CHEMICAL ENGINEERING TRANSACTIONS
VOL. 102, 2023
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Laura Piazza, Mauro Moresi, Francesco Donsì

ISBN 979-12-81206-01-4; **ISSN** 2283-9216
DOI: 10.3303/CET23102034

Potential of Germination to Improve the Properties of Lentils (Lens Culinaris Medik.)

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Lentils (*Lens culinaris Medikus*) are of interest to the food industry due to their affordability, nutritional value, associated health benefits, environmental benefits and agronomic productivity, including nitrogen fixation and drought resistance (Romano et al., 2021). The goal of this preliminary study was to investigate the impact of germination times (0, 24 and 48 hours) on some physico -chemical, functional and aromatic properties of lentils samples.Germinated samples showed some significant differences ($p < 0.05$) on results of properties of control sample (0 hours). The total starch of germinated samples decreased compared to the control sample (0 hours). Germination time affected properties, leading to a significant increase in techno - functional properties such as water absorption (WHC), oil absorption (OHC) capacities and swelling index (SI). The control had the smallest WHC (1.65 g /g), OHC (1.39 g /g) and SI values at all temperatures (60- 90°C). Flavour attributes were significantly influenced by the germination times. Overall, a total of 13 (control sample) and 16 (germinated samples) aromatic compounds were identified.

1. Introduction

Pulses, the dry edible seeds of the legume family, are consumed worldwide and are desired for their high protein quality and quantity. In particular, lentils (*Lens culinaris Medikus*) are a pulse crop belonging to the Fabaceae family and are grown in > 70 countries, ranking fourth in the global grain legumes production after bean (*Phaseolus vulgaris L*.), pea (*Pisum sativum L*.) and chickpea (*Cicer arietinum L*.) (Romano et al., 2021). Lentils are nutritionally beneficial to all, including vegetarian and vegan diets. Lentils are usually used for consumption in the form of cooked whole seeds or split cotyledons or processed into various ingredients (e.g., flour) for the uses in different food applications. They have been studied for application in various product formulations, including bakery (bread, cake, crackers), extruded (pasta, snacks) and other products (dressings, soups, dairy and meat products) (Romano et al., 2021). The effective use, attributes and consequent acceptance of lentils or pulses by consumers are correlated with its nutritional, physicochemical and functional properties (Adedeji et al., 2014).
Several works have been done on germination (sprouting) as an alternative to genetic engineering in improving the nutritive values (such as amino acids, vitamins, minerals etc.), functional (gelatinization, swelling, and solubility) and chemical (pH, total starch etc.) properties of cereals and pulses (Adedeji et al. 2014; Frías et al., 2002).
Germination is a traditional and inexpensive method, that involves chemical changes such as the hydrolysis of starch, protein and fat by amylolytic, proteolytic and lipolytic enzymes, respectively. When seeds are hydrated (soaked) and then held (sprouted) under ambient conditions (20 - 25 °C), both endogenous and newly synthesized enzymes begin to modify seed constituents and consequently their properties (Romano et al., 2021). The germination time and temperature are the most important factors influencing the nutritional quality and properties in sprouted seeds (Adedeji et al. 2014; Aparicio-García et al., 2020). However, the impact of germination on the physico - chemical and functional properties of pulse flours has not been fully explored.

Paper Received: 25 February 2023; Revised: 24 April 2023; Accepted: 27 June 2023
Please cite this article as: Romano A., Romano R., De Luca L., Masi P., 2023, Potential of Germination to Improve the Properties of Lentils (Lens Culinaris Medik.), Chemical Engineering Transactions, 102, 199-204 DOI:10.3303/CET23102034

Therefore, the aim of this preliminary work was to evaluate the effects of germination times (0, 24 and 48 hours) on some physico -chemical, functional and aromatic properties of green lentils samples.

2. Materials and Methods

2.1 Materials

Approximately 2 – 3 kg of lentil seeds (*Lens culinaris Medik.*) (control) from the 2021 crop year were gifted from producers (Terre di Altamura, Bari, Apulia, Italy). Seeds were harvested in Altamura (Bari, Italy) and had an average mass of 0.03 g and the diameter of 4.38 ± 0.14 mm.

2.2 Preparation of germinated samples

Lentil seeds were washed with 0.07 % (w/v) sodium hypochlorite solution for 30 min at room temperature. Then, they were washed with distilled water several times until reaching neutral pH. Afterwards, they were soaked in the 1:3 (w/v) ratio of seeds to distilled water overnight in a dark chamber under ambient laboratory conditions (22 - 24°C). In the end, the water was drained, and seed samples were ready to be germinated under a wet cloth in a dark condition for 24 (G) and 48 (H) hours. The sprouted seeds were oven-dried at 60 °C for 17–18 h. The rootlets and hulls were removed and the germinated samples was packed in polypropylene airtight containers at 4 ± 3°C.

2.3 Milling of lentil seeds

Lentil seeds - raw (control, 0h) and germinated for 24h (G) and 48h (H) - were milled into flour samples using a Laboratory mill (mod. 3100, Perten instruments Ab, Finland) after ten days and then sifted to obtain a particle size < 300 µm (Giuliani sifter, Turin, Italy). The flour samples were packed in polypropylene airtight containers at 4 ± 3°C.

2.4 Properties of samples

The moisture content of each sample was determined by the AACC method (number 44-15.02, 1999). Three samples of flour, weighing approximately 3 g, were dried for 24 h at 105 ºC. Samples were removed from the oven and immediately placed in a desiccator prior to weighing after cooling and within 30 min. The dried samples weight was subtracted to the respective initial weight. The results were calculated as percentage of water per sample weight (%).
Total starch (TS) (g/100g) was determined using an enzymatic assay kit (Total Starch Assay Kit, Megazyme International Ireland) by AACC method (number 76.13, 2009).
The pH of samples was measured by using a calibrated digital pH meter (XS instruments pH-8, Carpi, Italy).
Samples of flour were used for Water Holding Capacity (WHC) and Oil Holding Capacity (OHC) analyses, according to method of Romano et al (2016).
The swelling index of flour samples (SI) was measured following the method described by Yadav and colleagues (Yadav et al., 2012), with modifications. Suspensions (2% w/v) were heated 60, 70, 80 and 90 ◦C for 1 h, cooled at 30 ◦C for 30 min and were then centrifuged at 8000 rpm for 20 min. The weight of the resulting pellet was determined. SI was calculated as the ratio between sediment and fresh sample weights. Each average value represents the mean of 3 independent measurements.

2.5 Volatile organic compounds

Volatile organic compounds (VOCs) were extracted and analyzed from lentils following the method proposed by Xu et al. (2019), with modifications. Briefly, 2 g of sample were weighed into a 20 mL vial and 5 mL of saturated NaCl solution was added. The vial was placed in a thermal bath at a temperature of 60 °C for 10 min. Subsequently, the fiber DVB/CAR/PDMS (50/30 µm layer of divinylbenzene / carboxen / polydimethylsiloxane) was inserted for 50 min in headspace of sample.
Subsequently, the SPME fiber was introduced directly into the GC injector, where the thermal desorption of the analytes was performed at 250 °C for 3 min. A 6890N GC system equipped with a 5973 mass detector was used. VOCs were separated on a 30 m × 0.250 mm capillary column coated with a 0.25 µm polymer of 5% diphenyl 95% dimethylpolysiloxane. Splitless injection was used for the samples.
The oven temperature was held at 40 °C for 5 min and increased from 40 °C to 85 °C at 45 °C/min, from 85 to 200 °C at 9 °C/min and from 200 to 250 to 45 °C/min, the temperature of 250 °C was maintained for 3 min. The injection source and ion temperatures were 250 and 230 °C, respectively. Helium was used as a carrier gas at a flow rate of 1 mL/min. The ionizing electron energy was set to 70 eV and the scanned mass range was set to 40–450 amu in the full scan acquisition mode.
Compounds were identified comparing the mass spectra fragmentation patterns with the spectra data from the

NIST Atomic Spectra Database version 1.6 and the retention indices with those reported in literature. The relative content of VOCs was calculated on the basis of peak area ratios.
Each average value represents the mean of 3 independent measurements.

2.6 Statistical analysis

All experiments were performed in triplicate and data were expressed as mean values ± SD. Statistical analyses were performed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). Analysis of variance was carried out on the data gathered from the different evaluations. Significant differences between the detected parameters were compared by Duncan's multiple comparison test at the 95% confidence level ($p \leq 0.05$).

3. Results and Discussion

3.1 Effect of germination time on properties of lentils

Table 1 shows the moisture content, Total Starch (TS), pH, Water Holding Capacity (WHC) and Oil Holding Capacity (OHC) values for the raw green lentils (control) and germinated samples (G and H).

Table 1: Physicochemical and functional properties of control, G (24 h), H (48 h) samples. Each value is expressed as mean ± SD

Parameter	control (0h)	G (24h)	H (48h)
moisture content (%)	7.71 ± 0.51a	7.91 ± 0.57a	8.90 ± 0.31b
TS (%)	34.83 ± 0.82b	32.95 ± 0.19a	32.89 ± 0.53a
pH	6.04 ± 0.01 b	5.97 ± 0.09 ab	5.91 ± 0.03 a
WHC (g/g)	1.65 ± 0.06 a	1.84 ± 0.10 b	1.92 ± 0.17 b
OHC (g/g)	1.39 ± 0.10 a	1.71 ± 0.09 b	2.49 ± 0.18 c

Means followed by a different letter within a row are significantly different ($p < 0.05$)

Raw and germinated lentil flours had moisture contents in the range of 7.7 to 8.9%, which the highest amount is for H, the long term germination sample. The moisture content in the green lentils was 7.7%, which is in good agreement with those reported earlier (Alkaltham et al., 2022).
Effect of soaking / germination was significant ($p < 0.05$) on the moisture contents of the samples after one day, exhibiting an upward trend with increase in germination time (Table 1).
Total starch content in raw lentils (control) was 35%. Over 1 and 2 days of germination, total starch content in lentils decreased at 33%. As is well known, a whole spectrum of hydrolytic enzymes, including α-amylase, glucosidase, and dextranase are generated from the aleurone layer of pulse seeds, and β-amylase existing in the endosperm, is activated during pulse seeds germination (Olaerts et al., 2016). These hydrolytic enzymes are responsible for the conversion of starch into oligosaccharides or monosaccharides, resulting in the reduction of starch content.
The pH decreased significantly ($p \leq 0.05$) as the germination time increased. Lentils grains that germinated for 48 h had the lowest pH value of 5.9. The decrease observed in pH might have been as a result of secretion of enzymes resulting in the hydrolysis of complex organic molecules such as phytin and protein into simpler and more acidic compounds such as phosphoric acid and amino acids, respectively. Evans et al. (2003) reported a marked increase in alpha amylase and other amylases during cereal germination.
The water holding capacity (WHC) increased significantly after germination (Table 1), with the control (ungerminated) sample having the least value ($p \leq 0.05$). This variation in WHC is mainly related to differences in chemical composition of samples. In fact, the increase in WHC may be attributed to increase in compounds having good water holding capacity such as soluble sugars and increase in number of polar groups on low molecular weight proteins upon germination. According to Okaka and Potter (1997), water holding capacity depends on the water bounding capacities of food components. Increase in WHC is a useful functional property in foods such as processed meats, where protein mixed with water imparts thickening power and viscosity to the food (Wanasundara and Shahidi, 1994).
Oil Holding Capacity (OHC) varied significantly ($p \leq 0.05$) among the samples. In particular, samples with long – term germination (48 h) having the highest OHC value (2.5 g/g). Giani and Bekebain (1992) reported that germination of grains enhances the OHC due to the entrapment of oil related to the non-polar side chains of proteins.Swelling Index (SI) defines the water absorbed and trapped in the gel network created by starch granule hydrogen bonds during heating and stirring in excess of water (Li et al., 2014). At low temperatures, thermal energy swells starch granules without disruptions; greater thermal energy with temperature increases induces crystalline structure breakdown and increased SI (Li et al., 2014).

Figure 2 shows the impact of germination on the swelling properties of the lentil flour samples.

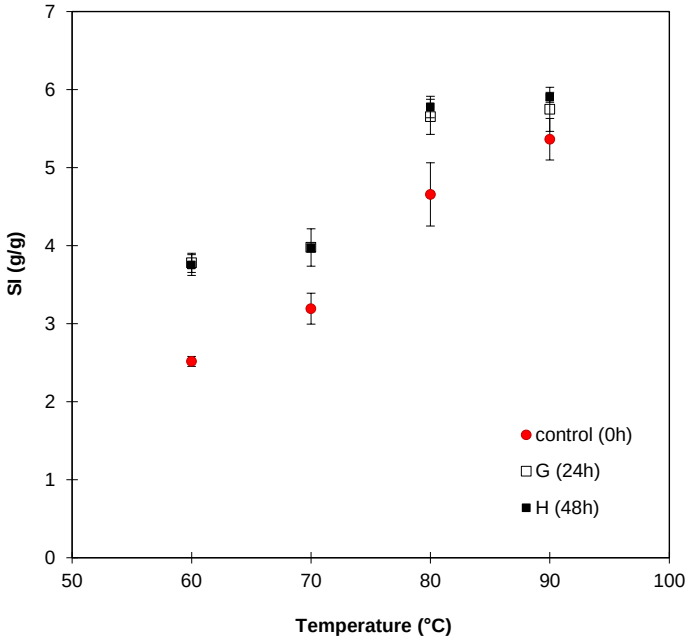

Figure 1: The swelling index of lentil samples: control (●, 0h), G (□, 24 h), H (■, 48 h).

With the increase of temperature, the SI of all samples increased due to the starch taking up water and swelling substantially. In particular, SI of control increased with rising temperature until 90°C, while the SI of germinated samples increased until 80°C, and then remained constant as previously reported (Boukid et al., 2019).

Among samples, control showed SI values lower ($p \leq 0.05$) than those of G and H at all swelling temperature (60, 70, 80 and 90°C). This behaviour might be explained by a greater degree of amylose and amylopectin interactions in control which, prevent starch molecules from releasing amylose during gelatinization (Wani et al., 2016). Overall, SI depends on several factors, e.g., amylose/amylopectin ratio, size, morphology and ultrastructure of starch granules and cell wall intactness, temperature, and pH (Boukid et al., 2019; Wani et al., 2016).To describe Volatile Organic Compounds (VOCs) profiles, control, G and H were analysed. A total of 13 (C) and 16 (G and H) aromatic compounds were identified through SPME-GC/MS analysis (Figure 2).

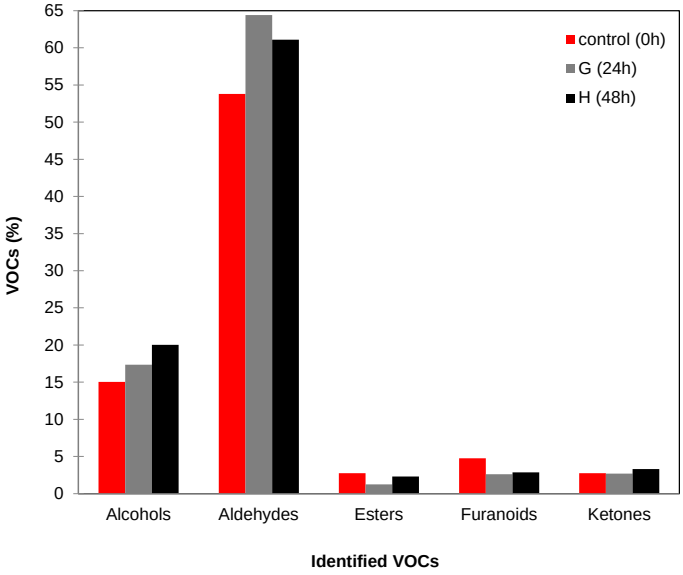

Figure 2: Volatile organic compounds (VOCs) (%) of lentil samples: control (●, 0h), G (■, 24 h), H (■, 48 h).

VOCs profiles appeared affected by the length of the germination. Aldehydes (53.78 % - 64.33 %), alcohols (15.05 % - 20.03 %) esters (1.24 % - 2.75 %), ketones (2.73 % - 3.33 %) and furanoids (2.61 % - 4.72 %) were identified in the germinated lentils. In this way, aldehydes, alcohols, ketones and furanoids have been identified as volatile compounds in lentils (Roland et al., 2017; Xu et al., 2019).
The aldehydes concentration increased during the germination. The hexanal was the most present and ranging between 32.62 % in control to 43.11 % in G with an increase during the germination showing the highest content after 24 h of germination. The presence of hexanal has previously been reported in beans, soybeans, and peas. In the raw seeds, linoleic acid is oxidized to hydroperoxides in the presence of oxygen and the hexanal may be formed by the cleavage of 13-hydroperoxylinoleic acid by lyases (Ma et al., 2016). The hexanal influences the greeny and grassy flavour of legumes (Paucean et al., 2018).
The total alcohols concentration increased during the germination. Among alcohols 1-hexanol, 1-octen-3-ol and benzyl alcohol were found. Free fatty acid breakdown is the major origin identified in the production of alcohols, but the degradation of amino acids also leads to the formation of these molecules (Karolkowski et al., 2021). The alcohols influence the green, mushroom and fruity aroma (Xu et al., 2020; Kaczmarska et al., 2018; Yilmaztekin, 2014)
Among ketones the 3,5-octadien-2-one was found and such compound ranged from 2.73 % in control to 3.33 % in H sample (48h), so to increase during the germination and its presence in germinated pulses was reported also by Xu et al. (2019). In the pulses the ketones derived from amino acid degradation, carotenoid breakdown, but the free fatty acid oxidation is predominant way (Karolkowski et al., 2021).
Finally, among furanoids, the 2-pentylfuran was found with a reduction of its concentration during the germination from 4.7 % in control to 2.85 % in H. This compound is a marker of beany aroma (Rajhi et al., 2022).

4. Conclusions

It can be inferred from the present work that germination has some important effects on the composition, functional and aromatic properties of green lentils.
Germinated samples performed better in terms of water absorption capacity, oil absorption capacity and swelling index than control. In particular, sample that germinated for 48 h performed best in water absorption capacity and oil absorption capacity. Moreover, germination of lentils can be a practical and effective treatment to improve aromatic profile.
Although the interactions of the flavour compounds with the properties of the samples should be more systematically investigated, the preliminary findings of this work could have useful implications. Firstly, the results may support technologists in the development and improvement of highly nutritional bakery products based on germinated lentil flour, with beneficial effects for both producers and consumers.

Acknowledgments

The contribution made by Carmela Piemonte is gratefully acknowledged.

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