Decorticated Lentil Malt Flour: Production Process and Use

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In this work, the malting process of lentil seeds (*Lens culinaris*) was set-up to minimize their anti-nutrient content. The first (water steeping) and second (germination) process steps were studied in a 1.2-kg bench-top plant at 25 °C. After 2-h steeping about 98.8% of seeds sprouted. As the germination process was prolonged for 72 h, the flatulence-inducing raffinose or phytic acid content was reduced by 94% or 63%, respectively. The third process step (kilning), carried out under fluent dry air at 50 °C for 48 h and at 75 °C for 3 h, gave rise to a gold metallic yellow-lentil malt, the cotyledons of which were cyclonically recovered and finally milled. The resulting decorticated yellow-lentil malt flour was used to prepare a fresh egg pasta high in raw protein (28±2 g/100 g), low in phytate (0.46±0.03 g/100 g) and *in vitro* glycemic index (38%), and approximately zero oligosaccharides.
KEYWORDS: Dehulled lentil malt flour, lentil malting, gluten-free fresh egg pasta, antinutrient removal, glycemic index.

1. Introduction

Legume seeds are valuable sources of protein, dietary fibers, and micronutrients, generally recommended as healthy replacers of foods of animal origin in the diet. Despite their positive nutritional profile and low environmentally impacting cultivation (Nemecek et al., 2008), their average per capita consumption (~21 g/day) has on a global scale stagnated over the last three decades (Rawal and Navarro, 2019), probably for their long cooking times, unpleasant flavor, low-digestible proteins, and gastrointestinal problems (De Almeida Costa et al., 2006). Their richness in anti-nutritional factors (e.g., phytic acid, tannins, enzyme inhibitors, and flatulence-inducing oligosaccharides) is another factor limiting the consumption of pulses (Gebrelibanoset et al., 2013). Among them, lentil (*Lens culinaris*) was probably the first domesticated crop and still represents quite an important traditional food in many developing and underdeveloped countries. The annual global production of lentils is up to 6.32 million metric tons (Mg), Canada and India being the first and second producers with around 3.23 and 1.06 million Mg, respectively (Atlasbig, 2022). Lentil varieties differ in size from extra-small to large, and in color from yellow and red orange to green, black, and brown (Asif et al., 2013). Since lentils are gluten-free and have a low glycemic index even when mixed with other grains, they have been used to formulate vegetarian diets, as well as bakery products for people with celiac disease or diabetes (Asif et al., 2013). To minimize the main anti-nutritional effects of pulses, several thermal and non-thermal processing techniques have been tested (Sharma et al., 2022). Malting, a processing method commonly used in the beer industry, might be used to reduce the content of anti-nutrients, such as phytic acid responsible for mineral malabsorption and flatulence-causing oligosaccharides, as well as improve the taste acceptability and healthiness of pulses. Thus, the main aim of this work was to determine the most proper operating conditions for each of the three stages (i.e., seed steeping, germination, and kilning) of a yellow-lentil malting process in a lab-scale plant to obtain a decorticated yellow-lentil malt flour low in phytate and α-galactosides useable for preparing a gluten-free fresh egg pasta with a low *in vitro* glycemic index.

2. Materials and methods

The lentil line 6002/ILWL118/1-1 (*Lens culinaris* ssp *orientalis*) used in this work was produced in an experimental station in Marchouch (Morocco: lat. 33.567, long. 6.333, alt. 255 m a.s.l.). It was characterized by Romano et al. (2022) and kindly supplied by the International Center for Agricultural Research in the Dry Areas (ICARDA, Rabat, Morocco). These yellow-lentil seeds as such or malted were characterized by means of the

following physical tests: seed weight and volume, mean radius (R_S), density, hydration capacity (HC), and swelling capacity (SC), as previously described by Cimini et al. (2021). Their hydration kinetics was studied in a bench-top plant, previously described (Cimini et al., 2021). It consisted of an insulated chamber provided with six stainless-steel perforated baskets each one containing 200 g of seeds, fan ventilators, compressed-air injectors, water drainage system, and sensors to monitor the relative humidity (RH) and temperature (T) of air (CJMCU-1080 HDC1080, Texas Instruments, Dallas, Texas, USA) and seed temperature (DS18B20, Maxim Integrated, San Jose, CA, USA). A Geekcreit® ATmega328P Nano V3 microcontroller enabled the automatic operation of solenoid valves and data recording. To assess the seed germination degree (G), 40 seeds undergoing steeping at 25 °C for times ranging from 0 to 8 h were collected from the above baskets, and placed over an absorbent paper sheet, pre-soaked in 50 mL of deionized water, into a (20 cm x 14.5 cm x 2.5 cm) box, that was hermetically sealed and housed in a dark chamber kept at 25 °C for 24 or 48 h. By counting all the seeds with an evident root regardless of its length, it was possible to determine the germination degree after 24 (G_{24}) or 48 (G_{48}) h, and thus the steeping time (t_S) associated with the minimum number of non-sprouted seeds. As the seeds had been hydrated at 25 °C for t_S, the steeping water was discharged to let the moist seeds germinate for as long as 96 h. The degradation of their main antinutrients was monitored using the Phytic Acid and Raffinose/Sucrose/D-Glucose Assay Kits (Megazyme Ltd, Bray, Ireland), respectively. In particular, the α-galactosides were expressed as raffinose equivalent on a dry matter basis. Germinated lentils at a moisture content of 48-52% on a wet basis, were then dehydrated till a residual moisture of 10% (w/w) at 50 °C for 48 h and 75 °C or 3 h using the Nobel Pro 6 ventilated dryer (Vita 5, Gronsveld, The Netherlands). The color of the malted product was measured in the CIELAB color space using a portable color-measuring instrument mod. D25-PC2 (Hunterlab, Restow, Virginia, USA) with a diffuse (0/45°) illuminating viewing geometry. A cyclone separator, specially designed and produced using a 3D printer, was used to separate the cuticle fragments from the cotyledons, once malted seeds had been submitted to slight abrasion. The cotyledon-rich fraction was ground using a multipurpose electric coffee bean grinder (Veotech, Vannes Cedex, France), thus obtaining a decorticated yellow-lentil malt flour. The Pasta Machine PF40E (Fimar Spa, Villa Verucchio, Italy), equipped with a water-cooled extrusion die and a pasta cutting knife, was used to mix such a flour or all-purpose wheat flour with whole egg in a weight ratio of 63:37 g/g and extrude two fresh egg pastas in the long-shaped format of 3-mm wide *tonnarelli*. Fifty grams of each egg pasta were cooked in a lidded stainless-steel pot using a 2-kW induction-plate hob (Melchioni INDU, Melchioni Spa, Milan, Italy) and a water-to-pasta ratio of 10 L/kg. Cooked pasta strands were recovered using a colander and cooled according to Method 66-50.01 of the American Association of Cereal Chemists (2009) and submitted to *in vitro* digestion according to the method developed by Zou et al. (2015). All the tests were replicated at least 3 times. The concentration of glucose (C_G), released by the simulated digestion of starch in the mouth and stomach, was determined at different times (t) ranging from 0 to 300 min via the enzymatic kit D-Glucose Assay Procedure, K-GLUC 07/11 (Megazyme Ltd, Bray, Ireland). The area (AUC) under the C_G-vs.-t curve (digestogram) for a digestion time ranging from 0 to 180 min for each fresh pasta sample was numerically calculated according to the Trapezoidal Rule and normalized with respect to the corresponding area for a reference product (i.e., white bread), as suggested by Giuberti et al. (2015) to yield the percentage starch hydrolysis index (SHI). The latter was used to calculate the *in vitro* glycemic index (GI) by using the empirical formula given by Granfeldt et al. (1992). The moisture content of lentil seeds undergoing steeping, germination, or kilning, as roughly divided in parts, was assessed at 110 °C for ~20 min using a Kern DAB 100-3 thermostatic scale (Kern&Sohn GmbH, Balingen, Germany). Total starch (TS) and resistant starch (RS) fractions in seeds, flours, and cooked pasta samples were determined using the total starch (amyloglucosidase/α-amylase method) and resistant starch kits by Megazyme Ltd (Bray, Ireland), respectively. Crude protein content was measured by the AOAC 992.23 method (Association of Official Analytical Chemists, 1998) using a nitrogen conversion factor of 6.25.

2.1 Data analysis

The lentil hydration kinetics was described using the empirical model developed by Peleg (1988):

$$M(t) = \frac{x_W}{1 - x_W} = M_0 + \frac{t}{k_1 + k_2\, t} \tag{1}$$

where $M(t)$ and M_0 are the instantaneous and initial moisture ratios, x_w is the current moisture content on a wet basis, t the hydration time, k_1 the Peleg rate constant, and k_2 the Peleg capacity constant. While the initial water uptake rate ($R_{W0}=dM/dt|_{t=0}$) is inversely proportional to the Peleg rate constant k_1, the equilibrium moisture ratio (M_e) coincides with its initial value (M_0) plus the reciprocal of the Peleg capacity constant k_2. Upon linearization of Eq. (1), it would be possible to determine both k_1 and k_2 using the least squares method.

The degradation kinetics of either the reference oligosaccharide (e.g., raffinose) or phytic acid was described as a first order reaction:

$$\frac{d\,C_i}{dt} = -\,k_i\,C_i \tag{2}$$

where k_i is the degradation kinetic rate constant of the i-th anti-nutrient. By separating the independent variables and integrating, the following was obtained:

$$C_i = C_{i0}\, e^{-k_i t} \qquad\qquad \text{for } t \geq 0 \qquad\qquad (3)$$

where C_{i0} is the initial concentration of the i-th component.

All the trials were triplicated to estimate the mean values (μ) and standard deviation (sd) of all data collected. The Tukey's test was applied for the statistical comparison of means at the probability level (p) of 0.05.

3. 3. Results and Discussion

3.1. Physical properties

The chemico-physical properties of the lentil seeds under study are listed in Table 1. The crude protein, total starch, phytic acid and raffinose contents on a dry matter basis fell within the same range of several lentil varieties (Johnson et al., 2013; Najib et al., 2022; Rawal and Navarro, 2019; Xu et al., 2019). The seed weight (m_S) and volume (V_S) were intermediate between those of large green lentils, such as Greenland, and those of small green lentils, such as Imvincible (Najib et al., 2022). On the contrary, its density (ρ_S) was smaller than that determined for the aforementioned green lentils (Najib et al., 2022). The equivalent spherical diameter of the yellow lentils was just smaller than that estimated for the Greenland variety (0.24 cm/seed).

Table 1: Lentil seeds as such or malted: main chemico-physical properties and CIELab coordinates (L^*, a^*, b^*).

Chemico-Physical property	Lentil	Lentil Malt	Unit
Raw protein	22.82±1.90 [a]	24.01±2.00 [a]	g/100 g dm
Total Starch (TS)	44.98±4.22 [a]	51.10±3.09 [a]	g/100 g dm
Resistant Starch (RS)	1.44±0.27 [a]	1.62±0.15 [a]	g/100 g dm
Phytic Acid (PA)	1.36±0.12 [a]	0.76±0.06 [b]	g/100 g dm
Raffinose (R)	4.26±0.46 [a]	0.20±0.14 [b]	g/100 g dm
Seed weight (m_S)	0.059±0.001 [a]	0.0438±0.0001 [b]	g/seed
Seed volume (V_S)	0.050±0.000 [a]	0.0348±0.0000 [b]	cm^3/seed
Mean seed radius (R_S)	0.229±0.000 [a]	0.2025±0.000 [b]	cm/seed
Seed density (ρ_S)	1.18±0.02 [a]	1.259±0.004 [b]	g cm^3
Hydration capacity (HC)	0.058±0.001 [a]	0.0468±0.0003 [b]	g/seed
Swelling capacity (SC)	0.105±0.005 [a]	0.086±0.003 [b]	cm^3/seed
L^*	65.8±0.9 [a]	69.4±1.8 [b]	-
a^*	3.4±1.0 [a]	2.2±0.4 [b]	-
b^*	38.3±2.2 [a]	35.5±2.1 [b]	-

In each row, values with the same letter have no significant differences at $p < 0.05$.

3.2. Hydration kinetics of dry lentils

Figure 1 shows the evolution of the hydration isotherm at 25 °C for the lentil seeds under study. Such isotherm was characterized by an initial quick increase for t varying from 0 to 3 h. After that, there was a slower growth up to reach a saturation moisture ratio. The experimental moisture ratio was appropriately reconstructed using Eq. (1), the Peleg constants k_1 and k_2 being estimated via the least squares method:

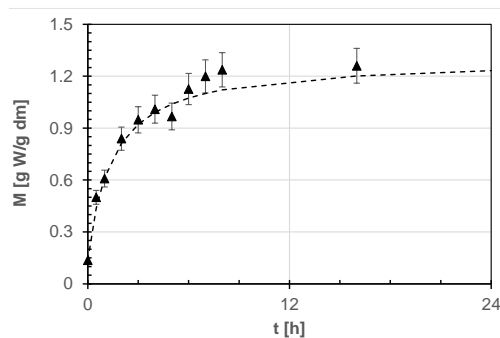$k_1 = 1.25 \pm 0.17$ h g dm/g W; $\qquad k_2 = 0.81 \pm 0.03$ g dm/g W $\qquad (r^2 = 0.99)$

Figure 1: Time course of the experimental moisture ratio M during the steeping of lentils at 25 °C (▲). The broken line curve was plotted using the Peleg model and the constants listed in the text.

Thus, the estimated value of the equilibrium moisture ratio (M_e) or weight fraction (x_{We}) was equal to 1.38 ± 0.18 g W/g dm or 58 ± 3% (w/w), respectively.

3.3. Lentil germinability

During the hydration of the lentil seeds at 25 °C for as long as 8 h, at 1-h intervals 40 seeds were collected and let germinate for 24 or 48 h at the same steeping temperature. Figure 2 shows the percentage of sprouted seeds. Just 1.2% of the lentil seed imbibed with water for 2 h exhibited no rooting after 24- and 48-h germination. Since the degree of inhomogeneity in germinating seeds was minimum, the steeping process at 25 °C was carried out for just 2 h, allowing the seeds to reach an average moisture content of 45.6±0.5 % (w/w).

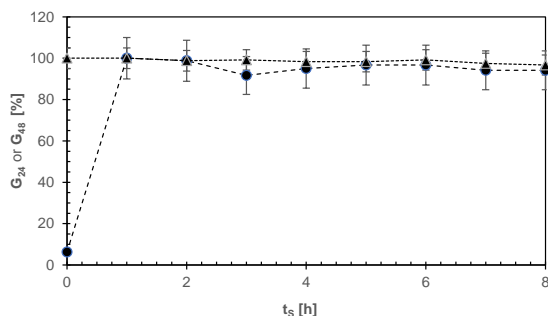*Figure 2: Average percentage of lentil grains germinated after 24 (G_{24}: ●) or 48 (G_{48}: ▲) h, once they had been submitted to water steeping at 25 °C for different steeping times (t_S).*

3.4. Lentil germination

The above soaked seeds were kept germinating at 25 °C for as long as 96 h, while monitoring the time course of the concentration (dry matter basis) of raffinose and phytic acid. Figure 3 shows that the natural logarithm of the ratio (C_i/C_{i0}) between the current and initial concentrations of each antinutrient was approximately a linear function of the germination time (t_G). Thus, the degradation kinetics of both antinutrients was retained of the first order with respect to C_i (Eq. 2), its kinetic rate constant (k_i) being equal to 0.0058±0.0005 or 0.035±0.004 h^{-1} for raffinose or phytic acid, respectively, as determined via the least squares method.
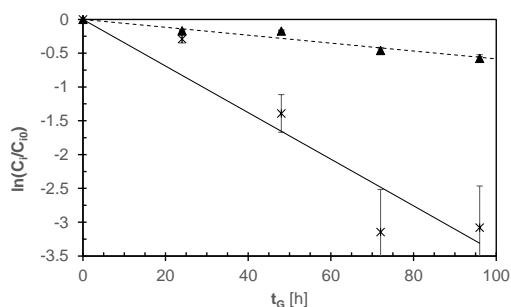

Figure 3: Effect of the germination time (t_G) on the natural logarithm of the instantaneous-to-initial concentration ratio for the i-th antinutrient (∗, raffinose; ▲, phytic acid) during lentil germination at 25 °C. The continuous and broken lines were plotted using the first-order kinetic model and the kinetic constant rates reported in the text.

3.5. Lentil malt flour production

Germinated lentils were dried to obtain a yellow-lentil malt with a moisture content of 5-8% (w/w). The split malted seeds, as characterized by the CIELab color coordinates shown in Table 1, exhibited a positive value of b*, which indicates a yellow hue. The lentil malt color was a light one quite near to the gold metallic color (//convertingcolors.com). The latter was also near to the color of split lentils as such, even if their color was darker for the smaller L* value (Table 1). The cyclonic removal of cuticle fragments from the input lentil malt gave rise to a cotyledon-rich fraction (~0.85 g/g), which was ground for two or three cycles, thus yielding a de-hulled lentil malt flour (~0.965 g/g).

3.6. Potential new use of decorticated yellow-lentil malt flour in egg pasta production

Two fresh egg pastas containing 37% whole egg and 63% wheat flour type 00 or dehulled yellow-lentil malt flour were prepared, their proximate composition being shown in Table 2.

Table 2: Proximate composition of egg pasta obtained by mixing whole egg with common bread wheat flour type 00 or de-hulled yellow-lentil malt flour in a ratio of 37/63 g/g, and of a commercial lentil pasta.

Component (g/100 g db)	Egg Pasta	Lentil Malt Egg Pasta	Commercial Lentil Pasta
Raw protein	16.6±1.5 [b]	28.4±1.5 [a]	26±1.0 [a]
Total Starch (TS)	71.4±1.5 [a]	45.3±1.9 [b]	50.6±1.0 [b]
Resistant Starch (RS)	0.54±0.04 [b]	1.45 ±0.06 [a]	nd
Phytic Acid (PA)	0.03±0.01 [c]	0.46±0.03 [b]	0.90±0.07 [a]
Raffinose (R)	nd	0 [b]	0.61±0.30 [a]

In each row, values with the same letter have no significant differences at $p < 0.05$; nd – not determined.

The conventional fresh egg pasta was richer in total starch, but poorer in crude protein, resistant starch and phytic acid. Both fresh egg pastas were devoid of α-galactosides, but the commercial lentil spaghetti, acquired in a local supermarket and examined here, had a crude protein content not statistically different at 95% confidence level, but quite a high level of flatulence-inducing sugars and a phytate content about the double of that detected in the fresh egg pasta enriched with yellow-lentil malt (Table 2).

To describe the simulated starch digestion kinetics of these fresh pasta samples, the average concentration of glucose freed by the enzymatic treatments (C_G) was plotted against the incubation time (t), as shown in Figure 4.
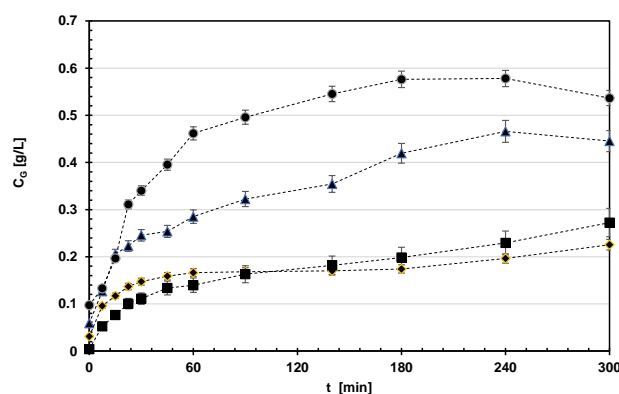

Figure 4: Time course of simulated in vitro starch digestion using white bread (●), cooked fresh egg pasta samples containing 63% (w/w) common bread wheat flour (▲) or lentil malt flour (◆), or dry lentil pasta (■).

The cooked fresh egg pasta enriched with yellow-lentil malt flour appeared to be digested as the commercial lentil pasta, but in a significantly slower way than conventional fresh egg pasta and white bread. The area under each digestogram up to an overall incubation time of 180 min (AUC) amounted to about 81.2, 54.6, 28.4 or 26.8 g min/L in the case of white bread, conventional fresh egg pasta, lentil malt flour-enriched fresh egg pasta and dry lentil pasta, respectively. According to the classification system recently reviewed by Atkinson et al. (2021), the conventional fresh egg pasta exhibited a medium in vitro GI value (66.2%), while that enriched with yellow-lentil malt flour a low one (38.3%), not statistically different from that of dry lentil pasta (36.6%). Therefore, the consumption of such a novel fresh egg pasta would just slightly increase the post-prandial level of glucose in the blood, thus lessening the long-term risk of type 2 diabetes mellitus and preventing obesity and metabolic risk factors, such as coronary heart disease (Brand-Miller et al., 2003).

4. Conclusions

The key outcomes of this research work demonstrated the applicability of the lentil seeds' malting process to reduce the antinutrient content of the resulting malted products, which might be used to balance better the diet of the final consumer. More specifically, all the steps of the production process of a decorticated yellow-lentil malt flour were defined. A two-h steeping process at 25 °C was sufficient to activate the metabolic processes of lentil germination, which after 72 h yielded 94% or 63% reduction in the flatulence-inducing raffinose or phytic acid, respectively. The subsequent kilning at 50 °C for 48 h and at 75 °C for 3 h gave rise to a gold metallic-type yellow-lentil malt. The decorticated yellow-lentil malt flour, as resulting from the grinding of the yellow cotyledons recovered via cyclonic separation, was used to prepare a fresh egg pasta, devoid of flatulence inducing oligosaccharides and with a crude protein content of 28.4 g/100 g dm, and low phytate content (0.46 g/100 dm)

and *in vitro* glycemic index (38%). Further studies are needed to confirm the acceptability of the novel yellow-lentil malt egg pasta by instrumental and sensory analyses.

Acknowledgments

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