Solid-state fermentation of lentil (*Lens culinaris* L.) with *Aspergillus awamori*: Effect on phenolic compounds, mineral content, and their bioavailability

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Abstract
Microorganisms have long been used in the production of a variety of foods, alcoholic beverages, additives, and supplements due to their cost effectiveness and environmental advantages. Solid-state fermentation (SSF) reproduces the natural microbiological process that can be utilized in a controlled way to produce the desired product. In the present study, modulation of phenolic compounds, antioxidant potential, and mineral content during SSF of three lentil cultivars, namely, HM-1, LL-931, and Sapna, were explored. The total phenolic content (TPC) for 6th day *Aspergillus*-fermented lentil (AFL) flour increased by 79.2% for cv. HM-1, 78.8% for cv. LL-931, and 122.8% for cv. Sapna. High-performance liquid chromatography (HPLC) results also showed that SSF not only improved the phenolic content of lentil cultivars but also resulted in the formation of some new phenolic compounds (resorcinol and cinnamic acid). The condensed tannin content, DPPH (2,2-diphenyl-1-picrylhydrazyl) inhibition activity, hydroxyl free radical scavenging activity, reducing power activity, and total antioxidant capacity of aqueous ethanolic extracts from all AFLs also increased significantly (*p* ≤ 0.05) up to 6th day of fermentation. Mineral content differed significantly (*p* ≤ 0.05), with AFL extracts exhibiting higher mineral content than their unfermented counterparts. Among different minerals, Cu content of all AFL extracts was the highest with an increase of 46.4% to 60.0% upon fermentation. All minerals showed a significant (*p* ≤ 0.05) increase in their concentrations upon fermentation except for K in which the increase was less than 0.1%. However, in vitro bioavailability of iron and zinc was significantly (*p* ≤ 0.05) higher in AFL as compared with their unfermented counterparts, with the highest level being observed on the 6th day of fermentation. Thus, biotransformed lentils could be utilized in the preparation of functional foods and novel nutraceuticals for their health-promoting properties.
INTRODUCTION

Legumes, a main component of human diet worldwide, provide protein, energy, and other essential nutrients. A number of experimental, epidemiological, and clinical studies have proved that apart from their nutritional properties, they are also a rich source of natural antioxidants (Dhull et al., 2019; Xu & Chang, 2007). Lentil (Lens culinaris L.) belongs to family Fabaceae, a rich source of fibers, resistant starch, oligosaccharides, proteins, and vitamins (Bautista-Expósito, Peñas, Silván, Frias, & Martínez-Villaluenga, 2018; Faris & Attlee, 2017; Faris, Takruri, & Issa, 2013; Siva & Thavarajah, 2018). In the last decade, the use of lentils has increased in food industry due to their promising functional and nutritional properties (Faris et al., 2013; Paucean et al., 2018; Zhang et al., 2015). Lentils are composed of 20%–30% protein, 50%–58% carbohydrates, 19% dietary fiber, less than 2% fat (Stolova, Petrova, Penov, & Krastanov, 2013), and appreciable amount of minerals such as magnesium, phosphorus, calcium and sulfur (Paucean et al., 2018). Protein malnutrition and micronutrient deficiencies among people living in developing countries can be alleviated by adopting lentils as an inexpensive alternative source of protein and minerals as lentils are high in iron and zinc with relatively small quantities of copper, calcium, and magnesium (Ramírez-Ojeda, Moreno-Rojas, & Cámara-Martos, 2018). Lentils are also receiving great deal of attention due to presence of bioactive compounds in them such as polyphenols, phytosterols, bioactive peptides, saponins, etc., with antioxidant and anti-diabetic properties (García-Mora et al., 2017; Mirali, Purves, & Vandenberg, 2016). Furthermore, lentils have been reported to have highest phenolic content as compared with other pulses such as chickpeas, yellow and green peas, red kidney beans, and black and yellow soybeans (Fratianni et al., 2014; Xu & Chang, 2007, 2008). This rich antioxidant profile of lentils makes them a suitable functional food (Alshikh, de Camargo, & Shahidi, 2015; Singh, Singh, Shevkani, Singh, & Kaur, 2016).

Environmental factors and unhealthy human lifestyle generate free radicals including reactive oxygen species (ROS) and reactive nitrogen species (RNS) spontaneously through metabolism (Aseervatham, Sivasudha, Jeyadevi, & Arul, 2013). In a normal healthy body, these prooxidants are kept in check through various antioxidant defense systems, such as glutathione, catalase, and superoxide dismutase (Ighodaro & Akinloye, 2018). Exposure to unhealthy environmental conditions and habits result in an imbalance of antioxidants, which causes severe oxidative stress leading to reactive oxygen species-mediated tissue damage (Videla, 2009). To deal with this excess stress, body requires additional strategies such as diet supplements loaded with exogenous antioxidants, which can help in scavenging free radicals (Aseervatham et al., 2013; Dhull & Sandhu, 2018). The use of synthetic antioxidants has been restricted because of their suspected carcinogenic effects (Valentão et al., 2002). Therefore, there is increasing interest in the intake of natural antioxidants such as vitamin C, polyphenols, and flavonoids from plant sources in place of synthetic antioxidants.

Phenolic compounds are primarily produced by plants, but some other sources can also produce these compounds as secondary metabolites such as green algae (Onofrejová et al., 2010), yeasts (Banach & Ooi, 2014), endophytes (de Carvalho, Silva, Chagas-Paula, Luiz, & Ikekagi, 2016), and mushroom basidiomycetes (Palacios et al., 2011; Reis, Martins, Barros, & Ferreira, 2012). Fermentation has been receiving considerable attention for the extraction and production of phenolic compounds due to the fast growth rate, cost effectiveness, easy cultivation, recovery, and eco-friendly nature of microbial generation of phenolic compounds (Fowler & Koffas, 2009). During solid-state fermentation (SSF), various enzymes such as amylases, proteases, and lipases are produced by fermenting microorganisms. These enzymes hydrolyze carbohydrates, protein, and lipids to more digestible compounds with a pleasant aroma, flavor, and texture. Moreover, many antinutritional compounds such as phytic acid, tannins, protease inhibitors, etc., are significantly reduced during fermentation (Soetan & Oyewole, 2009).

These days, SSF has been projected as a good process to improve the nutritional profile and antioxidant quality of a variety of cereals and legumes (Dey, Chakraborty, Jain, Sharma, & Kuhad, 2016; Magro, Silva, Rasera, & de Castro, 2019). Previous studies carried out by many researchers have reported that SSF improved the protein, fat, free phenolics, antioxidant activity of African locust beans (Oboh, Alabi, & Akindahunsi, 2008), and some underutilized legumes such as pigeon pea, bambara groundnut, African yam bean, and kidney bean (Oboh, Ademiluyi, & Akindahunsi, 2009). SSF of chickpeas with fungi Cordyceps militaris SN-18 significantly increased various phenolic compounds including chlorogenic acid, shikimic acid, rutin, genistein, diadzein, and biochanin A (Xiao et al., 2014).

Though many studies on modulation of nutritional and functional attributes of lentils due to germination and fermentation have been carried out, to the best of our knowledge, changes in phenolic acids and mineral bioavailability of Indian lentil cultivars due to SSF have not been discussed so far. In this perspective, the present study was carried out to compare the phenolic profile of lentil cultivars and to analyze the changes in antioxidant potential through biotransformation of lentil cultivars with Aspergillus awamori. Moreover, the effect of fermentation on the mineral content and bioavailability of iron and zinc in fermented lentil flours were also evaluated.

KEYWORDS
antioxidant, Aspergillus awamori, fermentation, lentil, mineral bioavailability
2 | MATERIALS AND METHODS

2.1 | Substrate

Certified seeds of three lentils cultivars, namely, HM-1, LL-931, and Sapna, were procured from Chaudhary Charan Singh Haryana Agricultural University, Hisar, India. The seeds were thoroughly cleaned and stored in airtight containers until further use.

2.2 | Starter culture for SSF

Starter culture, that is, A. awamori (MTCC 548), purchased from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India, was maintained on czapekdox agar (CDA) and czapekdox broth (CDB) at 30 ± 2°C. Spore suspension was prepared by washing a 4-day-old mycelium with an aqueous solution of 0.1% (W/V) Tween 80, which was further used for inoculating the autoclaved lentil grains.

2.3 | Solid-state fermentation

The method described by Salar, Purewal, and Sandhu (2017) was adapted for SSF of lentil grains. Shade dried lentil grains (50 g) were taken in Erlenmeyer flasks (250 ml) as substrate for each of SSF and soaked in CDB at room temperature overnight. Next day excess of CDB was decanted; lentil grains in the Erlenmeyer flasks were autoclaved (121°C for 15 min) and then cooled. The spore suspension prepared with double distilled water was sprayed (5 ml) on to the surface of autoclaved grains, mixed thoroughly and incubated at 25 ± 2°C for a period of 7 days. The lentil grains were stirred and mixed after 24 and 36 h of inoculation to release the fermentation heat. The unfermented lentils were prepared without the addition of spore suspension.

2.4 | Extraction of bioactive compounds from unfermented and fermented lentils

Aspergillus-fermented lentil (AFL) samples were withdrawn out of the Erlenmeyer flasks at every 24-h intervals and dried in an oven at 40°C for 48 h. The dried AFL and unfermented control (UFL) were ground in an electric grinder (Sujata, India). All flour samples (AFL and UFL) were defatted with hexane (1:5 w/v, 5 min, thrice) at ambient temperature and air dried. The defatted samples (1 g) were extracted with aqueous ethanol (50%) in the ratio (1:3 w/v) at 60°C for 60 min in a water bath. After extraction, samples were filtered (Whatman No. 1) and then vacuum evaporated. Extracts were prepared with the solvent in ratio (1:2) and frozen at −4°C in dark until further analysis (Bhanja, Kumari, & Banerjee, 2009).

2.5 | Total phenolic and condensed tannin contents

For the determination of the total phenolic content (TPC) of AFL and UFL extracts, method as given by Gao, Wang, Oomah, and Mazza (2002) was adapted. Briefly, 100 μl of extracts and 0.5 ml of FC reagents were mixed in a 10-ml volumetric flask followed by mixing with 1.5-ml aqueous solution (20%, w/v) of anhydrous sodium carbonate, vortexing and incubation for 15 min at room temperature. After incubation, the flask was filled with distilled water to volume. The absorbance was read at 765 nm, and the results were calculated from the standard calibration curve and expressed as mg gallic acid equivalents per gram (mg GAE/g) of sample.

The condensed tannin content (CTC) of the extracts was estimated according to the method given by Julkunen-Titto (1985). The absorbance against blank was recorded at 500 nm. Catechin was used to make standard curve (0.05 to 1 mg/ml). The results were expressed as milligrams of catechin equivalent per g (mgCE/g) dry weight basis (dwb). All analyses were performed in triplicates.

2.6 | Antioxidant potential

2.6.1 | 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity of sample extracts was analyzed by the adapting the method described by Yen and Chen (1995). Briefly, 100 μl of extract was taken in spectrophotometric cell and then 3 ml of 100-μM DPPH was added. The changes in absorbance at 517 nm were recorded after 30 min. Percent (%) DPPH scavenging activity was calculated using the following formula:

\[
\text{DPPH Scavenging Activity (\%)} = \left(\frac{A_C - A_E}{A_C}\right) \times 100, 
\]

where \(A_C\) and \(A_E\) are the absorbance of control and extracts, respectively.

2.6.2 | Hydroxyl free radical scavenging activity

The hydroxyl free radical scavenging activity (HFRSA) of sample extracts against hydroxyl radicals was evaluated using the method of Smirnoff and Cumbes (1989). Briefly, 100 μl of extracts was mixed with 3 ml of Smirnoff reagent and incubated at 37°C for 30 min. Extraction phase was used as a negative control to check the antioxidant potential of different extracts. The percentage of HFRSA by extracts was calculated using the following formula:

\[
\text{HFRSA (\%)} = \left(\frac{A_C - A_E}{A_C}\right) \times 100, 
\]
where \( A_C \) and \( A_E \) are the absorbance of control and extracts, respectively.

### 2.6.3 Reducing power activity

The reducing power activity (RPA) of the extracts was estimated by adapting the method of Oyaizu (1986). Briefly, 100 \( \mu l \) of extract was mixed with 100 \( \mu l \) of potassium ferricyanide (1%) and incubated at 50°C for 30 min. After that, 100-\( \mu l \) trichloroacetic acid (1%) and 100-\( \mu l \) ferric chloride (0.1%) were added, and the mixture was further incubated for 20 min at ambient temperature. The mixture was diluted with double distilled water to prepare final volume of 10 ml, and the absorbance was recorded at 700 nm. Quercetin was used as standard to compare the reducing power potential of extracts, and the results were expressed as mg quercetin equivalents per gram (mg QE/g) of sample.

### 2.6.4 Total antioxidant capacity

The method described by Prieto, Pineda, and Aguilar (1999) was adapted using ascorbic acid as standard to evaluate the total antioxidant capacity (TAC) of sample extracts. The reagent to assess antioxidant activity of extracts was prepared by mixing concentrated sulfuric acid (0.6 M), ammonium molybdate (4 mM), and sodium hydrogen orthophosphate (28 mM) in the ratio of 1:1:1. Further, 100 \( \mu l \) of sample extract was mixed with 3 \( \mu l \) of prepared reagent and incubated in water bath at 95°C for 90 min. Absorbance was recorded at 695 nm using ascorbic acid as standard to compare the antioxidant activity of extracts and the results were expressed as mg ascorbic acid equivalents per gram (mg AAE/g) of sample.

### 2.7 High-performance liquid chromatography

High-performance liquid chromatography (HPLC) was carried out to detect the presence of specific bioactive compounds in AFL (6th day sample) and UFL by the method as described by Dhull, Kaur, and Purewal (2016). All HPLC experiments were carried out on a Shimadzu10 AVP HPLC system comprising a SCL10 AVP system controller, two LC-10 AVP pumps CTO-10 AVP column oven with Rheodyne 7,120 injection value (20-\( \mu l \) sample loop), and SPD-M10 AVP photodiode-array detector (all from Shimadzu, Tokyo, Japan). Gemini-NX C18 analytical HPLC column (250 \( \times \) 4.6 mm, 3 \( \mu m \)) with a guard column (40 \( \times \) 3 mm, 3 \( \mu m \)), both from Phenomenex (Torrance, CA, USA) were used. Analysis was performed at a rate of 0.6 ml/min using 2% v/v acetic acid (solvent A) and methanol: acetonitrile (40:50 v/v) mixture (solvent B) under the following gradient program: 0–8 min 70% acetic acid, 8–19 min 60% acetic acid, and 19–30 min 30% acetic acid. Injection volume was 10 \( \mu l \), and the analytes were detected at 280 nm. For the quantification purpose, standard graphs were prepared, and the equation generated by the data was used to quantify the studied bioactive constituents.

### 2.8 Mineral estimation and in vitro bioavailability

The UFL and AFL flour samples were analyzed for their calcium, iron, zinc, and copper contents in atomic absorption mode and sodium and potassium contents in emission mode using atomic absorbance spectrophotometer (AAS; AA-7000, Shimadzu, Tokyo, Japan) following the methods reported by the Association of Official Analytical Chemists (2005). Samples were subjected to ashing (at 550°C for 8 h), solubilized in triacid mixture, and heated to complete the dissolution. All the samples were diluted to a suitable dilution before analysis by AAS. The in vitro bioavailability of iron and zinc was determined by adapting the simulated gastrointestinal model as described by Herrero-Barbudo, Granado-Lorencio, Blanco-Navarro, Pe'rez-Sacrista'n, and Olmedilla-Alonso (2009). Compositions and concentrations of inorganic and organic solutions, saliva, gastric juice, duodenal juices, and bile constituents were carefully duplicated as described by Granado-Lorencio et al. (2007).

### 2.9 Statistical analysis

The data were reported as the mean ± standard deviation of three replicates (\( n = 3 \)) for all experiments except for HPLC analysis (\( n = 2 \)). Further, using the commercial statistical package (SPSS Inc, Chicago, IL), an analysis of variance with a significance level of 5% was done, and Duncan’s test was applied to determine significant difference between mean values.

### 3 RESULTS AND DISCUSSION

#### 3.1 Effect of SSF on total phenolics and condensed tannin content

The effect of fermentation time on TPC and CTC of UFL and AFL extracts is shown in Table 1. The TPC of flours increased significantly (\( p \leq 0.05 \)) upon fermentation with the maximum content being observed on 6th day of fermentation, thereafter it decreased for all the cultivars. The unfermented flours showed TPC in the range between 15.8% to 17.5%. Upon fermentation, it was increased to 31.0%–35.2% with an increase of 78.8% to 122.8% in concentration for various cultivars. In a previous study carried out by Magro et al. (2019) for fermentation of lentil with A. oryzae and A. niger, it was observed that TPC increased linearly during fermentation with the highest TPC reaching after 96 h of fermentation. SSF of pearl millet by A. sojae (Salar et al., 2017) and wheat with A. awamorinakazawa (Sandhu, Punia, & Kaur, 2016) also resulted in an increase in their phenolic contents. This increase has been reported to the production of some active hydrolases such as \( \beta \)-glucosidase, \( \beta \)-xylosidase, and...
**TABLE 1** Effect of SSF on total phenolic and condensed tannin content of extracts from different unfermented and Aspergillus-fermented lentil cultivars

| Fermentation time | Total phenolic content (mg GAE/g dwb) | Condensed tannin content (mg CE/g dwb) |
|-------------------|--------------------------------------|----------------------------------------|
|                   | HM-1 | Variation (%) | LL-931 | Variation (%) | Sapna | Variation (%) | HM-1 | Variation (%) | LL-931 | Variation (%) | Sapna | Variation (%) |
| Control           | 17.3 ± 0.21<sup>a</sup>            | -         | 17.5 ± 0.19<sup>b</sup> | -         | 15.8 ± 0.36<sup>c</sup> | -         | 156 ± 0.13<sup>e</sup> | -         | 2.40 ± 0.16<sup>g</sup> | -         | 1.66 ± 0.12<sup>i</sup> | -         |
| 1st day           | 18.6 ± 0.15<sup>c</sup>            | +7.5      | 18.4 ± 0.15<sup>c</sup> | +5.1      | 16.3 ± 0.25<sup>a</sup> | +3.1      | 158 ± 0.16<sup>b</sup> | +1.2      | 2.42 ± 0.11<sup>c</sup> | +0.8      | 1.70 ± 0.14<sup>e</sup> | +2.4      |
| 2nd day           | 19.4 ± 0.13<sup>c</sup>            | +12.1     | 24.6 ± 0.21<sup>c</sup> | +40.5     | 18.4 ± 0.29<sup>a</sup> | +16.4     | 164 ± 0.10<sup>c</sup> | +5.1      | 2.50 ± 0.15<sup>c</sup> | +4.1      | 1.86 ± 0.11<sup>c</sup> | +12.0     |
| 3rd day           | 20.8 ± 0.37<sup>d</sup>            | +20.2     | 28.1 ± 0.34<sup>d</sup> | +60.5     | 23.9 ± 0.31<sup>d</sup> | +51.2     | 182 ± 0.12<sup>c</sup> | +16.7     | 2.61 ± 0.18<sup>c</sup> | +8.7      | 2.18 ± 0.14<sup>c</sup> | +31.3     |
| 4th day           | 21.9 ± 0.13<sup>d</sup>            | +26.6     | 28.7 ± 0.10<sup>d</sup> | +64.0     | 29.3 ± 0.17<sup>d</sup> | +85.4     | 226 ± 0.17<sup>c</sup> | +44.8     | 2.48 ± 0.15<sup>d</sup> | +11.6     | 2.81 ± 0.15<sup>d</sup> | +69.2     |
| 5th day           | 26.3 ± 0.11<sup>d</sup>            | +52.0     | 30.4 ± 0.17<sup>d</sup> | +73.7     | 34.8 ± 0.19<sup>d</sup> | +120.2    | 286 ± 0.15<sup>d</sup> | +83.3     | 3.86 ± 0.13<sup>d</sup> | +60.8     | 3.30 ± 0.19<sup>d</sup> | +98.7     |
| 6th day           | 31.0 ± 0.19<sup>d</sup>            | +79.2     | 31.3 ± 0.16<sup>d</sup> | +78.8     | 35.2 ± 0.27<sup>d</sup> | +122.8    | 316 ± 0.24<sup>d</sup> | +102.5    | 4.52 ± 0.07<sup>d</sup> | +88.3     | 4.21 ± 0.14<sup>d</sup> | +190.0    |
| 7th day           | 28.7 ± 0.24<sup>e</sup>            | +65.9     | 28.2 ± 0.27<sup>e</sup> | +61.1     | 30.5 ± 0.18<sup>e</sup> | +93.0     | 220 ± 0.11<sup>e</sup> | +41.0     | 3.24 ± 0.10<sup>e</sup> | +35.0     | 3.08 ± 0.12<sup>e</sup> | +85.5     |

Note. Mean ± SD, n = 3, followed by different superscripts (a–h) in a column differ significantly (p ≤ 0.05) and show variation among Aspergillus scripts p, q, and r in a row show variation among different cultivars. Variation (%) denotes the percentage increase from control samples for corresponding properties.

3.2 Effect of SSF on antioxidant potential

SSF can induce some biological changes in the substrate leading to the production of biologically active secondary metabolites, ultimately enhancing their antioxidant activity. The theoretical principle of every assay is different for measurement of various antioxidants. Therefore, the antioxidant potential of unfermented flours was observed. Salar et al. (2017) reported maximum DPPH radical scavenging activity of lentil extracts (90.2%) in 6th day fermentation. The highest CTC reached on the 6th day of fermentation with an increase of 88.3% to 159% in concentration. On the 7th day of fermentation, the CTC of all AFL extracts decreased to the range of 2.20 to 3.24 mg CE/g. A substantial increase in the values of total CTC was observed by Salar et al. (2017) in Aspergillus-fermented pearl millet.

The electron or hydrogen donating capability of an extract can be assessed by DPPH radical scavenging assay. The DPPH radical scavenging activity by single assay. Therefore, in the present study, four different assays were explored to analyze the antioxidant potential of unfermented and fermented extracts. The results also showed that SSF significantly (p ≤ 0.05) improved the antioxidant capacities of the different extracts.

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produce hydrolytic enzymes catalyzing the release of aglycones, thereby increasing the phenolics, anthocyanin content, and the antioxidative capability of fermented flours (Bhanja et al., 2009; Lee, Hung, & Chou, 2007, 2008).

Free radical scavenging activities of the AFL extracts were measured in comparison with their respective unfermented controls (UFL). HFRSA for UFL extracts ranged from 22.8% to 33.2%, the highest and the lowest values observed for cv. HM-1 and cv. LL-931, respectively.

**FIGURE 1** Effect of SSF on antioxidant potential of lentil cultivars: (a) DPPH (2,2-diphenyl-1-picrylhydrazyl) inhibition activity; (b) Hydroxyl free radical scavenging activity (HFRSA); (c) Reducing power activity (RPA); (d) Total antioxidant capacity (TAC)

**FIGURE 2** Chromatogram of unfermented lentil (UFL) and 6th day Aspergillus-fermented lentil (AFL) extracts from different cultivars: (a) HM-1 (UFL); (b) HM-1 (AFL); (c) LL-931 (UFL); (d) LL-931 (AFL); (e) Sapna (UFL); (f) Sapna (AFL)

[Correction added on 07 May 2020, after first online publication: Figures 2c and 2d have been corrected.]
HFRSA was also found to be the maximum on 6th day of the process, showing the highest value for cv. LL-931 (43.1%) with an increase of 89% in relation to their respective unfermented substrate. However, Salar et al. (2017) observed a constant increase (31.5%) in HFRSA until 10th day of SSF in pearl millet. In an earlier study it was observed that enzymatic activity of β-glucosidase increased significantly during fermentation, transforming less active isoflavone glucosides to more active isoflavone aglycones antiradicals (Kim et al., 2011).

RPA of AFL extracts continued to improve up to 6 days of SSF with an increase of 26.0% to 30.4%, and thereafter, it decreased (Figure 1c). Salar et al. (2017) reported an increase in RPA up to 8 days during SSF of pearl millet, which they attributed to increased xylanase activity upon fermentation. Also, the hydrogen donating ability of reductants was found to be directly related with the reducing power (Lee et al., 2008). During fermentation, the reductant formation starts, which terminates the radical chain reaction by reacting with the free radicals, resulting in high RPA of the fermented extracts (Lin, Wei, & Chou, 2006).

The TAC of unfermented flours was 8.0, 8.1, and 5.6 mgAAE/g, for cv. HM-1, cv. LL-931, and cv. Sapna, respectively. Likewise other assays, TAC showed a gradual and significant ($p \leq 0.05$) increase up to 6 days of fermentation (ranging between 10.4 to 19.0 mgAAE/g) and decreased thereafter (Figure 1d). Some of previous studies pointed...
out that Aspergillus species are excellent producers of various hydrolytic enzymes including proteases, cellulases, amylases, and lipases (de Castro & Sato, 2013; Gottschalk et al., 2010; Ohara et al., 2018). Therefore, there is a possibility of hydrolysis of polymers during fermentation, thereby releasing the conjugated phenolic compound present in the cell walls of legumes, making these compounds soluble, thereby increasing their concentration and antioxidant potential of the extracts.

### 3.3 HPLC of bioactive compounds

Among different legumes, lentils have been reported to have the highest oxygen radical absorption and antioxidant potential (Fratianni et al., 2014) that can be attributed to a wide range of phenolic as well as nonphenolic antioxidants (Xu & Chang, 2008). Different standards (ascorbic acid, quercetin, vanillin, resorcinol, p-coumaric acid, catechin, cinnamic acid, and gallic acid) were selected for bioactive constituent screening in the extracts. The results of HPLC qualitative as well as quantitative analysis of UFL and AFL are presented in Figure 2a–f and Table 2, respectively. Ascorbic acid and two phenolic compounds (quercetin and catechin) were observed mainly in both UFL and AFL extracts. The amount of bioactive compounds was found to be higher in AFL in comparison with their control counterparts. Some previous studies also demonstrated the role of SSF in the modulation of nutrient profile, polyphenolic content, and antioxidant potential of different legumes, cereals, and other natural sources (Oboh et al., 2008; 2009; Juan & Chou, 2010; Wu & Chou, 2009; Sandhu et al., 2016; Salar et al., 2017). Also, some new phenolic compounds not originally detected in UFL extracts were found to be present in AFL extracts such as resorcinol (for cv. HM-1 and LL-931) and cinnamic acid (for cv. LL-931), which provided clear evidence in support of microbial synthesis of these phenolic compounds. During microbial fermentation, carbohydrate cleavage due to different enzymatic activities are increased which result in change in the glycosides or fragmentary cleavage and release of potent antioxidants (Chiou et al., 2013; McCue & Shetty, 2003). On the other hand, compounds such as vanillin and gallic acid originally present in UFL extracts were not observed in their AFL counterparts. Bhat et al. (1998) also postulated that gallic acid may be degraded to aliphatic compounds during the process of fermentation resulting in a decrease in phenolic content.

#### 3.4 Effect of SSF on mineral content and bioavailability

A number of key functions such as building strong bone to transmitting nerve impulse are performed by minerals for a healthy and lengthy life. Different macro (Ca, K, Na) and micro (Fe, Zn, Cu) minerals were analyzed in the extracts using AAS but the results for control (UFL) and 6th day fermented AFL are only reported (Table 3). Among different lentil cultivars, mineral content differed significantly \((p \leq 0.05)\), with AFL extracts exhibiting higher mineral content than their unfermented counterparts. The mineral content of AFL increased until the 6th day of fermentation, thereafter it decreased. Zn content ranged from 31.8 to 32.5 ppm for UFL and 34.2 to 35.5 ppm for AFL, thereby showing an increase of 7.5% to 9.2% in concentration. Cu content of all AFL extracts was the highest with an increase of 46.4% to 60.0%, the highest increase was observed for cv. LL-931. All minerals showed significant increase in their concentrations upon fermentation except for K in which the increase was less than 0.1%. Sadh, Chawla, Bhandari, Kaushik, and Duhan (2017) and Chawla, Bhandari, Sadh, and Kaushik (2017) also reported increase in mineral content in biotransformed peanut oil cakes and black-eyed pea seed flour, respectively.

The total quantity of mineral present in a food does not reflect total amount of available mineral and adsorbed by human body, as only a certain quantity is bioavailable (Jafari & McClements, 2017). The bioavailability of a mineral can be defined as the fraction of the consumed mineral, which absorbed and utilized in different physiological functions of body (Fairweather-Tait & Hurrell, 1996). In vitro bioavailability of iron and zinc of AFL was significantly \((p \leq 0.05)\) higher in comparison with their unfermented counterparts (Table 3) and increased with increasing time of SSF (highest on 6th day of fermentation).

### Table 2 Quantification profile of bioactive compounds using high-performance liquid chromatography (HPLC) from different unfermented (UFL) and 6th day Aspergillus-fermented (AFL) lentil cultivars

| Phenolic compounds (mg/g dwb) | HM-1 UFL | HM-1 AFL | LL-931 UFL | LL-931 AFL | Sapna UFL | Sapna AFL |
|-----------------------------|----------|----------|-----------|-----------|-----------|-----------|
| Ascorbic acid               | 9.81a    | 10.96d   | 8.80b     | 12.04e    | 7.58a     | 18.10f    |
| Quercetin                   | 0.12a    | 1.14c    | nil       | 0.47b     | nil       | nil       |
| Vanillin                    | 0.68a    | nil      | nil       | nil       | 1.04b     | nil       |
| Resorcinol                  | nil      | 0.19a    | nil       | 0.31b     | nil       | nil       |
| p-coumaric acid             | nil      | nil      | 0.18a     | nil       | nil       | 2.14b     |
| Catechin                    | 0.56a    | 2.37c    | nil       | 0.96b     | 3.32d     | 4.61e     |
| Cinnamic acid               | nil      | nil      | nil       | 1.05      | nil       | nil       |
| Gallic acid                 | nil      | nil      | 1.22      | nil       | nil       | nil       |

Note. The values followed by different superscripts in a row differ significantly \((p \leq 0.05)\).
factors such as phytates and polyphenols in legume flours that form complexes with Zn and Fe and remarkably reduce their absorption (Gupta, Gangoliya, & Singh, 2015; Kiewlicz & Rybicka, 2020). After fermentation, these antinutritional factors complexing with proteins and minerals are reduced, thereby increasing their bioavailability in fermented flours (Adegbehingbe, 2015).

### 4 CONCLUSIONS

SSF using *A. awamori* (MTCC 548) significantly improved the antioxidant profile of fermented flours from all lentil cultivars. Fermentation with *A. awamori* is a cost effective, reliable, and efficient method to improve the TPC, CTC, and antioxidant potential of lentils in a short period of time and, therefore, may prove to be an important process for industrial usage. New phenolic compounds (resorcinol, cinnamic acid) synthesis was also confirmed by HPLC. Different macro (Ca, K, Na) and micro (Fe, Zn, Cu) mineral content improved during fermentation. However, in vitro bioavailability of iron and zinc of AFL was significantly \( p \leq 0.05 \) higher in comparison with their unfermented counterparts. Thus, biotransformed Aspergillus-fermented lentils could be used in preparation of nutritious functional foods.

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### DATA AVAILABILITY STATEMENT

Due to technical limitations, the full dataset is unable to be published at this time. However, it is available upon request from the authors.

### CONFLICT OF INTEREST

The authors have no conflicts of interest with respect to this manuscript.

### COMPLIANCE WITH ETHICAL REQUIREMENTS

Due to technical limitations, the full dataset is unable to be published at this time. However, it is available upon request from the authors.

### TABLE 3 Mineral (Fe, Zn, Ca, Cu, Na, K) content and in vitro bioavailability (Fe, Zn) of different unfermented (UFL) and 6th day Aspergillus-fermented (AFL) lentil cultivars

| Mineral (ppm) | HM-1 UFL | AFL | Variation (%) | LL-931 UFL | AFL | Variation (%) | Sapna UFL | AFL | Variation (%) |
|--------------|----------|-----|--------------|------------|-----|--------------|------------|-----|--------------|
| Fe           | 64.5 ± 0.2a | 70.4 ± 0.5d | +8.6        | 65.2 ± 0.2c | 70.8 ± 0.4d | +8.5        | 64.2 ± 0.3a | 69.4 ± 0.2c | +8.0        |
| Zn           | 32.5 ± 0.3b | 35.5 ± 0.3c | +9.2        | 31.8 ± 0.2b  | 34.2 ± 0.3a | +7.5        | 32.5 ± 0.2b | 35.1 ± 0.2c | +8.0        |
| Ca           | 557.6 ± 2.0a | 564.3 ± 2.1b | +1.2        | 555.9 ± 3.1a | 562.5 ± 2.2b | +1.1        | 556.9 ± 3.0a | 563.2 ± 2.1b | +1.1        |
| Cu           | 2.8 ± 0.1a  | 4.1 ± 0.2a  | +46.4       | 2.5 ± 0.3a  | 4.0 ± 0.3a  | +60.0       | 2.7 ± 0.2a  | 4.2 ± 0.1b  | +55.5       |
| Na           | 60.6 ± 1.5a | 65.6 ± 1.4b | +8.2        | 61.3 ± 1.3a | 65.7 ± 1.3b | +7.2        | 60.5 ± 1.2a | 65.2 ± 1.4b | +7.6        |
| K            | 6,773 ± 3.2a | 6,778 ± 2.2b | +0.07       | 6,773 ± 2.1a | 6,779 ± 3.0b | +0.09       | 6,771 ± 2.5a | 6,778 ± 2.8b | +0.09       |
| In vitro bioavailability (%) | Fe | 18.3 ± 1.02a | 32.5 ± 1.01b | +77.6       | 17.2 ± 1.05a | 32.8 ± 1.10b | +90.6       | 18.0 ± 1.0a  | 30.3 ± 1.20a | +68.3       |
|              | Zn | 15.1 ± 0.91a | 28.2 ± 1.02b | +86.7       | 15.5 ± 0.85a | 30.5 ± 1.11b | +96.7       | 14.7 ± 0.95a | 29.5 ± 0.80b | +100.6      |

Note: Mean ± SD, n = 3, followed by different superscripts (a–f) in a row differ significantly \( p \leq 0.05 \). Variation (%) denotes the percentage increase from control samples for corresponding properties.
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