applied sciences

MDPI

Article

Brewing with 10% and 20% Malted Lentils—Trials on Laboratory and Pilot Scales

Jonas Trummer [1,*], Hellen Watson [2], Jessika De Clippeleer [2,3] and Aleksander Poreda [1]

[1] Department of Fermentation Technology and Microbiology, University of Agriculture in Krakow, Balicka 122, 30-149 Krakow, Poland; a.poreda@urk.edu.pl
[2] Innovation Centre for Brewing & Fermentation (IBF), Faculty of Bioscience Engineering, Department of Biotechnology, Ghent University, Valentin Vaerwyckweg 1, B-9000 Ghent, Belgium; hellen.watson@ugent.be (H.W.); jessika.declippeleer@ugent.be (J.D.C.)
[3] AgroFoodNature, Innovation Centre for Brewing & Fermentation (IBF), HOGENT University of Applied Sciences and Arts, Valentin Vaerwyckweg 1, B-9000 Ghent, Belgium
* Correspondence: j.trummer@urk.edu.pl

Abstract: Lentils, a popular foodstuff worldwide, are gaining more interest for their use in alternative diets. In addition, we are observing an ever-growing demand for new raw materials in the malting and brewing industry and an overall rising interest in a low-gluten lifestyle. Therefore, in this study, malt was produced from green lentils and used in both laboratory- and pilot-scale brewing trials. Malted lentils were used as 10% and 20% adjuncts at the laboratory scale, following the Congress mash procedure, and the most important parameters (e.g., filtration time, pH, color, extract, fermentability) of the wort and beer samples were analyzed with a special focus on the concentrations of metal ions (Mg^{2+}, Ca^{2+}, Zn^{2+}, Fe) in wort. The production of beer with lentil malt as an adjunct was then scaled up to 1 hl, and several beer parameters were analyzed, including the gluten content and foam stability. The results showed that the gluten content was decreased by circa 35% and foam stability was enhanced by approximately 6% when adding 20% lentil malt. Furthermore, the use of lentil malt reduced the filtration time by up to 17%. A trained panel evaluated the sensorial qualities of the produced beers. Overall, the use of green lentil malt shows promising results for its potential use in brewing.

Keywords: lentil malt; beer; adjunct; novel raw material

Citation: Trummer, J.; Watson, H.; De Clippeleer, J.; Poreda, A. Brewing with 10% and 20% Malted Lentils—Trials on Laboratory and Pilot Scales. *Appl. Sci.* **2021**, *11*, 9817. https://doi.org/10.3390/app11219817

Academic Editor: Maria Kanellaki

Received: 31 August 2021
Accepted: 19 October 2021
Published: 20 October 2021

1. Introduction

In a steadily growing market of craft breweries and specialty beers, new raw materials for brewing are in demand by maltsters and brewers [1–5]. These new materials can be used to create novel beers with distinctive flavors, for technological purposes such as a processing aids or as marketing tactics. The most common starchy raw materials used in beer production are barley, wheat, rice and corn in both malted and unmalted forms. However, alternative cereals (e.g., oat and spelt) and pseudocereals (e.g., buckwheat and quinoa) can also be used [3,6–13]. Each raw material is unique with a distinctive composition that can have both positive and negative effects on beer production (e.g., the high viscosity of oat malt wort). Usually, the cereals and pseudocereals used in brewing can be malted. However, in current beer production, only barley and wheat are malted, with the others typically used in their raw forms as so-called adjuncts, especially rice and corn. At present, it is estimated that around 85–90% of the global beer production uses adjuncts [14]. The use of other seeds, such as legumes, is not common in brewing. However, brewing with beans or peas as adjuncts was not unusual in medieval times [15]. In this research, we investigated the use of lentil malt in brewing. Lentils consist of 43.4–74.9% carbohydrates, which qualifies them as a carbohydrate-rich food [16–21]. This carbohydrate fraction is mostly composed of starch (up to 63%), but it also includes soluble sugars (1–2.5%), galactooligosaccharides (2–8%), and non-starch polysaccharides (around 20%) [19,22–25].

Compared to other plant kernels, such as cereal grains, legume seeds are usually higher in protein content. The reported protein values for lentils range from 15.9 to 31.4 g/100 g d.m., depending on the variety [16]. Most of the seed protein is stored in the cotyledons, with the majority of the protein content consisting of 70% salt-soluble globulins (storage proteins) that are stored in protein bodies, as well as 16% albumins, 11% glutelins and 3% prolamins [26,27]. Lentils lack a gluten protein fraction, which makes them an attractive food for alternative diets. Currently, there is a rising interest in gluten-free beverages, which contain no more than 20 mg/L gluten according to the World Health Organization [28]. Traditionally, beers are not often gluten-free, as they are made from gluten-containing barley malt [29]. Legumes such as lentils are naturally gluten-free. Therefore, the use of lentil malt as a brewing adjunct to barley malt in the mash bill could be an interesting approach to lower the overall gluten content and produce low-gluten beer.

Another focus of this research was on the metal ion content in wort. Metal ions play important roles in the brewing process and can either support yeast metabolism (Mg^{2+}, Ca^{2+}, Zn^{2+}) or negatively affect the overall quality (Fe) of the beer [30–33].

In this research manuscript, we propose malted lentils as a new raw material and adjunct in beer production. Our research focused on the production of wort and beer with 10% and 20% addition of malted lentils in both laboratory- and pilot-scale brewing trials. Important standard parameters, as well as the concentrations of Mg^{2+}, Ca^{2+}, Zn^{2+} and Fe in the resultant wort samples, were analyzed. Finally, a trained panel evaluated the sensorial qualities of the produced beers. Our study is one of the first to evaluate lentil malt as a suitable brewing adjunct and the first to consider lentil malt in the production of gluten-reduced beer.

2. Materials and Methods

Raw materials: Green lentils (*Lens culinaris*), which were cropped and harvested in Canada and obtained from a Polish supplier (Tesco Polska Sp. z o.o., Krakow, Poland), were used for malting. Barley malt used for brewing was obtained from IREKS GmBH (Kulmbach, Germany) and the hops used were obtained from PolishHops Sp. z o.o. (Karczmiska, Poland).

Malting: Lentils were steeped and germinated in an aerated cabinet (Q-Cell 240, Pol-Lab sp. K., Wilkowice, Poland). A Memmert SLL 400 drying oven (Memmert GmbH & Co. KG, Schwabach, Germany) was used for drying and kilning. The steeping process was performed at 20 °C for 16 h, and germination was performed at the same temperature for 48 h, with subsequent drying and kilning at a temperature of 50 °C for 5 h and 120 °C for 3 h. The moisture content of the lentil malt was 4.62% *w*/*w* d.m. The lentils were frequently turned during germination and kilning. The moisture content after kilning was measured with a Radwag MAC 50 moisture analyzer (Radwag, Radom, Poland). Steeping, germination and kilning regimes were chosen after several pre-trials in order to achieve maximum lentil malt extract content and satisfactory aroma attributes (see Trummer et al. [34])

Brewing at the laboratory scale: Fine milling of both barley and lentil malt was performed with a WZ 1 knife mill (Zaklad Badawczy, Bydgoszcz, Poland) Wort and beer production with lentil malt adjuncts at the laboratory scale was carried out according to the Congress mash procedure (EBC 4.5.1).

Brewing at the pilot scale: Both barley and lentil malt were milled with a two-roll malt mill (Maltmill®, Jack Schmidling Productions Inc., Marengo IL, USA). Wort and beer with lentil malt adjuncts were produced in a 100 L experimental brewhouse (Oxeria, Sp. z o.o., Krakow, Poland) of the University of Agriculture in Krakow. The equipment consists of a mash tun, lauter tun, wort kettle, whirlpool, plate heat exchanger and three 45 L cylindro-conical unitanks (SS Brewtech, Wildomar CA, USA). Three different brews in the style of a light dark beer [35], were produced in triplicate. The aim was to produce a dark-colored beer with an extract around 10 °P. The intention was to have lower gluten content without diluting the aroma. The reference brew was produced with 96.2% Pilsner

malt and 3.8% Chocolate malt. With the addition of 10% or 20% lentil malt, the amount of Pilsner malt was decreased accordingly. The brews were calculated to have a pre-boil volume of 80 L. The mashing regime was: 50 °C/10 min rest, heating to 62 °C/20 min rest, heating to 72 °C/30 min rest and heating to 78 °C/mashing off. The heating rate of the brewhouse, as set by the manufacturer, was 0.75 °C/min. Hops were added during boiling at 60, 30 and 5 min before the end, as well as at the end of boiling. The hop varieties used were Polish Marynka at the beginning and Polish Lubelski for the other additions in order to reach an overall bitterness of 20 international bitter units (IBU). The wort was cooled to 20 °C and pitched with 1.5 g/L of W34/70 dry yeast (Lesaffre, Marcq-en-Baroeul, France). The young beer was cooled to 14 °C in the first 24 h and fermented for 9 days, after which it was transferred to kegs for maturation. The pitching rate and fermentation temperatures were chosen according to standard procedures of industrial breweries. Maturation was performed for 6 weeks at 8 °C cellar temperature.

Mash, wort and beer analysis: The brewing analyses were performed according to the standard procedures in the latest versions of the European Brewery Convention (EBC) [36] or the Mitteleuropäische Brautechnische Analysekommision (MEBAK) [37]. The specific procedures are reported in Table 1.

Table 1. Overview of mash, wort and beer analyses and method specifications.

Type of Analysis	Analysis	Method
Mash analyses	Congress mash	EBC 4.5.1
	Odor	EBC 4.5.1
	Saccharification rate	EBC 4.5.1
	Filtration	EBC 4.5.1
Wort analyses	Free amino nitrogen	EBC 8.10
	pH	EBC 8.17
	Color	EBC 8.5
	Extract	EBC 8.3
Beer analyses	Color	EBC 9.6
	Bitterness	EBC 9.8
	Free Amino Nitrogen	EBC 9.10
	Total Polyphenols	EBC 9.11
	Total Carbohydrates	EBC 9.26
	pH	EBC 9.35
	Foam Stability	EBC 9.42
	Extract	EBC 9.43.2
	Apparent degree of fermentation	MEBAK 2.9

Metal ion concentrations of wort were determined using atomic absorption spectrometry with flame atomization using a Varian AA240FS spectrophotometer (VARIAN Inc., Palo Alto CA, USA) with an automatic sample-dispensing system (SIPS-20, Varian Inc., Palo Alto CA, USA). The wort samples were mineralized in a Mars Express microwave oven (CEM Corp., Matthews NC, USA) with the addition of 3 mL of concentrated HNO_3 using sealed pressure vessels (170 °C, 15 min, 1200 W). The gas flow rate was 3.5 L/min for acetylene and 14 L/min for air. To determine the metal ions present, the rapid sequence mode (Fast Sequential mode) was used. A standard solution was prepared from 1000 mg/L Sigma-Aldrich standards (Sigma-Aldrich, St. Louis, MO, USA) containing the metal ions Mg^{2+}, Ca^{2+}, Zn^{2+} and Fe at 100, 40 and 10 mg/L, respectively. The absorbance of the samples was determined at the following wavelengths (in nm): 202.6 (Mg^{2+}), 213.9 (Zn^{2+}), 248.4 (Fe) and 422.7 (Ca^{2+}). Calibration algorithm to obtain calibration curves was the parabolic rational function provided by the manufacturer of the spectrophotometer.

Beer gluten contents were determined by competitive R5-ELISA and measured by the Ridascreen Gliadin competitive R7021 Enzyme-Linked Immunosorbent Assay (ELISA) kit (R-Biopharm, Darmstadt, Germany). The extraction of gluten from the beer samples, the

ELISA and data analysis by RIDA® SOFT Win software Z999 were carried out according to the manufacturer's instructions (R-Biopharm, Darmstadt, Germany).

The turbidity of wort was analyzed with a turbidity meter (Eutech TN 100, Fisher Scientific GmbH, Heiligen, Germany). The displayed NTU units were converted to EBC units (4 NTU = 1 EBC).

Sensory analyses were performed by a sensory panel consisting of eight people, all of whom were experienced assessors trained with the use of FlavorActiV standards (FlavorActiV Limited, Aston Rowant, United Kingdom). The eight panellists were five males and three females. All panelists were instructed in the methods and evaluation sheets used. For sample preparation, 150 mL of the sample was measured in a plastic cup with a maximum volume of 420 mL. Samples were prepared no more than 15 min before degustation and sensorial evaluation. The plastic cups were labelled with random three-digit codes throughout the degustation sessions to minimize handling and expectation errors. All samples and reference solutions were prepared in a food-safe environment. Sensory evaluation was conducted with descriptive analysis according to the flavor/aroma wheel for beer which was invented in order to simplify and standardize the description of beers [36,38]. More than 100 different attributes are normally represented on the flavor/aroma wheel for beer. Therefore, only the most important attributes, plus a range of additional characteristics (e.g., peaish), were chosen for the evaluation of the beers in this research. These attributes were evaluated with a score from zero to five (with five being the most intense) and expressed in a radar chart.

Statistical Analysis: The results of all analyses performed in this work are reported as the average of three independent experiments. The data were analyzed using one-way analysis of variance (ANOVA). Values given in tables are the means of three determinations ± standard deviation. Significant differences between means were verified by the Duncan test, determined at a 5% significance level, and are labelled with different letters. Gluten concentrations are expressed as means (biological replicates, n = 6) ± standard deviation (SD). One-way analysis of variance (ANOVA) (IBM SPSS Statistics® version 24.0 software) was carried out to determine the hypothesized gluten-lowering effect of lentil malt as a brewing adjunct to barley malt in the mash bill. If significant differences were found, a post-hoc Tukey test was performed. The significance threshold was set at $\alpha = 0.05$. Scores of sensory analyses were processed, and the calculated average was taken as the result.

3. Results and Discussion

3.1. Laboratory-Scale Brewing Trial

Results of the analyses performed are shown in Table 2.

Table 2. Results of analyses during and after the brewing process—Brewing with 10% and 20% malted lentils at the laboratory scale.

	BM	BM + 10% LM	BM + 20% LM
Mash analyses			
Odor	Normal	Normal	Normal
Saccharification time (min)	10–15	20–25	25–30
Filtration time (min)	$25^{b} \pm 1.5$	$22^{a} \pm 1.5$	$21^{a} \pm 0.6$
Wort analyses			
pH	$5.58^{a} \pm 0.01$	$5.82^{b} \pm 0.01$	$5.98^{c} \pm 0.01$
Color (EBC unit)	$4.66^{a} \pm 0.04$	$5.70^{b} \pm 0.10$	$7.55^{c} \pm 0.09$
Extract (°Plato)	$8.87^{c} \pm 0.06$	$8.60^{b} \pm 0.02$	$8.30^{a} \pm 0.04$
Turbidity (EBC unit)	$4.0^{c} \pm 0.3$	$3.1^{b} \pm 0.1$	$1.5^{a} \pm 0.4$
Beer analyses			
pH	$4.74^{a} \pm 0.03$	$4.83^{b} \pm 0.01$	$4.90^{c} \pm 0.01$
Color (EBC unit)	$4.56^{a} \pm 0.05$	$5.40^{b} \pm 0.08$	$7.11^{c} \pm 0.14$
Apparent extract (°Plato)	$1.90^{a} \pm 0.04$	$1.89^{a} \pm 0.07$	$1.98^{b} \pm 0.04$
Apparent degree of fermentation (%)	$78.57^{b} \pm 0.28$	$78.03^{b} \pm 0.72$	$76.11^{a} \pm 0.51$

Values are means ± standard deviation (n = 3). Superscript letters indicate significantly different groups ($p \leq 0.05$), starting from [a] for lower values (BM = Barley Malt; LM = Lentil Malt).

3.1.1. Odor

Odor was considered normal for all mashes and no unwanted odors were detectable. Mashes with lentil malt adjuncts had the same characteristic wort odor as all-barley malt mashes.

3.1.2. Saccharification Time

Saccharification occurred within the expected time range of 10–15 min for pale barley malt [39]. With the addition of 10% and 20% lentil malt, the saccharification time increased to 20–25 and 25–30 min, respectively. As the lentil malt was kilned at a higher temperature (120 °C) compared to Pilsner-type barley malt (80 °C), increased saccharification time was expected, as higher temperatures decrease the overall enzymatic activity, including that of starch-degrading enzymes [40]. Moreover, enzymatic activity may decrease with the addition of lentil malt due to the low enzyme activity in lentils and the presence of amylase inhibitors [16,41,42]. As shown in previous research, wort production with 100% lentil malt is only possible with exogenous enzymes when a product containing α-amylase, β-glucanases and endopeptidase is used [34]. However, when using 10% and 20% lentil malt as an adjunct, the barley malt enzymes are sufficient to saccharify the mash in under 30 min. Gasiński et al. [43] reported a saccharification time of 10 min with the addition of 30% lentil malt, but they included a pre-gelatinization step. After gelatinization, starch is already accessible for enzymatic breakdown and saccharification occurs faster.

3.1.3. Filtration Time

At less than 30 min, the filtration time was considered to be within the normal range (<1 h) for all of the mashes [39], but the filtrations of the adjunct mashes were statistically significantly shorter than those for the 100% barley malt mash. As lentils typically contain less glucan than barley (0.4–1.1% d.m. [44] for lentils and 3.6–9.0% d.m. for barley [45]), the use of lentil malt as an adjunct reduces the overall amount of glucans in the mash and can therefore reduce filtration time.

3.1.4. pH

The pH values for all of the Congress wort samples were below 6, which is considered good according to Pfenninger [39]. Wort with a pH value that is too high can have a negative impact on fermentation performance [46]. The lowest pH value of 5.58 ± 0.01 was measured for all-barley malt (BM) Congress wort. An increase in pH was observed when lentil malt was added, with pH values of 5.82 ± 0.01 with the addition of 10% malted lentils (BM + 10% LM) and 5.98 ± 0.01 with 20% malted lentils (BM + 20% LM). This was predicted, as the pH of Congress wort made from 100% lentil malt showed a pH close to 6 in previous research [34]. When adding 30% pre-gelatinized lentil malt, Gasinski et al. [43] reported a pH of 5.34. This lower pH can be explained by the lower pH of the barley malt used as well as a pH drop due to the gelatinization step applied to the lentil malt. A similar pH drop (between 0.84 and 1.08) was measured in our fermented beers made from the Congress wort samples. These decreases in pH are normal and indicate healthy fermentation [38]. Stable and volatile organic acids are formed during fermentation, and the pH value shifts towards more acidity [47].

3.1.5. Color

The color of Congress wort produced with 100% barley malt was 4.66 ± 0.04 EBC and therefore in the expected range of around 4 EBC units [37]. With the addition of lentil malt, the Congress wort color increased to 5.70 ± 0.10 (BM + 10% LM) and 7.55 ± 0.09 EBC (BM + 20% LM). In previous research, a wort color of approximately 25 EBC units was obtained when brewed with 100% lentil malt [34]. Therefore, an increase in the color was expected. The measured color values were still in the range of pale wort (<10 EBC for Pilsner beer style); hence, the production of pale beer styles with the addition of 10% or

20% lentil malt is possible [35]. As expected, the color of the beers was slightly lighter after fermentation.

3.1.6. Turbidity

The turbidity of Congress wort is an atypical measurement. Other factors, such as hop compounds, trub formation during and after wort boiling and the subsequent fermentation, will have a profound impact on the colloidal stability of beer and therefore the turbidity of the final product [48]. Nevertheless, measuring the wort turbidity can provide insight into the quality of the filtration and the impact of the lentil malt as an adjunct. The turbidity of Congress wort made from all-barley malt was 4.0 ± 0.3 EBC. As the amount of lentil malt adjunct increased, the turbidity of the Congress wort decreased significantly, with values of 3.1 ± 0.1 (BM + 10% LM) and 1.5 ± 0.4 EBC (BM + 20% LM). This could be due to the generation of fewer small particles when milling lentil malt. The milling of barley malt always results in significant amounts of flour (15–20%), whereas this was not observed when milling lentil malt. This may be due to a smaller overall endosperm volume and therefore less extensive modification of the lentil malt seed.

3.1.7. Extract and Apparent Extract

The Congress wort produced from all-barley malt had the highest extract content with 8.87 ± 0.06 °Plato (°P). This value was expected, as the malt extract in %, as given by the supplier, is 81% or more. As the amount of the lentil malt adjunct increased, the extract of the Congress wort samples decreased to 8.60 ± 0.02 (BM + 10% LM) and 8.30 ± 0.04 °P (BM + 20% LM). As the lentil malt used showed an extract content of 59.9% in previous research, this drop in extract content was expected [34]. The measured extract contents are still in the range of all-barley malt Congress wort samples and even higher than Congress wort samples produced with some other raw materials, such as spelt or oats [7,49]. When applying 30% lentil malt that had been germinated for 6 days, Gasinski et al. [43] reported an extract content of 6.69 °P. Such low values can be due to too long of a germination period, as this causes the seed to consume large amounts of starch compounds required for rootlet growth. Additionally, the lower extract reported by Gasinski et al. [43] can be caused by the use of barley malt with low extract content.

The apparent extract, which is the extract measured after fermentation, was similar between all-barley malt beer (1.90 ± 0.04 °P) and beer with the addition of 10% lentil malt (1.89 ± 0.07 °P). With the addition of 20% lentil malt, a slightly higher apparent extract of 1.98 ± 0.04 °P was observed. As the initial extracts of the Congress wort samples with lentil malt adjuncts were lower than those with all-barley malt, the apparent extract was expected to be lower. The higher apparent extract left in the beer with 20% lentil malt adjunct is presumably due to different protein fractions transferred to the wort sample. These are also detected by the density meter and can therefore slightly change the extract value.

3.1.8. Apparent Degree of Fermentation

This value is calculated from the initial extract and the apparent extract and shows the limit of fermentation when the process occurs at industrial standards. The calculated values were 78.57 ± 0.28 (BM) and 78.03 ± 0.72% (BM + 10% LM). With the addition of 20% lentil malt, a small but statistically significant difference was observed, with an apparent degree of fermentation of 76.11 ± 0.51%. This lower degree of fermentation is due to the slightly higher apparent extract left in the beer brewed with 20% lentil malt. Overall, degrees of fermentation close to 80% are considered acceptable values that imply a good wort composition for the yeast [47].

3.1.9. Metal Ion Analyses

The concentrations of the measured metal ions (Mg^{2+}, Ca^{2+}, Zn^{2+}, Fe) were similar in all wort samples (Table 3). The only exception was a slightly higher zinc content of 0.56 ± 0.05 mg/L for Congress wort produced with 20% lentil malt.

Table 3. Metal ion concentrations of the different Congress wort samples produced from 100% barley malt (BM) with a 10% or 20% lentil malt (LM) addition.

	BM	BM + 10% LM	BM + 20% LM
Mg^{2+} (mg/L)	77.9 ^{a} ± 4.1	71.9 ^{a} ± 8.2	78.1 ^{a} ± 5.5
Ca^{2+} (mg/L)	21.3 ^{a} ± 4.1	21.7 ^{a} ± 1.6	23.2 ^{a} ± 5.5
Zn^{2+} (mg/L)	0.43 ^{a} ± 0.07	0.48 ^{a} ± 0.1	0.56 ^{b} ± 0.05
Fe (mg/L)	0.11 ^{a} ± 0.05	0.13 ^{a} ± 0.06	0.16 ^{a} ± 0.07

Values are means ± standard deviation (n = 3). Superscript letters indicate significantly different groups ($p \leq 0.05$), starting from ^{a} for lower values (BM = Barley Malt; LM = Lentil Malt).

Most of the metal ions that occur naturally in seeds are removed by the malting process and then further depleted during the different steps of the brewing process, especially with the spent grains after lautering [50,51]. The variety of the cereal or legume also has an impact on metal content. Wang and Daun [52] showed that different varieties of lentils can differ in their metal ion content. The measured metal ion contents were all attributable to the malts used, as distilled water was used in accordance with the Congress mash procedure. This is also closer to industrial standards, where treated soft water with low mineral content is typical.

In the literature, Mg^{2+} levels of 40–100 mg/L have been reported in all-barley malt wort [46]. The Mg^{2+} levels measured in Congress wort samples were in this range, with values of 77.9 ± 4.1 (BM), 71.9 ± 8.2 (BM + 10% LM) and 78.1 ± 5.5 mg/L (BM + 20% LM). Similar values were reported by Poreda et al. [32]. The Mg^{2+} concentration influences phosphorylation and cannot be exchanged with another mineral. Additionally, it works as a coenzyme for carboxy- and decarboxylases [53].

The Ca^{2+} ion concentrations in the Congress wort samples were 21.3 ± 4.1 (BM), 21.7 ± 1.6 (BM + 10% LM) and 23.2 ± 5.5 mg/L (BM + 20% LM), which are within the desired range of 10–60 mg/L [46]. Calcium ions stimulate yeast multiplication and affect enzymatic activities and yeast flocculation [54]. On the other hand, excessive levels of Ca^{2+} are undesirable due to their potential reaction with oxalic acid. This reaction forms calcium oxalate which is known to promote gushing [47].

The Zn^{2+} concentration in the Congress wort samples were 0.43 ± 0.07 (BM), 0.48 ± 0.1 (BM + 10% LM) and 0.56 ± 0.05 mg/L (BM + 20% LM). The literature reports several suitable zinc levels ranging from 0.05 to 0.25 mg/L [46,47,55,56]. However, higher concentrations (up to 0.9 mg/L) have been reported [30]. Zinc levels have important physiological effects, as Zn^{2+} is involved in protein synthesis, the multiplication of yeast cells and fermentation. Zn^{2+} ions are actively translocated into the yeast cell to carry out important physiological roles and are mostly retained in the cell [57]. A lack of zinc (less than 0.1 mg/L), can lead to poor multiplication of yeast cells, slow fermentation and the partial reduction of diacetyl [47]. The Zn^{2+} contents of the Congress wort samples were measured before boiling and without the addition of hops. Therefore, hot trub was not formed and separated from the wort before pitching. As large amounts of Zn^{2+} ions are removed with the hot trub, the slightly higher measured zinc contents by the omission of this step [50,58]. Pitching wort zinc concentrations that are higher than 0.6 mg/L can lead to yeast autolysis and a sulphury off-flavor [47]. However, the need for zinc depends on the yeast strain used [53]. Wort with the addition of 20% lentil malt showed significantly higher zinc content compared to all-barley malt Congress wort and the wort with 10% malted lentils. Therefore, lentil malt could be used as a supplementary zinc source when brewing with raw materials that have low zinc content.

The iron ion contents of the Congress wort samples were 0.11 ± 0.05 (BM), 0.13 ± 0.06 (BM + 10% LM) and 0.16 ± 0.07 mg/L (BM + 20% LM), which all fall within the required range of 0.10–0.27 mg/L [47,55,59]. In the brewing process, most iron is removed with the spent grains after filtration [30,60]. Iron ions are important for enzymes involved in respiratory metabolism, as well as for cellular multiplication, and iron ion concentrations less than 0.1 mg/L can limit the synthesis of these enzymes. However, more than 1 mg/L can cause the degeneration of yeast. Additionally, if the iron content is higher than 1 mg/L,

oxidation processes and haziness are enhanced [47]. Iron is one of the so-called transition metals with pro-oxidative effects in beer and plays a key role in radical reactions, e.g., the Fenton and Haber–Weiss reaction [61]. Therefore, the iron content in the finished product is ideally as low as possible. The negative influence of metals, such as iron, was also reported by Zufall and Tyrell [62].

3.2. Pilot-Scale Brewing Trial

Results of the analyses performed are shown in Table 4.

Table 4. Results of analyses during and after the brewing process—Brewing with 10% and 20% malted lentils at the pilot scale.

	BM	BM + 10% LM	BM + 20% LM
Mash analysis			
Odor	Normal	Normal	Normal
Saccharification time (min)	<10	<10	<10
Lautering time (min)	93.6 ^{c} ± 1.2	81.7 ^{b} ± 1.5	77.3 ^{a} ± 2.1
Wort analysis			
Free amino nitrogen (mg/L)	390.3 ^{c} ± 15.1	311.5 ^{b} ± 13.9	280.8 ^{a} ± 14.2
pH	5.92 ^{a} ± 0.02	5.96 ^{ab} ± 0.04	6.01 ^{b} ± 0.04
Color (EBC unit)	41.3 ^{a} ± 1.1	48.9 ^{b} ± 0.8	54.1 ^{c} ± 0.5
Extract (°Plato)	10.57 ^{c} ± 0.12	10.20 ^{b} ± 0.12	9.48 ^{a} ± 0.12
Beer analysis			
Free amino nitrogen (mg/L)	191.5 ^{a} ± 12.6	202.1 ^{a} ± 14.7	200.0 ^{a} ± 5.9
pH	4.51 ^{a} ± 0.10	4.63 ^{ab} ± 0.09	4.72 ^{b} ± 0.07
Color (EBC unit)	37.7 ^{a} ± 0.6	46.4 ^{b} ± 0.9	51.5 ^{c} ± 0.5
Apparent extract (°Plato)	2.24 ^{a} ± 0.16	2.14 ^{a} ± 0.16	1.96 ^{a} ± 0.15
Apparent degree of fermentation (%)	78.75 ^{a} ± 1.39	79.05 ^{a} ± 0.72	79.10 ^{a} ± 1.66
Bitterness (IBU)	19.3 ^{a} ± 0.4	20.0 ^{a} ± 0.6	21.2 ^{b} ± 0.6
Total polyphenols (mg/L)	200.1 ^{a} ± 5.8	216.1 ^{b} ± 2.3	230.5 ^{c} ± 2.2
Total carbohydrates (g/100 mL)	2.66 ^{b} ± 0.14	2.40 ^{ab} ± 0.20	2.04 ^{a} ± 0.22
Foam stability (s)	297.9 ^{a} ± 0.5	307.0 ^{b} ± 0.3	316.3 ^{c} ± 0.3

Values are means ± standard deviation (n = 3). Superscript letters indicate significantly different groups ($p \leq 0.05$), starting from ^{a} for lower values (BM = Barley Malt; LM = Lentil Malt).

3.2.1. Odor

Odor was considered normal for all mashes and no unwanted odors were detectable. As intended, the addition of a small amount of chocolate malt produced a slightly roasted, chocolate-like odor. Mashes with lentil malt adjuncts had the same characteristic wort odor as all-barley malt mashes.

3.2.2. Saccharification Time

After reaching the 72 °C rest, iodine negativity was obtained in less than 10 min for all mashes. These results indicate the ideal breakdown of starch chains and good enzymatic activity. A longer saccharification time (up to 30 min) was observed in the laboratory-scale trial. At the pilot scale, the Congress mash procedure was adjusted to a mashing regime with several different temperature rests in order to achieve more intense breakdown of starch by amylolytic enzymes. To assure the breakdown of proteins potentially introduced to the mash by the lentil malt adjunct, mashing-in was carried out at 50 °C and the temperature was held for 10 min. At this temperature, cytolytic enzymes, such as β-glucanases, are also active. Specific mashing rests at 62, 68 and 72 °C were implemented to directly target the amylolytic enzymes. The highest enzyme activity of β-amylase is at around 62 °C, whereas that of α-amylase is at around 72 °C [40]. The mashing rest at 68 °C, where both enzymes are active, was performed for additional reassurance in order to achieve complete and rapid saccharification.

3.2.3. Lautering Time

Filtration of the produced wort samples was completed in less than 95 min for all the mashes with filtration times of 93.6 ± 1.2 (BM), 81.7 ± 1.5 (BM + 10% LM) and 77.3 ± 2.1 min (BM + 20% LM). As previously observed at the laboratory scale, filtration with the addition of lentil malt decreased the time of this crucial and normally time-consuming process step. The addition of 10% lentil malt reduced the filtration time by approximately 12%; with the addition of 20% lentil malt it was reduced by 17%. This could be explained by the higher glucan content in barley malt (see Section 3.1.3). With the decrease in the amount of glucan, the viscosity of the mash decreases and filtration is faster. Gasiński et al. [43] also showed a lower viscosity for Congress wort made with 30% malted lentils compared to all-barley malt wort. The results suggest that lautering time may be reduced by approximately 15 min when adding 20% malted lentils. The industry standard for lautering time is around 1.5 h and it is often the bottleneck in the brewhouse. Saving 15 min per brew could potentially add the option of up to two additional brews per day (assuming 10 brews/day on average).

3.2.4. Free Amino Nitrogen

The measured FAN values of wort exceeded the required range of 200–250 mg/L [38,46,63,64], especially for all-barley malt mash which had a FAN value of 390.3 ± 15.1 mg/L. FAN values of 311.5 ± 13.9 and 280.8 ± 14.2 mg/L were measured in samples with the addition of 10% and 20% malted lentils. In beer from a 12°P wort, standard FAN values between 100 and 120 mg/L are required [38]. FAN levels in the produced beers did not drop to these expected levels, and all of them showed similar values of around 200 mg/L (191.5 ± 12.6 (BM), 202.1 ± 14.7 (BM + 10% LM) and 200.0 ± 5.9 mg/L (BM + 20% LM)). As the fermentation and the degree of fermentation were very satisfactory for all beers, the higher FAN contents did not appear to have significant negative effects. The similar FAN values in the beer samples show that the yeast was taking up the FAN to a limit of around 200 mg/L. The more extract was in the wort, the more FAN was also available and demanded to a higher extent by the yeast during fermentation.

3.2.5. pH

The pH values of the wort and beer samples were a bit higher than recommended in the literature (pH 5.3–5.6 for wort and pH 4.2–4.6 for beer) [40,46]. Wort produced with 100% barley malt showed the exact value given by the supplier, i.e., 5.92 ± 0.02. Slightly higher pH values were observed for wort made with the addition of lentil malt, with pH values of 5.96 ± 0.04 (BM + 10% LM) and 6.01 ± 0.04 (BM + 20% LM). As an ideal wort pH is between 5.3 and 5.6, acidification of the mash or wort could be a solution. For example, a lower pH would enhance enzymatic activity in the mash and subsequently lead to a more pronounced bitterness and enhanced protein coagulation during boiling [46]. With fermentation, a drop in pH is expected and was also observed in this research. The observed decreases in pH were around 1.29–1.41, indicating good fermentation, and resulted in beer pH values of 4.51 ± 0.10 (BM), 4.63 ± 0.09 (BM + 10% LM) and 4.72 ± 0.07 (BM + 20% LM), which are close to the recommended value of 4.2–4.6 [40,46]. The pH was slightly higher in beer produced with 20% lentil malt, with a pH of 4.72, which could make it more vulnerable to microbial contamination. An additional pasteurization step after bottling of the beer would be recommended. Overall, the addition of lentil malt resulted in an increase in pH. This correlated with the lower extract values in these wort samples, which therefore have less sugars available for fermentation.

3.2.6. Color

With color values between 37.7 and 54.1 EBC, the produced wort and beer samples were in the color range for dark beers [35]. As expected, an increase in color was observed with an increase in the added lentil malt, as more dark malts were in the malt bill overall. A decrease in color from wort to beer samples was observed, as during fermentation, the pH

drop leads to the decolorization of certain substances. Additionally, some color compounds adsorb to the surface of the yeast and are subsequently removed with the settling yeast. The decrease in color was around the expected value of 2–3 EBC (see Table 4) [40,46].

3.2.7. Extract and Apparent Extract

The measured value of the all-barley malt wort was 10.57 ± 0.12 °P. With the increasing addition of lentil malt, the initial wort extract decreased to 10.20 ± 0.12 (BM + 10% LM) and 9.48 ± 0.12 °P (BM + 20% LM). This drop in extract was expected, as lentil malt was previously reported to have lower extract content [34]. In addition, Congress wort measured at the laboratory scale showed the same decrease in extract with the addition of lentil malt.

The apparent extract, measured after fermentation, showed similar results for all of the produced wort samples, with values of 2.24 ± 0.16 (BM), 2.14 ± 0.16 (BM + 10% LM) and 1.96 ± 0.15 °P (BM + 20% LM). Normal values for commercial beers with an initial extract of 11.5–12.5 °P should be between 2.2 and 2.5 °P [40]. With apparent extract values of around 2 °P, the fermentation of available fermentable sugars occurred at the expected intensity, as the wort extract was lower than in commercially produced beer.

3.2.8. Apparent Degree of Fermentation

The results for the apparent degree of fermentation were only slightly higher than those obtained at the laboratory scale, with values of 78.75 ± 1.39 (BM), 79.05 ± 0.72 (BM + 10% LM) and 79.10 ± 1.66% (BM + 20% LM). This increase compared to trials at the laboratory scale can be explained by the different mashing regimes. As specific mashing rests for the activity of the amylolytic enzymes were performed (62 °C: β-amylase; 68 °C: both α- and β-amylase; 72 °C: α-amylase), more fermentable sugars were produced. In the production of Congress wort, longer dextrins are left in the beer, as the mashing temperature is increased directly to 70 °C [39].

3.2.9. Bitterness

The measured international bitterness units (IBU) for the beer samples were 19.3 ± 0.4 (BM), 20.0 ± 0.6 (BM + 10% LM) and 21.2 ± 0.6 IBU (BM + 20% LM). The bitterness in beer is mainly attributable to iso-α-acids. α-Acids are extracted from hops during the wort-boiling step, and their structure re-arranges to form iso-α-acids, resulting in bitterness [65]. Measurements of bitterness levels showed similar results, with a slightly higher IBU value for the beer produced with 20% lentil malt. This is due to a better isomerization rate of α-acids in wort with a higher pH [40].

3.2.10. Total Polyphenols

Polyphenols in the final beer are important compounds that can act as antioxidants and have been linked to health aspects. Additionally, polyphenols contribute to mouthfeel and can prevent beer staling [48]. As they are derived from the raw materials, most of the polyphenols remain in the spent grains or sediment with the hot/cold trub. Measured total polyphenol contents were 200.1 ± 5.8 (BM), 216.1 ± 2.3 (BM + 10% LM) and 230.5 ± 2.2 mg/L (BM + 20% LM). Typical values for pale lagers are between 130 and 200 mg/L [40,46]. Bhatty [17] reported that the polyphenol content in raw lentils is around 0.41–0.53 *w*/*w* %, most of which are flavan-3-ols. In raw barley, 0.1–0.3 *w*/*w* % polyphenols are found, most of which are also flavan-3-ols. This can explain the higher amount of total polyphenols in the beers brewed with the addition of lentil malt. Additionally, Samaras et al. [66] reported that kilning at a higher temperature increases the polyphenol content of malt. As the lentil malt was kilned at 120 °C, higher polyphenol contents are expected. A crude extract of green lentils was produced by Amarowicz et al. [67], and its phenolic compounds were investigated. Twenty compounds were identified, among which catechin glucoside, quercetin diglycoside and a procyanidin dimer were determined to be the dominant phenolic compounds [67]. When compared to six other legumes, lentils

showed the highest polyphenol content and even higher oxygen radical scavenging potential than other fruits, potatoes and wheat [68]. That oxygen radical scavenging potential can be beneficial in beer storage and may lead to the prolonged sensory stability of beer. Additionally, phenolic compounds are known to be able to bind and/or chelate iron ions, which have pro-oxidative effects in beer [69,70].

3.2.11. Total Carbohydrates

The total carbohydrates measured in beer correlated directly with the measured apparent extract of beers. The measured values were 2.66 ± 0.14 (BM), 2.40 ± 0.20 (BM + 10% LM) and 2.04 ± 0.22 g/100 mL (BM + 20% LM) The difference between the two measurements is the measurement type (density versus spectrophotometric measurement). The measured amounts were in the expected range of carbohydrates left in a fermented beer [40,47].

3.2.12. Foam Stability

Along with the color or clarity of a beer, the foam head is on of the first qualities perceived by a consumer. Therefore, brewers strive for a stable, long-lasting foam. Values of up to 300 sec are considered good, whereas values higher than 300 sec are very good [40]. The measured foam stability values were 297.9 ± 0.5 (BM), 307.0 ± 0.3 (BM + 10% LM) and 316.3 ± 0.3 sec (BM + 20% LM). Therefore, the foam stability of all-barley malt beer is considered good. With the addition of lentil malt, the foam stability increased to very good values. The higher the amount of the lentil malt adjunct, the better the foam stability. More bitterness in a beer is associated with better foam stability, as bitter substances are very surface active [40]. This is associated with the measured IBU values of the beers (see 3.2.9). Additionally, phenolic compounds are foam-positive. Beers with lentil malt were determined to have a higher total polyphenol content and therefore had more stable foam. Another important factor affecting foam stability is lipoxygenase. This enzyme produces trihydroxy octadecenoic acid, which can diminish foam stability [71,72]. Extensive denaturation of lentil lipoxygenase by heat treatment was reported by Pathiratne et al. [73]. Kilning at 120 °C can be considered a heat treatment, and therefore, lower lipoxygenase contents can be expected in the lentil malt used in this study. The significantly higher values of foam stability when brewing with lentil malt can be useful for the brewing industry. The use of lentil malt could be used to compensate for new brewing techniques with greater shear force or the use of barley malt with lower protein content.

3.2.13. Gluten Content

Results of gluten content in the beers are shown in Figure 1.

Gluten is a prolamin protein that can be found in several cereals. People who suffer from celiac disease experience allergic reactions when consuming foods or beverages containing gluten or peptides derived from gluten [40,46,48,49]. A gluten-free beverage contains no more than 20 mg/L gluten, whereas a low-in-gluten beverage cannot contain more than 100 mg/L gluten [28]. The gluten contents of the reference all-barley malt beers and the 10% lentil malt adjunct beers were around the 'low-in-gluten' threshold (≤100 mg/L), with similar gluten concentrations of 97 ± 3 mg/L and 95 ± 5 mg/L, respectively. Thus, substituting 10% barley malt with lentil malt in the mash bill did not produce an evident gluten-lowering effect. However, following the substitution of 20% barley malt with lentil malt in the mash bill, a significant decrease in the gluten content was observed. The 20% lentil malt adjunct beer contained 63 ± 3 mg/L gluten; therefore, the beer was 'low in gluten' but still far from the gluten-free threshold (≤20 mg/L). The use of lentil malt adjuncts combined with specific gluten-minimizing treatments (e.g., prolyl-endopeptidase from *Aspergillus niger* and silica gel [74]) could possibly result in gluten-free lentil malt adjunct beers.

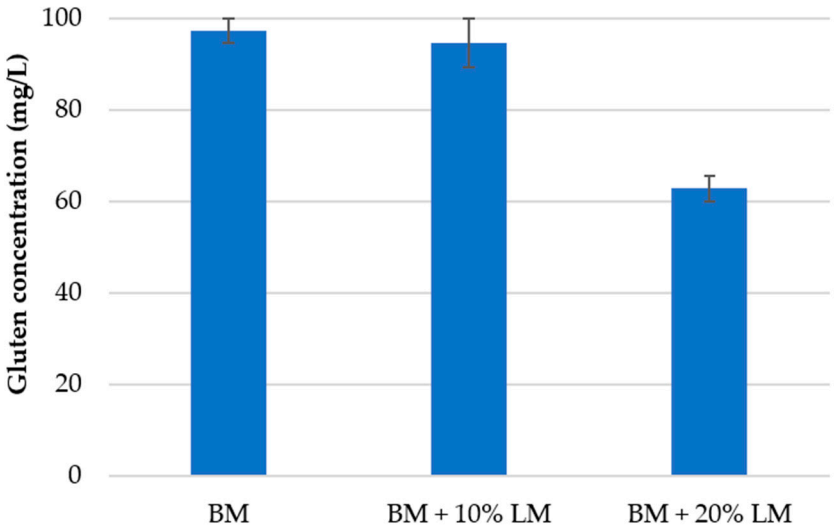

Figure 1. Gluten concentration of the pilot-scale beers (mg/L). Mean values labelled with the same letter are not significantly different ($p < 0.05$). Error bars represent standard deviation of mean (n = 6).

3.2.14. Sensory Analyses

The descriptive analysis targeted a series of possible sensory attributes. This evaluation focused entirely on the aroma, and the results are visualized in a radar chart (Figure 2). Similar results were obtained for the beers, with the main difference being a fruitier aroma in the reference. Additionally, a peaish aroma was detected for the beers made with lentil malt.

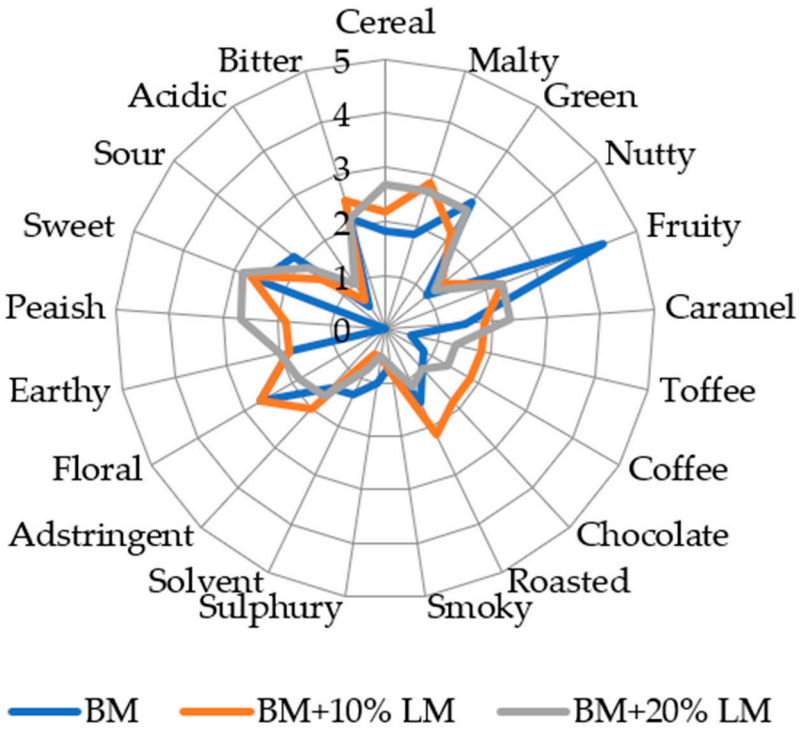

Figure 2. Radar chart of chosen quality attributes organoleptically assessed by a trained panel of eight evaluators. Each beer was assessed separately, and the final marks were calculated as an average value (n = 8).

Overall, the results of the radar chart show only minor differences in the various sensorial attributes. The basic tastes—sweet, sour, bitter and acidic—were similar for all beers. A slight sweetness was noted for all beers, which could have been interpreted from

fruity and malty aromas detected by retro-nasal olfaction. Typical malt aromas, such as cereal and malty, where expected, but caramel, coffee, toffee and roasted aromas were also introduced by the dark malts used. The mentioned coffee, chocolate and roasted aromas were more noticeable in beer with 10% lentil malt. A possible explanation is that the intensity of the 10% lentil malt is less than that of the 20% adjunct but greater than that of the barley malt. As shown in the radar chart, the strongest peaish aroma was detected for beer with 20% LM adjunct, followed by 10% LM adjunct. No such aroma was detected in all-barley malt beer. A peaish aroma is common in legumes and can be regarded as an off-flavor, alongside beany, green and hay-like aromas in legumes [75]. Alcohols, aldehydes and ketones have been reported as volatile compounds in lentils, but to date, there is no literature on other particular volatile compounds that can generate off-flavors in lentils [76]. On the one hand, lentils have lipoxygenase (LOX) activity, and therefore, off-flavors can develop from lipid oxidation, as previously reported [17]. On the other hand, most of the LOX enzymes are denatured during the kilning process [40,64,73,77]. A beany flavor was reported in lentils at the beginning of germination, which changed to a peaish off-flavor after 7 days of germination. Additionally, an unpleasant astringency, which could be connected to the glycosides present, vanished after germination [78]. Nevertheless, soaking (as in the steeping step) and heat treatment (as in kilning and subsequent wort boiling) diminish these off-flavors [75]. Therefore, the sensory panel only detected the peaish-like aroma to a minimal degree. As this aroma was not detected at all in the all-barley malt beer, the panelists were more focused on its fruity aroma, which was scored higher for this beer type. In conclusion, the use of lentil malt can definitely be detected sensorially as a peaish aroma. Possible changes in the kilning temperature of the lentils could further improve the aroma in order to produce a more acceptable beer.

4. Conclusions

As evidenced by our results in both laboratory- and pilot-scale brewing, this research indicates that it is possible to use lentil malt as a 10% or 20% adjunct. No change in metal ion content (Mg^{2+}, Ca^{2+}, Zn^{2+}, Fe) compared to 100% barley malt wort was found. Gluten content decreased with the increasing addition of malted lentils with a visible trend; thus, with the addition of even higher lentil malt content, a gluten-free beer could be produced. Furthermore, the addition of lentil malt resulted in significantly faster filtration, reducing the time required for this process step by up to 17%, which could increase productivity for the beer brewing industry. With the potential gain of two additional brews per day (assuming 10 brews/day on average), this would result in more efficient and cost-effective brewery operations and may be a promising development for future beer production. Nevertheless, the flavor of beers produced with lentil malt can still be enhanced. Sensory evaluations by a trained panel showed minor aroma differences, with a slight peaish aroma noticeable when lentil malt was added. Other germination times and kilning regimes might result in a better aroma. Furthermore, the use of lentil malt in more flavorful and hoppy beers may be an option. Overall, the addition of malted lentils to the brewing process is a novel, innovative brewing approach with promising results.

Author Contributions: Conceptualization, J.T. and A.P.; methodology, J.T. and H.W.; formal analysis, J.T. and H.W.; writing—original draft preparation, J.T. and H.W.; writing—review and editing, J.T., A.P. and J.D.C. All authors have read and agreed to the published version of the manuscript.

Funding: This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant agreement No 722166.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Black, K.; Barnett, A.; Tziboula-Clarke, A.; White, P.J.; Iannetta, P.P.M.; Walker, G. Faba bean as a novel brewing adjunct: Consumer evaluation. *J. Inst. Brew.* **2019**, *125*, 310–314. [CrossRef]
2. Bogdan, P.; Kordialik-Bogacka, E. Alternatives to malt in brewing. *Trends Food Sci. Technol.* **2017**, *65*, 1–9. [CrossRef]
3. Kordialik-Bogacka, E.; Bogdan, P.; Diowksz, A. Malted and unmalted oats in brewing. *J. Inst. Brew.* **2014**, *120*, 390–398. [CrossRef]
4. Steiner, E.; Auer, A.; Becker, T.; Gastl, M. Comparison of beer quality attributes between beers brewed with 100% barley malt and 100% barley raw material. *J. Sci. Food Agric.* **2012**, *92*, 803–813. [CrossRef] [PubMed]
5. The Brewers of Europe Beer Statistics 2018 Edition. Available online: www.brewersofeurope.org (accessed on 10 August 2021).
6. Muñoz-Insa, A.; Gastl, M.; Zarnkow, M.; Becker, T. Optimization of the malting process of oat (Avena sativa L.) as a raw material for fermented beverages. *Span. J. Agric. Res.* **2011**, *9*, 510–523. [CrossRef]
7. Klose, C.; Mauch, A.; Wunderlich, S.; Thiele, F.; Zarnkow, M.; Jacob, F.; Arendt, E.K. Brewing with 100% oat malt. *J. Inst. Brew.* **2011**, *117*, 411–421. [CrossRef]
8. Schnitzenbaumer, B.; Arendt, E.K. Brewing with up to 40% unmalted oats (Avena sativa) and sorghum (Sorghum bicolor): A review. *J. Inst. Brew.* **2014**, *120*, 315–330. [CrossRef]
9. Zarnkow, M.; Keßler, M.; Back, W.; Arendt, E.K.; Gastl, M. Optimisation of the mashing procedure for 100% malted proso millet (Panicum miliaceum L.) as a raw material for gluten-free beverages and beers. *J. Inst. Brew.* **2010**, *116*, 141–150. [CrossRef]
10. Zarnkow, M. Optimisation of malting conditions of quinoa. *Brauwelt* **2008**, 374–379.
11. Deželak, M.; Zarnkow, M.; Becker, T.; Košir, I.J. Processing of bottom-fermented gluten-free beer-like beverages based on buckwheat and quinoa malt with chemical and sensory characterization. *J. Inst. Brew.* **2014**, *120*, 360–370. [CrossRef]
12. Bailliere, J.; Laureys, D.; Aelbrecht, G.; Vermeir, P.; Vanderputten, D.; De Clippeleer, J. Unmalted alternative (pseudo) cereals for the production of specialty beers. In Proceedings of the World Brewing Congress 2020, American Society of Brewing Chemists, Master Brewers Association of the Americas, Online, 13–17 August 2020.
13. Bailliere, J.; De Clippeleer, J.; De Leyn, I.; Huys, J.; Laureys, D.; Vanderputten, D.; Vermeir, P. Comparison of Congress Mash with Final 65°C Mash for Production of Wort with Unmalted Barley, Tritordeum and Quinoa with or without Pregelatinizing and/or Enzyme Addition. In Proceedings of the ASBC Meeting 2021, American Society of Brewing Chemists, Online, 7–9 June 2021.
14. Martenka, J. Reis und Mais in der Brauindustrie. Master's Thesis, Technical University Munich-Weihenstephan, Freising, Germany, 2003.
15. Unger, R. *Beer in the Middle Ages and the Renaissance*, 1st ed.; University of Pennsylvania Press: Philadelphia, PA, USA, 2004; p. 181. ISBN 978-0-8122-1999-9.
16. Erskine, W.; Muehlbauer, F.J.; Sarker, A.; Sharma, B. *The lentil: Botany, Production and Uses*, 1st ed.; CAB International: Wallingford, UK, 2009; pp. 382–383. ISBN 9781845934873.
17. Bhatty, R.S. Composition and quality of lentil (Lens culinaris Medik): A review. *Can. Inst. Food Sci. Technol. J.* **1988**, *21*, 144–160. [CrossRef]
18. Ereifej, K.I.; Haddad, S.G. Chemical composition of selected Jordanian cereals and legumes as compared with the FAO, Moroccan, East Asian and Latin American tables for use in the Middle East. *Trends Food Sci. Technol.* **2000**, *11*, 374–378. [CrossRef]
19. El-Adawy, T.A.; Rahma, E.H.; El-Bedawey, A.A.; El-Beltagy, A.E. Nutritional potential and functional properties of germinated mung bean, pea and lentil seeds. *Plant Foods Hum. Nutr.* **2003**, *58*, 1–13. [CrossRef]
20. Shahwar, D.; Bhat, T.M.; Ansari, M.Y.K.; Chaudhary, S.; Aslam, R. Health functional compounds of lentil (Lens culinaris Medik): A review. *Int. J. Food Prop.* **2017**, *20*, 1–15. [CrossRef]
21. Joshi, M.; Timilsena, Y.; Adhikari, B. Global production, processing and utilization of lentil: A review. *J. Integr. Agric.* **2017**, *16*, 2898–2913. [CrossRef]
22. Frias, J.; Vidal–Valverde, C.; Bakhsh, A.; Arthur, A.E.; Hedley, C. An assessment of variation for nutritional and non-nutritional carbohydrates in lentil seeds (Lens culinaris). *Plant Breed.* **1994**, *113*, 170–173. [CrossRef]
23. Frias, J.; Diaz-Pollan, C.; Hedley, C.L.; Vidal-Valverde, C. Evolution and kinetics of monosaccharides, disaccharides and α-galactosides during germination of lentils. *Zeitschrift fur Leb. -Untersuchung und -forsch.* **1996**, *202*, 35–39. [CrossRef]
24. Frias, J.; Prodanov, M.; Sierra, I.; Vidal-Valverde, C. Effect of light on carbohydrates and hydrosoluble vitamins of lentils during soaking. *J. Food Prot.* **1995**, *58*, 692–695. [CrossRef]
25. Vidal-Valverde, C.; Frías, J.; Valverde, S. Changes in the carbohydrate composition of legumes after soaking and cooking. *J. Am. Diet. Assoc.* **1993**, *93*, 547–550. [CrossRef]
26. Jarpa-Parra, M. Lentil protein: A review of functional properties and food application. An overview of lentil protein functionality. *Int. J. Food Sci. Technol.* **2018**, *53*, 892–903. [CrossRef]
27. Boye, J.; Zare, F.; Pletch, A. Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Res. Int.* **2010**, *43*, 414–431. [CrossRef]
28. World Health Organization. *Codex Alimentarius: Codex Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten*; World Health Organization: Geneva, Switzerland, 2008.
29. Watson, H.G.; Decloedt, A.I.; Vanderputten, D.; Van Landschoot, A. Variation in gluten protein and peptide concentrations in Belgian barley malt beers. *J. Inst. Brew.* **2018**, *124*, 148–157. [CrossRef]
30. Poreda, A.; Bijak, M.; Zdaniewicz, M.; Jakubowski, M.; Makarewicz, M. Effect of wheat malt on the concentration of metal ions in wort and brewhouse by-products. *J. Inst. Brew.* **2015**, *121*, 224–230. [CrossRef]

31. Poreda, A.; Tuszyński, T.; Zdaniewicz, M.; Sroka, P.; Jakubowski, M. Support materials for yeast immobilization affect the concentration of metal ions in the fermentation medium. *J. Inst. Brew.* **2013**, *119*, 164–171. [CrossRef]
32. Aleksander, P.; Piotr, A.; Tadeusz, T.; Tuszyński, T.; Makarewicz, M. Accumulation and release of metal ions by brewer's yeast during successive fermentations. *J. Inst. Brew.* **2009**, *115*, 78–83. [CrossRef]
33. Boulton, C.M.; Quain, D. *Brewing Yeast and Fermentation*, 1st ed.; Blackwell Science: Oxford, UK, 2001; pp. 80–90. ISBN 0-632-05475-1.
34. Trummer, J.; Poreda, A.; Berski, W. Effect of malting on commercial green lentils and their suitability for wort production. *Przem. Ferment. I Owocowo-Warzywny* **2019**, *10*, 14–18. [CrossRef]
35. Strong, G.; Bach, R.; Garofalo, P.; Hall, M.L.; Houseman, D.; Tumarkin, M.; Zainasheff, J.; England, K.; Hieronymus, S.; Fitzpatrick, T.; et al. *BJCP Guidelines for Beer, Mead and Cider*; BJCP Inc.: St. Louis Park, MN, USA, 2008; p. 4.
36. European Brewing Convention. *Analytica EBC*; Fachverlag Hans Carl: Brussels, Belgium, 2010.
37. Anger, H.-M. *Mitteleuropäische Brautechnische Analysenkommission—Brautechnische Analysenmethoden—Rohstoffe: Gerste, Rohfrucht, Malz, Hopfen und Hopfenprodukte*; Selbstverlag der MEBAK: Freising-Weihenstephan, Germany, 2006; p. 137.
38. Miedaner, H. *MEBAK-Band 2 Bier.pdf*, 4th ed.; Selbstverlag der MEBAK: Freising-Weihenstephan, Germany, 2002; pp. 65, 212. ISBN 3-9805814-5-4.
39. Pfenninger, H. *Mitteleuropäische Brautechnische Analysenkommission—Brautechnische Analysenmethoden Band I*, 3rd ed.; Selbstverlag der MEBAK: Freising-Weihenstephan, Germany, 1997; pp. 226, 234–235. ISBN 3-9805814-0-3.
40. Kunze, W. *Technology Brewing and Malting*, 3rd ed.; VLB Berlin Int Edition: Berlin, Germany, 2004; pp. 161, 486, 583, 239–243. ISBN 3-921690-49-8.
41. Yadav, S.; McNeil, D.; Stevenson, P. *Lentil An Ancient Crop for Modern Times*, 1st ed.; Springer: Dordrecht, The Netherlands, 2007; pp. 75, 89. ISBN 9781402063121.
42. Tárrago, J.F.; Nicolás, G. Starch degradation in the cotyledons of germinating lentils. *Plant Physiol.* **1976**, *58*, 618–621. [CrossRef] [PubMed]
43. Gasiński, A.; Błażewicz, J.; Kawa-Rygielska, J.; Śniegowska, J.; Zarzecki, M. Analysis of physicochemical parameters of Congress worts prepared from special legume seed malts, acquired with and without use of enzyme preparations. *Foods* **2021**, *10*, 304. [CrossRef]
44. Demirbas, A. β-Glucan and mineral nutrient contents of cereals grown in Turkey. *Food Chem.* **2005**, *90*, 773–777. [CrossRef]
45. Arendt, E.K.; Zannini, E. *Cereal Grains for the Food and Beverage Industries*; Woodhead Publishing Limited: Sawston, UK; CRC Press: Cambridge, UK, 2013; pp. 155–201. ISBN 9780857094131.
46. Esslinger, H.M. *Handbook of Brewing: Processes, Technology, Markets*, 1st ed.; Wiley-VCH: Weinheim, Germany, 2009; pp. 222, 129–130, 209–213, 244–245. ISBN 978-3-527-31674-8.
47. Narziss, L. *Abriss der Bierbrauerei*, 7th ed.; Wiley-VCH: Freising-Weihenstephan, Germany, 2005; pp. 415, 204–205, 306–308. ISBN 0897910214.
48. Bamforth, C.; Russell, I.; Stewart, G. *Beer: A Quality Perspective*, 1st ed.; Elsevier Inc.: San Diego, CA, USA, 2009; pp. 114–120. ISBN 978-0-12-669201-3.
49. Krahl, M. Funktionelle Getränke auf Basis vermälzter Zerealien und Pseudozerealien. Ph.D. Thesis, Technical University Munich-Weihenstephan, Freising, Germany, 2010.
50. Wietstock, P.C.; Kunz, T.; Waterkamp, H.; Methner, F.J. Uptake and release of Ca, Cu, Fe, Mg, and Zn during beer production. *J. Am. Soc. Brew. Chem.* **2015**, *73*, 179–184. [CrossRef]
51. Lestienne, I.; Icard-Vernière, C.; Mouquet, C.; Picq, C.; Trèche, S. Effects of soaking whole cereal and legume seeds on iron, zinc and phytate contents. *Food Chem.* **2005**, *89*, 421–425. [CrossRef]
52. Wang, N.; Daun, J.K. Effects of variety and crude protein content on nutrients and anti-nutrients in lentils (Lens culinaris). *Food Chem.* **2006**, *95*, 493–502. [CrossRef]
53. Walker, G.M. Metals in yeast fermentation processes. *Adv. Appl. Microbiol.* **2004**, *54*, 197–229. [CrossRef]
54. Stratford, M. Yeast flocculation: Calcium specificity. *Yeast* **1989**, *5*, 487–496. [CrossRef]
55. Annemüller, G.; Manger, H.-J. *Gärung und Reifung des Bieres*, 1st ed.; VLB Berlin: Berlin, Germany, 2009; pp. 492–493. ISBN 978-3-921690-73-4.
56. Pfenninger, H. *Mitteleuropäische Brautechnische Analysenkommission—Brautechnische Analysenmethoden Band III*, 2nd ed.; Selbstverlag der MEBAK: Freising-Weihenstephan, Germany, 1996; p. 115. ISBN 3-9805814-2-X.
57. Walker, G.M.; De Nicola, R.; Anthony, S.; Learmonth, R. Yeast-metal interactions: Impact on brewing and distilling fermentations. In Proceedings of the Institute of Brewing & Distilling Asia Pacific Section Convention, Hobarts, Australia, 19–24 March 2006.
58. Poreda, A.; Stefaniuk, K.; Hoc, J.; Zdaniewicz, M. Improved efficiency of brewers wort supplementation with zinc ions. *Przem. Ferment. i Owocowo-Warzywny* **2014**, *2*, 4–8.
59. Krüger, E.; Anger, H.-M. *Kennzahlen zur Betriebskontrolle und Qualitätsbeschreibung in der Brauwirtschaft*, 1st ed.; Behr's Verlag—Hamburg: Hamburg, Germany, 1990; pp. 315–320. ISBN 978-3925673757.
60. Pagenstecher, M.; Maia, C.; Andersen, M.L. Retention of iron and copper during mashing of roasted malts. *J. Am. Soc. Brew. Chem.* **2021**, *79*, 138–144. [CrossRef]

61. Filipowska, W.; Jaskula-Goiris, B.; Ditrych, M.; Trueba, P.B.; De Rouck, G.; Aerts, G.; Powell, C.; Cook, D.; Cooman, L. De On the contribution of malt quality and the malting process to the formation of beer staling aldehydes: A review. *J. Inst. Brew.* **2021**, *127*, 107–126. [CrossRef]
62. Zufall, C.; Tyrell, T. The influence of heavy metal ions on beer flavour stability. *J. Inst. Brew.* **2008**, *114*, 134–142. [CrossRef]
63. Bamforth, C.W. *Brewing: New technologies*, 1st ed.; Woodhead Publishing Limited: Sawston, UK; CRC Press: Cambridge, UK, 2006; p. 54. ISBN 9781855734906.
64. Bamforth, C.W. *Brewing Materials and Processes: A Practical Approach to Beer Excellence*, 1st ed.; Elsevier Inc.: Davis, CA, USA, 2016; pp. 12, 80. ISBN 9780127999548.
65. Hanke, S. Untersuchungen zum Einfluss der Hopfungstechnologie auf die Geschmacksstabilität und Harmonie untergäriger Biere. Ph.D. Thesis, Technical University Munich-Weihenstephan, Freising, Germany, 2009.
66. Samaras, T.S.; Camburn, P.A.; Chandra, S.X.; Gordon, M.H.; Ames, J.M. Antioxidant properties of kilned and roasted malts. *J. Agric. Food Chem.* **2005**, *53*, 8068–8074. [CrossRef]
67. Amarowicz, R.; Estrella, I.; Hernandez, T.; Robredo, S.; Troszynska, A.; Kosinska, A.; Pegg, R.B. Free radical-scavenging capacity, antioxidant activity, and phenolic composition of green lentil (Lens culinaris). *Food Chem.* **2010**, *121*, 705–711. [CrossRef]
68. Ganesan, K.; Xu, B. Polyphenol-rich lentils and their health promoting effects. *Int. J. Mol. Sci.* **2017**, *18*, 2390. [CrossRef]
69. Khokhar, S.; Owusu Apenten, R.K. Iron binding characteristics of phenolic compounds: Some tentative structure-activity relations. *Food Chem.* **2003**, *81*, 133–140. [CrossRef]
70. Andjelković, M.; Van Camp, J.; De Meulenaer, B.; Depaemelaere, G.; Socaciu, C.; Verloo, M.; Verhe, R. Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. *Food Chem.* **2006**, *98*, 23–31. [CrossRef]
71. Hirota, N.; Kuroda, H.; Takoi, K.; Kaneko, T.; Kaneda, H.; Yoshida, I.; Takashio, M.; Ito, K.; Takeda, K. Development of Novel Barley with Improved Beer Foam and Flavor Stability—The Impact of Lipoxygenase-1-Less Barley in the Brewing Industry. In Proceedings of the 30th EBC, Prague, Czech Republic, 14–19 May 2005.
72. Yu, J.; Huang, S.; Dong, J.; Fan, W.; Huang, S.; Liu, J.; Chang, Z.; Tian, Y.; Hao, J.; Hu, S. The influence of LOX-less barley malt on the flavour stability of wort and beer. *J. Inst. Brew.* **2014**, *120*, 93–98. [CrossRef]
73. Pathiratne, S.M.; Shand, P.J.; Pickard, M.; Wanasundara, J.P.D. Generating functional property variation in lentil (Lens culinaris) flour by seed micronization: Effects of seed moisture level and surface temperature. *Food Res. Int.* **2015**, *76*, 122–131. [CrossRef]
74. Watson, H.G.; Vanderputten, D.; Van Landschoot, A.; Decloedt, A.I. Applicability of different brewhouse technologies and gluten-minimization treatments for the production of gluten-free (barley) malt beers: Pilot- to industrial-scale. *J. Food Eng.* **2019**, *245*, 33–42. [CrossRef]
75. Roland, W.S.U.; Pouvreau, L.; Curran, J.; Van De Velde, F.; De Kok, P.M.T. Flavor aspects of pulse ingredients. *Cereal Chem.* **2017**, *94*, 58–65. [CrossRef]
76. Lovegren, N.V.; Fisher, G.S.; Legendre, M.G.; Schuller, W.H. Volatile constituents of dried legumes. *J. Agric. Food Chem.* **1979**, *27*, 851–853. [CrossRef]
77. Briggs, D.; Boulton, C.; Brookes, P.; Stevens, R. *Brewing Science and practice*, 1st ed.; Woodhead Publishing Limited: Sawston, UK; CRC Press: Cambridge, UK, 2004; p. 156. ISBN 0849325471.
78. Troszyńska, A.; Estrella, I.; Lamparski, G.; Hernández, T.; Amarowicz, R.; Pegg, R.B. Relationship between the sensory quality of lentil (Lens culinaris) sprouts and their phenolic constituents. *Food Res. Int.* **2011**, *44*, 3195–3201. [CrossRef]