 ± 0.4^{bc})	30.4 ± 0.7^{c} (8.86 ± 0.02^{b})	23.8 ± 0.8^{de} (11.1 ± 0.2^{a})	29.3 ± 0.7^{c} (14.6 ± 0.4^{a})
12	23.1 ± 1.0^{a} (11.1 ± 0.6^{ab})	31.9 ± 0.5^{d} (8.26 ± 0.14^{a})	24.4 ± 0.4^{ef} (11.5 ± 0.5^{a})	29.36 ± 0.09^{c} (14.8 ± 0.3^{a})
14	23.8 ± 0.5^{ab} (11.5 ± 0.5^{bc})	33.3 ± 0.2^{e} (9.37 ± 0.09^{c})	25.2 ± 0.5^{f} (11.0 ± 0.3^{a})	27.4 ± 0.7^{b} (14.7 ± 0.3^{a})

* Values in parenthesis corresponds to protein fraction soluble in TCA.

The results represent the mean of three repetitions with their standard deviation. Different lowercase letters indicate significant differences between the different fermentation times with a 95% ($p < 0.05$) significance level.

An important aspect when it comes the nutritional evaluation of a food or ingredient is the content of some antinutrient compounds. It is known the contribution of phytates to decrease the absorption of essential micronutrients such as calcium, iron (Hurrell et al. 2003), zinc (Guttieri et al. 2006) or magnesium (Bohn et al. 2004; Peng et al. 2010) and have a negative impact on protein digestibility since they can bond to dietary protein or digestive enzymes (proteases and amylases) inhibiting their hydrolytic activity (Espinosa-Páez et al. 2017; Muñoz-Llandes et al. 2019). Since solid-state fungal fermentation is presented as a strategy to reduce the antinutrient content of certain substrates (Garrido-Galand et al. 2021), the evolution of phytic acid content was followed along the fermentation process and results are

shown in Fig. 2. According to literature quinoa seeds contain approximately between 1–2% of phytic acid (Hídvégi and Lásztity 2002; Febles et al. 2002) while lentils contain between 0.3–1.5% consistently with the initial values obtained in the raw materials used in this study (Table 1).

The degradation of phytate as consequence of fungal fermentation was observed, this being more pronounced in flours than in grains. A percentage of reduction of 27% and 89% was found in lentil grain and flour, respectively; while in quinoa, 45% in seed and 90% in flour was achieved. These changes begin to be significant ($p < 0.05$) from the tenth day of fermentation and depend on the substrate characteristics since the degradation of phytate after fermentation was greater in flours than in grains. These results are in accordance withCastro-Alba et al. (2019) who reported different level of phytate degradation in quinoa, canihua and amaranth according to their granulometry (seed or flour). The degradation rate of phytates seems to be also modulated by the pH reduction along fermentation because of organic acids production which depends on the inoculum employed at the same time. In this sense,Castro-Alba et al. (2019) reported differences between spontaneous fermentation and fermentation with a characterized specie such as *L. plantarum*. Furthermore, they suggest that the greater degradation of phytate in flour depends on both the exogenous phytase production of the microorganism and the activation of endogenous phytase. However, it must be considered that autoclaving prior to inoculation causes inactivation of endogenous phytase (Brejnholt et al. 2011). Therefore, the notable decrease in phytate in the last days of fermentation could be attributed mainly to the activity of the exogenous phytase from *P. ostreatus*, instead of the possible action of the endogenous phytase of the substrate.

On the other hand, *P. ostreatus* is known to be an excellent producer of hydrolytic enzymes, which contribute to the release of conjugated phenolic compounds chelated into the cell walls by hydrolysis during the fermentation. Phenolic compounds are the major contributors to antioxidant activity in fruit, vegetable, grain, or plant tissues. Thus, changes experimented by TPC along SSF time are shown in Fig. 3. Firstly, results evidenced a positive impact of thermal treatment on bound phenolic compounds release as TPC was much higher after autoclaving (time 0) than in the raw material (Table 1). Data are in agreement with those found by other authors (Bryngelsson et al. 2002; Madapathage Dona 2011). Hence, thermal treatment could promote cell walls disruption with the release of structural phenols and/ or the breakdown of insoluble polymeric phenols into smaller molecular weight compounds with enhanced extractability.

In contrast, a decreasing profile of the TPC was observed as long as fermentation progresses in lentil substrates, being higher in flour than in grain.Gebru & Sbhatu (2020) reported similar findings in white and brown teff solid-state fermented by *P.ostreatus* with a negligible, and even slightly decrease of TPC, after 6 days. Some authors, however, reported a significant increase of teff phenols when *G. lucidum* was used a starter and keeping the same condition of SSF. This fact evidences the relevance of each fungal mycelium metabolism, and enzymes synthesis, to conduct changes in the bioactive compounds.

In line, a negative correlation between TPC and fermentation time was also observed byXu et al. (2018) in 8 cereals and pseudocereals (wheat, corn, rice, millet, quinoa, oat, sorghum and buckwheat) and 2

legumes (soybean and pea) fermented with three different fungus and for 35 days. According to their results, only an increase of TPC were found at 14 days in oat. For the rest of the substrates, longer fermentation times were required to promote a significant increase of TPC content. In fact,Xu et al. (2018) reported a significant increase of TPC in fermented quinoa from 21 days of fermentation. This could be an explanation of the low TPC increase observed in the present study. It is well known that fermentation time is a kinetic parameter with importance for optimal enzyme production, thereby affecting transformation and production of particular compounds. The highest TPC value in quinoa seeds was detected after 8 days of fermentation (2.5 ± 0.3 mg GA/ g dry basis); while in flour, the highest value was reached after 10 days (3.36 ± 0.08 mg GA/ g dry basis).

The antioxidant activities of fermented lentil and quinoa are gathered in Table 3. Although SSF has been indicated with the aim of obtaining new ingredients with enhanced antioxidant properties, the capacity of the fermented substrates to scavenge free radicals such ABTS or DPPH, and to reduce ferric ions in FRAP assay decreased with fermentation time in this study. Besides, greater losses of antioxidant activities were achieved in lentils than quinoa. In addition to of total phenols, other metabolites formed, such as ergothionine, during the fermentation process may affect the antioxidant properties of the fermented products (Cai et al. 2011; Zhai et al. 2015; Bei et al. 2017). This fact could be responsible of the lack of correlation between TPC and extent of antioxidant activity (Torino et al. 2013; Alves Magro et al. 2019). Besides, competitive reactions between pro and antioxidant compounds can be done resulting in an increase or reduction of food’s antioxidant capacity. The ability of phenolic compounds to promote or inhibit oxidative damage depends on the different phenol concentration and pH, among other factors, Monohydroxylated phenols have been reported to exhibit a low radical scavenging activity (Briante et al. 2003; Villaño et al. 2005). Thus, (Fukumoto & Mazza (2000) found that benzoic and cinnamic acid derivatives, behave like prooxidants. According to this fact, a higher release of prooxidant phenols occurred in lentils than quinoa along fermentation time.

Table 3
Antioxidant activity (mg trolox/ g dry basis) and percentage of variation*, with respect to non-inoculated substrate (time 0), in lentil grain (LG) and flour (LF), and quinoa seed (QS) and flour (QF) at different SSF times.

Substrate	Fermentation time (days)	Antioxidant activity (mg trolox/ g dry basis)		
		ABTS	DPPH	FRAP
Lentil grain (LG)	0	10.7 ± 0.5^{dA} (0 ± 5)	5.6 ± 0.5^{fB} (0 ± 9)	7.3 ± 0.3^{fA} (0 ± 5)
	2	9.7 ± 0.4^{bcA} (-9 ± 4)	4.4 ± 0.2^{eB} (-21 ± 3)	4.8648 ± 0.0012^{eA} (-33.30 ± 0.02)
	4	8.7 ± 0.2^{aA} (-19 ± 2)	3.92 ± 0.08^{dB} (-30.1 ± 1.5)	4.17 ± 0.06^{dB} (-42.9 ± 0.9)
	6	9.3 ± 0.2^{abA} (-13 ± 2)	3.1 ± 0.2^{cB} (-44 ± 3)	3.03 ± 0.11^{cB} (-58.5 ± 1.6)
	8	8.99 ± 0.12^{aA} (-16.3 ± 1.2)	2.5 ± 0.2^{abB} (-55 ± 3)	2.31 ± 0.08^{bB} (-68.3 ± 1.1)
	10	10.2 ± 0.2^{cdB} (-5 ± 2)	2.79 ± 0.12^{bcB} (-50 ± 2)	2.54 ± 0.03^{bB} (-65.2 ± 0.5)
	12	9.9 ± 0.9^{bcA} (-7 ± 8)	2.21 ± 0.11^{aB} (-61 ± 2)	1.8 ± 0.2^{aB} (-75 ± 3)
	14	10.0 ± 0.3^{bcA} (-7 ± 3)	2.15 ± 0.03^{aB} (-61.7 ± 0.6)	1.72 ± 0.14^{aB} (-76 ± 2)
Lentil flour (LF)	0	12.90 ± 0.02^{dB} (0.00 ± 0.13)	4.307 ± 0.006^{fA} (0.00 ± 0.14)	7.53 ± 0.06^{fA} (0.0 ± 0.8)
	2	11.1 ± 0.8^{cA} (-14 ± 6)	3.1 ± 0.3^{eA} (-29 ± 7)	4.5 ± 0.6^{eA} (-41 ± 8)
	4	10.90 ± 0.12^{cB} (-15.5 ± 0.9)	2.65 ± 0.08^{dA} (-39 ± 2)	3.68 ± 0.11^{dA} (-51 ± 2)

Substrate	Fermentation time (days)	Antioxidant activity (mg trolox/ g dry basis)		
		ABTS	DPPH	FRAP
	6	10.3 ± 0.2^{abB} (-20.1 ± 1.5)	2.06 ± 0.02^{cA} (-52.2 ± 0.5)	2.52 ± 0.15^{cA} (-67 ± 2)
	8	10.06 ± 0.02^{aB} (-22.1 ± 0.2)	1.872 ± 0.014^{bA} (-56.5 ± 0.3)	1.92 ± 0.06^{bA} (-74.5 ± 0.7)
	10	9.81 ± 0.08^{aA} (-24.0 ± 0.6)	1.54 ± 0.02^{aA} (-64.3 ± 0.5)	1.19 ± 0.04^{aA} (-84.2 ± 0.6)
	12	9.99 ± 0.10^{aA} (-22.6 ± 0.8)	1.6087 ± 0.0008^{aA} (-62.65 ± 0.02)	1.43 ± 0.03^{aA} (-81.0 ± 0.5)
	14	10.67 ± 0.13^{bcB} (-17.3 ± 1.0)	1.66 ± 0.05^{aA} (-61.5 ± 1.1)	1.36 ± 0.12^{aA} (-82 ± 2)
Quinoa seed (QS)	0	11.54 ± 0.04^{bB} (0.0 ± 0.3)	3.08 ± 0.02^{eB} (0.0 ± 0.7)	2.74 ± 0.15^{dA} (0 ± 5)
	2	10.84 ± 0.04^{aB} (-6.1 ± 0.3)	2.76 ± 0.09^{dB} (-10 ± 3)	1.75 ± 0.03^{bA} (-36.2 ± 1.2)
	4	10.84 ± 0.04^{aB} (-6.1 ± 0.3)	2.576 ± 0.015^{cB} (-16.3 ± 0.5)	1.98 ± 0.05^{cA} (-28 ± 2)
	6	10.84 ± 0.03^{aB} (-6.1 ± 0.3)	2.48 ± 0.04^{bcB} (-19.3 ± 1.4)	1.55 ± 0.03^{aA} (-43.6 ± 1.0)
	8	12.83 ± 0.07^{cB} (11.1 ± 0.6)	2.30 ± 0.11^{aA} (-25 ± 4)	1.44 ± 0.07^{aA} (-47 ± 3)
	10	11.40 ± 0.08^{bB} (-1.3 ± 0.7)	2.28 ± 0.10^{aA} (-26 ± 3)	1.5 ± 0.2^{aA} (-45 ± 8)
	12	11.7 ± 0.4^{bB} (2 ± 3)	2.45 ± 0.02^{bB} (-20.3 ± 0.7)	1.42 ± 0.08^{aA} (-48 ± 3)

Substrate	Fermentation time (days)	Antioxidant activity (mg trolox/ g dry basis)		
		ABTS	DPPH	FRAP
	14	10.7 ± 0.4^{aB} (-7 ± 4)	2.555 ± 0.002^{bcB} (-17.00 ± 0.06)	1.458 ± 0.004^{aA} (-46.7 ± 0.2)
Quinoa flour (QF)	0	9.37 ± 0.03^{cA} (-0.0 ± 0.4)	2.81 ± 0.04^{eA} (0.0 ± 1.4)	4.87 ± 0.06^{fB} (0.0 ± 1.3)
	2	8.908 ± 0.007^{bA} (-4.94 ± 0.07)	2.55 ± 0.03^{dA} (-9.2 ± 1.2)	3.75 ± 0.08^{eB} (-23 ± 2)
	4	8.5 ± 0.2^{aA} (-10 ± 2)	1.98 ± 0.06^{aA} (-30 ± 2)	2.4 ± 0.3^{cB} (-50 ± 5)
	6	9.36 ± 0.04^{cA} (-0.2 ± 0.5)	2.291 ± 0.008^{cA} (-18.4 ± 0.3)	2.77 ± 0.02^{dB} (-43.0 ± 0.3)
	8	9.60 ± 0.13^{deA} (2.4 ± 1.4)	2.39 ± 0.02^{cA} (-15.0 ± 0.8)	2.31 ± 0.08^{bcB} (-53 ± 2)
	10	9.44 ± 0.09^{cdA} (0.7 ± 1.0)	2.12 ± 0.05^{bA} (-24 ± 2)	1.97 ± 0.11^{aB} (-60 ± 2)
	12	9.516 ± 0.002^{cdeA} (1.55 ± 0.02)	2.09 ± 0.14^{bA} (-26 ± 5)	2.34 ± 0.02^{bcB} (-51.8 ± 0.4)
	14	9.7 ± 0.2^{eA} (3 ± 3)	2.16 ± 0.06^{bA} (-23 ± 2)	2.21 ± 0.05^{bB} (-54.5 ± 1.0)

The results represent the mean of three repetitions with their standard deviation. Different lowercase letters indicate significant differences between the different fermentation times with a 95% ($p < 0.05$) significance level. Different capital letters indicate significant differences between grain and flour with a 95% ($p < 0.05$) significance level.

* Values in parenthesis corresponds to percentage of variation of antioxidant activity.

Nevertheless, and once more, autoclaving had a positive effect in terms of radical scavenging and reducing power activity of both substrates as a notable increase of mg trolox/ g dry basis at time 0 was found compared to the values found in raw materials (Table 1). These findings are in coherence with those of Rocchetti et al., (2019).

Conclusions

This study analysed the evolution on biomass production, total protein content and soluble fraction, phytic acid and phenolic contents of Pardina lentils and white quinoa, in grain/seed and flour, along fungal solid-state fermentation with the edible fungus *Pleurotus Ostreatus*. Based on the mentioned results, fungal solid-state fermentation could be considered an environmentally sustainable biotechnological strategy to obtain gluten-free fermented lentils and quinoa based-ingredients for new food formulations specially addressed to specific population groups with high protein requirements, such as the elderly, athletes, vegans, or subjects with gastrointestinal disorders. However, stable fermented ingredients production usually implies other unit operations such as conditioning, drying and/or milling. Therefore, the impact on these operations and the bioactive compounds of interest might be also assessed. Finally, it would be interesting to perform in vitro digestion studies that could help the decision-making to establish optimal conditions for fermented ingredients production with enhanced digestibility.

Abbreviations

SSF: Solid state fermentation

SmF: Sumerged fermentation

QS: Quinoa seed

QF: Quinoa flour

LG: Lentil grain

LF: Lentil flour

CECT: Spanish type culture collection

TCA: Trichloroacetic acid

ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)

DPPH: 2,2-diphenyl-1-picrylhydrazyl

FRAP: Ferric reducing antioxidant power

TPTZ: 2,4,6-tripyridyl-s-triazine complex

ºC: degrees Celsius

W: weight

V: Volume

rpm: revolutions per minute

g: gram

mg: milligram

l: liter

UV: ultraviolet

Vis: visible

GA: Galic acid

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors are consent for publication of this manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this article [and its supplementary information files]

Competing interests

Authors declare no competing interests

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Authors' contributions

Janaina Sánchez-García: Formal analysis, Writing and Editing; Andrea Asensio-Grau: Formal analysis, Writing and Editing; Jorge García-Hernandez: Writing, Review & Editing; Ana Heredia: Conceptualization, Writing - Review & Editing, Supervision, Funding acquisition; Ana Andres: Conceptualization, Writing - Review & Editing, Supervision, Funding acquisition.

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Figures

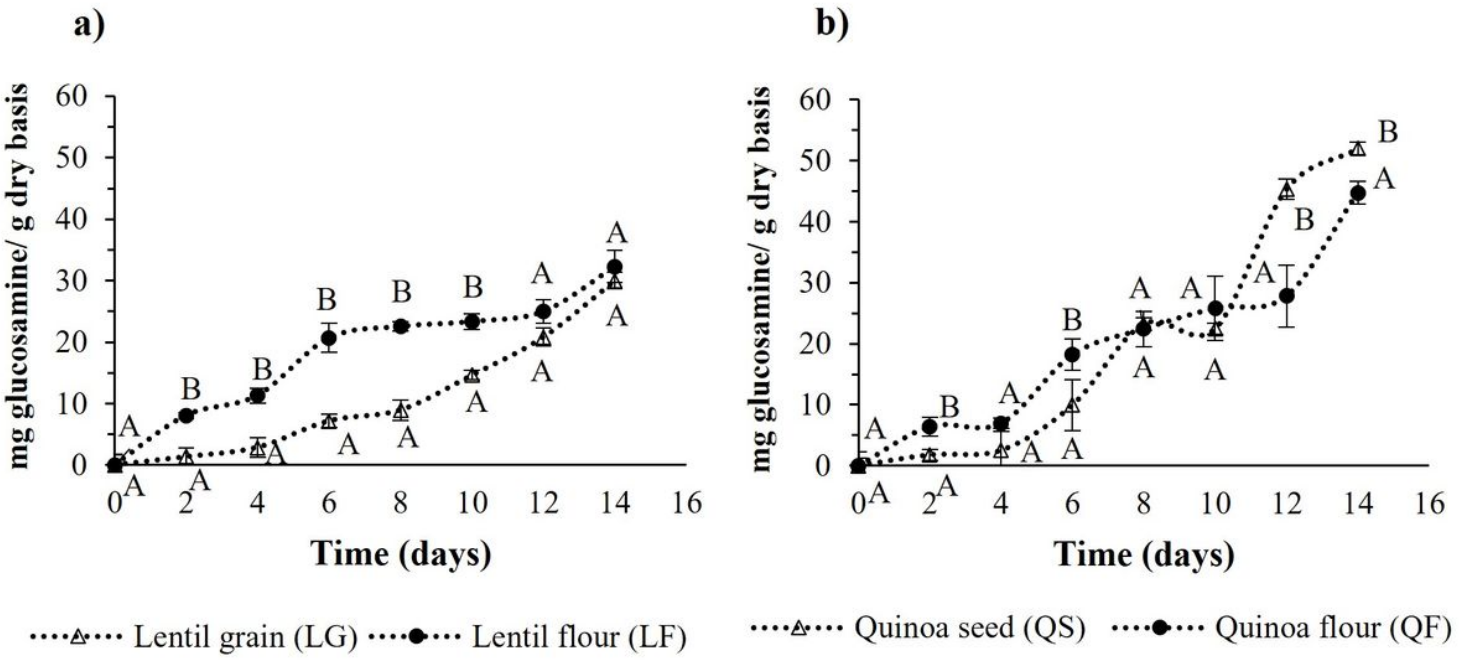

Figure 1

Biomass production (mg glucosamine/ g dry sample), with respect to non-inoculated substrate (time 0), in lentil grain (LG) and flour (LF), and quinoa seed (QS) and flour (QF) at different SSF times. Different capital letters indicate significant differences between grain/seed and flour with a 95% ($p < 0.05$) significance level.

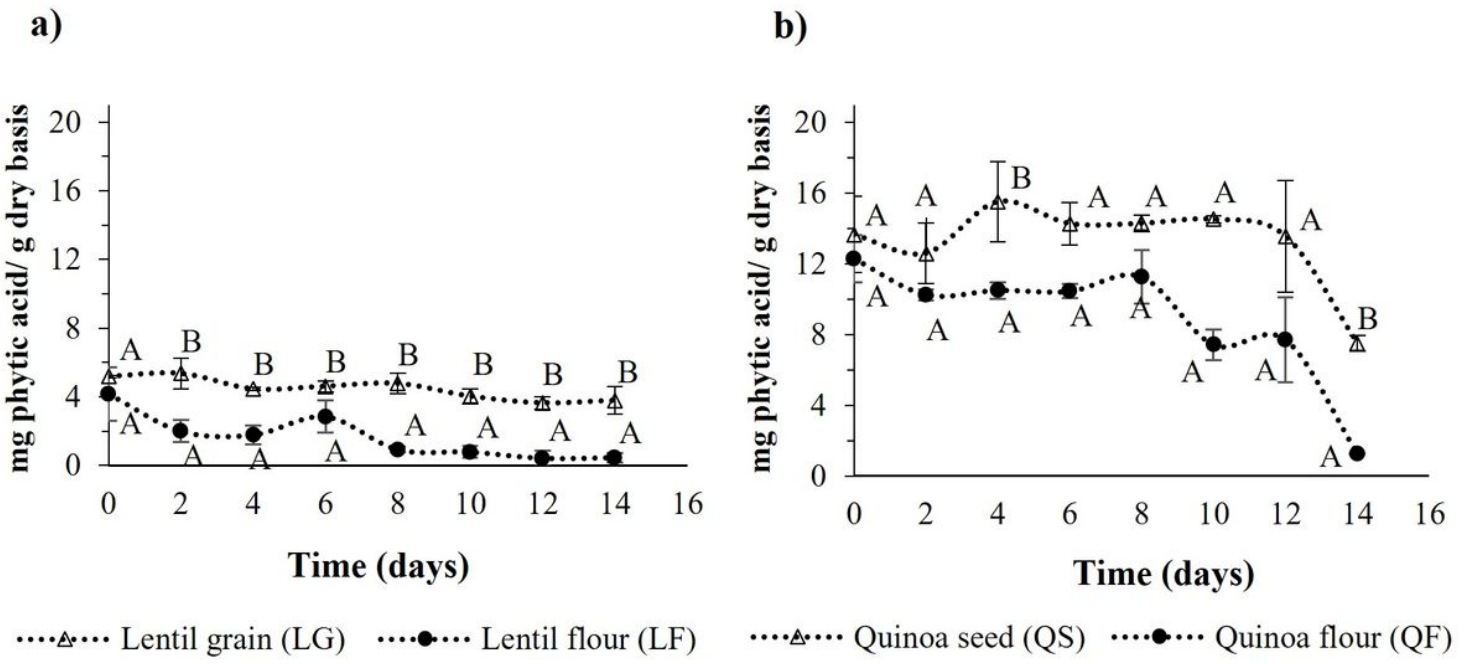

Figure 2

Evolution of phytic acid content (mg phytic acid/ g dry sample), with respect to non-inoculated substrate (time 0), in lentil grain (LG) and flour (LF), and quinoa seed (QS) and flour (QF) at different SSF times. Different capital letters indicate significant differences between grain/seed and flour with a 95% ($p < 0.05$) significance level.

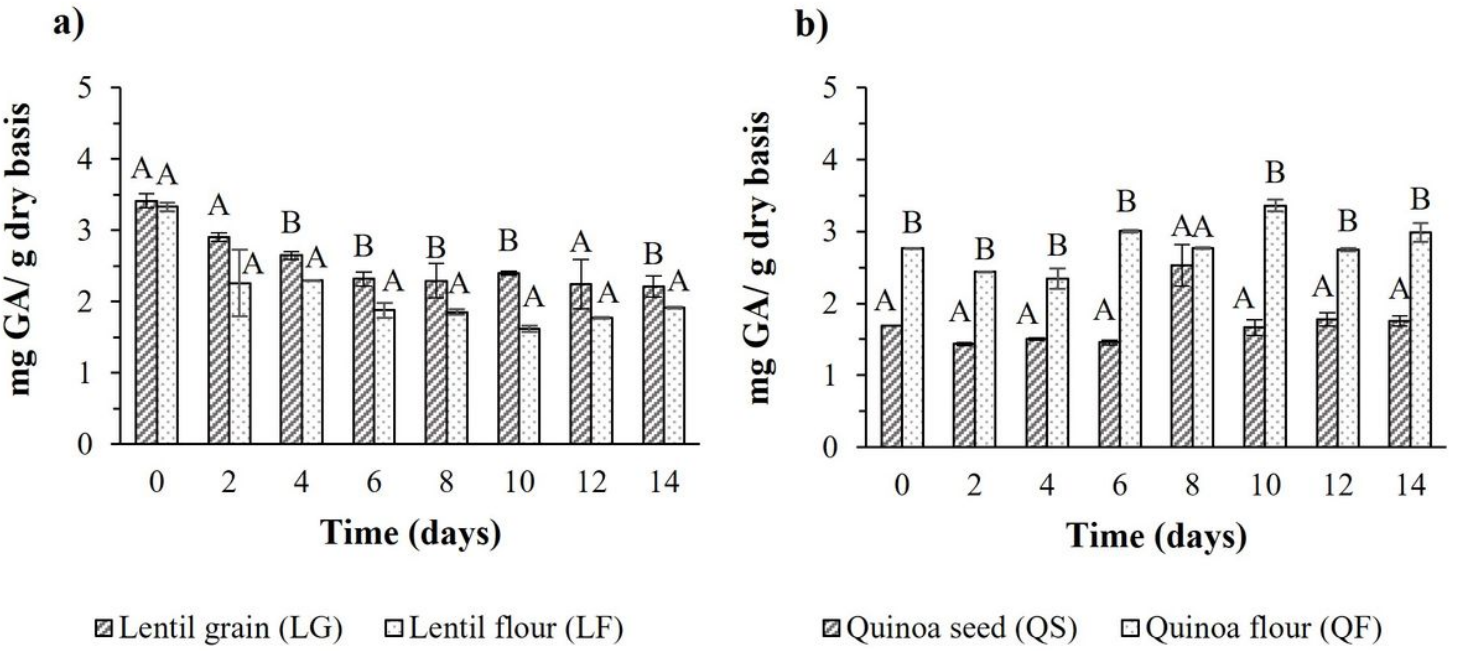

Figure 3

Total phenol compounds variation (mg GA/ g dry basis), with respect to non-inoculated substrate (time 0), in lentil grain (LG) and flour (LF), and quinoa seed (QS) and flour (QF) at different SSF times. Different capital letters indicate significant differences between grain/seed and flour with a 95% ($p < 0.05$) significance level.

Supplementary Files

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