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Review

Rootlets, a Malting By-Product with Great Potential

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Abstract: Barley rootlets are the most abundant by-product from the malting industry. Due to the inherent association of the malting industry with brewing and distilling industries, it is also considered a by-product of these industries. Barley rootlets are produced during the germination step of malting. These rootlets are a valuable source of nutrition, with protein and fibre holding a large proportion of their composition. Barley rootlets are generally pelletised and used as animal fodder; however, their usage may not be limited to this. Efforts have been made to utilise barley rootlets as food ingredients, sources of enzymes, antioxidants, raw materials in fermentations, and in biochar production. Conversion of this by-product into other/new applications would reduce waste production from their industry origin and reduce some of the impending environmental concerns associated with by-product production. The current review focuses on providing information on the formation, production, and processing of barley rootlets, while also highlighting the composition, quality, and potential applications of barley rootlets.

Keywords: malt rootlets; barley rootlets; malt culm/s; malt sprouts; by-product valorisation

1. Introduction

Barley is a cereal grain grass which is a member of the *Poaceae* family and the genus *Hordeum*. The most common form of barley is *Hordeum vulgare* [1]. It is the main raw material used in the production of standard beer, craft beer and malt whiskey [2]. The primary product of brewing is the beverage; however, the process also accumulates various by-products. These include brewers' spent grain, spent hops, and brewers' spent yeast [3,4]. Malt rootlets (sometimes referred to as malt culms/coombes/sprouts) constitute approx. 3–5% by weight of the malt produced [5,6]. However, due to the malting industries inherent association with the brewing industry it can also be considered a brewing by-product [2].

Barley has been considered the most suitable raw material for brewing for centuries. It provides the primary components to produce beer. The starch present in barley, approximately 50–65% of the barley dry weight, is converted to fermentable sugars for the yeast to consume during fermentation [6]. The protein present in barley, representing 11–16% of barley dry weight, makes a considerable contribution to the head retention in beer. Additionally, the husk of the barley grain is used as a filtering aid during the brewing process [6]. However, the starch present in barley, and ultimately the most important material in the grain for beer production, is trapped within the endosperm walls of the barley grain including the aleurone, seed coat, pericarp, and husk layers. Thus, malting is the process used to gain access to this starch.

The malting process induces germination which stimulates rootlet and acrospire production, enzyme formation, and what are collectively known as "modifications" in the barley grain. These "modifications" allow for the starch to be accessed. During the germination stage of malting, rootlets are produced and must be separated from the malted barley after the kilning stage of malting [2].

Currently, the vast majority of the rootlets produced are sold as animal feed and are implemented as a straight feed or a commodity in feed mixtures [2]. The rootlets are pelletised and sold to the animal feed industry. The pellets are often commonly referred to as "malt combings" because they can also include other by-products of the malting industry including: malt dust, small and broken barley grains, barley dust, acrospires and parts of the husk. Their use as animal feed originates from their composition, which is particularly high in protein and fibre.

The composition of barley rootlets can vary. They are rich sources of protein, amino acids (both essential and non-essential) and minerals. Fat levels in rootlets are comparable to fat contents found in the barley grain. Starch levels in the rootlets are very low in comparison to the level of starch found in the barley grain. Fibre is a predominant fraction of the rootlet composition, mainly insoluble dietary fibre, while also containing considerable levels of sugars (mono-,di-saccharides) and low levels of maltotriose [7]. Rootlets have also been found to be a source of phenolic compounds [7,8] and enzymes [9,10]. Due to this valuable composition, rootlets have been evaluated as food ingredients, as a source of enzymes such as 5'phosphodiesterase, as a fermentation substrate, and a source of phenolic compounds.

The utilisation of barley rootlets has been limited to date; however, this may be due to the limited research available. Increased utilisation of barley rootlets will help to reduce the by-products associated with the brewing/malting industry and help merge these processes into a more sustainable future. The purpose of this review is to provide an overview of the information available on barley rootlets in relation to their production, composition, and potential uses. It is important to note that in some studies reviewed, it was sometimes difficult to decipher the exact raw material utilised, as a universal term for barley rootlets has not been established. Terms such as malt sprouts were often used, particularly for studies involving lactic acid production and growth of lactic acid bacteria. Rootlets have also been referred to as "germs" in literature [11,12]. The authors used the terminology which was outlined in the studies they originated from, however where the term sprouts was used the authors cannot guarantee that this material is the rootlets alone. This term can imply that other parts of the grain are also present, such as the acrospires, barley dust and broken parts of the husk [5,13,14]. However, typically a high percentage of this mixture is comprised of the rootlets and is a good indication of how the rootlets may perform in the analysis. The need for an exact term for rootlets in the future is necessary to avoid confusion.

2. Biological Steps in Grain Germination and Relationship to the Malting Process

The aim of malting for the maltster is to gain access to the desired starch trapped within the endosperm of the barley grain. This is achieved by stimulating germination and exploiting the endogenous enzymes in the barley grain. Malting may potentially be one of the oldest biotechnologies recorded, and is believed to have been practiced in conjunction with brewing for at least 6000 years [5]. Evidence suggests that barley malting has occurred since ancient Egyptian times and was used in beer and bread production [15]. Malting has evolved over time due to the greater understanding of the physiology and biochemistry of the grain. The process is not confined to one type of grain, however historically for numerous reasons, barley malt has proved to be the most suitable malt for beer production [6].

Water is key in starting biological processes within the barley grain [16]. Water uptake initiates respiration and allows germination to occur. Respiration rates are highly dependent on water content and increase greatly once moisture content of the barley grain surpasses 15% [6]. As respiration rates increase, so too does the demand for oxygen. This must also be supplied to the barley grain in order to avoid intramolecular respiration and the formation of cell poisons (alkanals, alkanols) which can

ultimately kill the barley grain [6]. The grain then draws on its own supply of nutrients to germinate; however, such nutrients are locked within the endosperm and must be accessed. These nutrients become accessible as a consequence of the biological changes occurring in the grain, including enzyme activation, enzyme formation and metabolic changes [17,18]. Rootlets and acrospires are also produced in this process. Enzymes are formed and activated mainly in the aleurone layer as a result of the uptake of water and the release of the complex growth promoting hormone called gibberellic acid. Gibberellic acid is comprised of several classes, and various forms of the hormone are released during grain germination [19]. Alpha-amylase is produced, and beta-amylase is released, which is already present in large amounts in the endosperm. The level of alpha- and beta-amylase (particularly alpha-amylase) produced strongly correlates with respiration rates and requires oxygen for its formation [6]. A series of other enzymes are also released and activated, some of which include phosphatases, lipases, proteinases and saccharolytic (xylanases, beta-glucanases) enzymes, and are also of huge importance to the grain. These enzymes break down the long chain macromolecules in the grain, supplying energy for the new plant during germination, until the roots are formed [6,20]. These changes to the endosperm are collectively known as grain "modifications" [2,18]. Modifications begin in the starchy endosperm beside the scutellum and continues to the distal end of the grain (Figure 1). The growth element of germination, i.e., the production of rootlets and acrospires from the barley grain, is the visual representation of the extent of germination [21]. To maintain the composition of the barley grain and reap the benefits of the activated enzymes for the brewing process, germination must be stopped. This is achieved by the removal of the water, which previously ignited the life processes to occur [2].

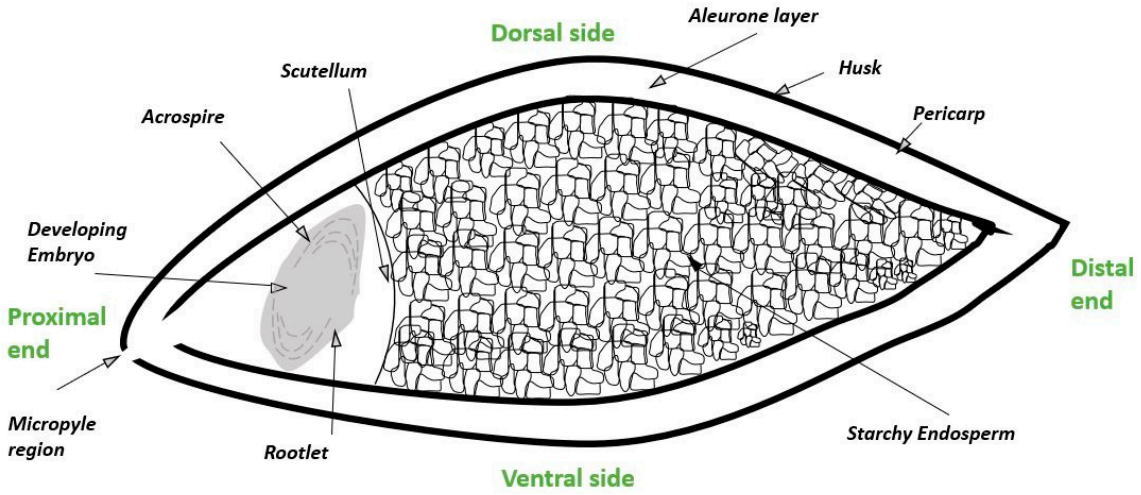

Figure 1. Structure of the barley grain. Adapted from Mosher et al. [22].

Malting can generally be broken into three stages: steeping, germination, and kilning. Although, in reality, a lot more steps are associated with the malting process and the divisions between such stages are not exactly definitive.

Steeping is the first step in malting. The barley grains are immersed in temperature-controlled water in the steeping vessel which promotes the grain to swell, soften and entice living tissues to resume metabolism [5]. As mentioned, the grain requires oxygen for respiration, therefore the barley grain needs to be aerated. In many malting plants, water is drained out and grains are left exposed to the air [5]. The water content of the barley grain needs to reach approx. 45% during steeping [22]. Following this, the grain is transferred to a germination vessel.

The germination process can vary with numerous techniques practiced, however most malting plants carry out germination using circular or rectangular boxes (Saladin boxes) [6]. In short, the basic steps to germination include barley grains spread to a certain depth, held under a controlled temperature, and rotated regularly. This allows for uniform ventilation of the grains, which stimulates even germination as well as preventing the rootlets from entangling [5,16,23]. The temperature and air

circulating the barley grains is controlled to manipulate respiration rates [6]. This is done to avoid large compositional losses from the barley grain which would have a negative effect on brewing yield [5]. As germination proceeds, the enzymes are produced/activated, rootlet and acrospire growth progresses due to the increased metabolic activity, and the endosperm "modifications" progress. Figure 2 shows the progression of the rootlets and acrospire growth during germination. Germination generally takes five days to complete and is terminated when conditions of the malt are met. Modifications and malt quality can be assessed by the eye of the maltster along with their crumbly, chalk like texture [6] and through the use of various technological techniques. Some of the technological techniques used include assessment of the malt extract (hot water extract (HWE), cold water extract (CWE), fine and course grind extract), the Kolbach index, friability, viscosity of malt extract/wort, diastatic power, soluble protein, free amino nitrogen (FAN) and β-glucan content [17,24,25].

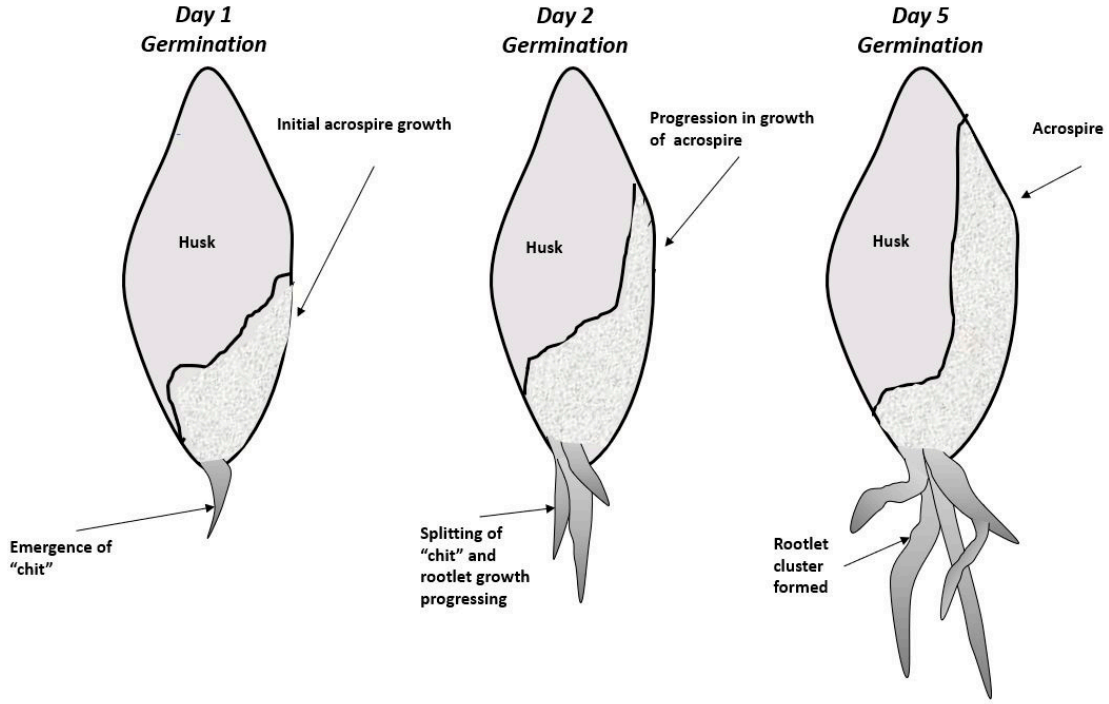

Figure 2. Growth of acrospire and rootlets during germination. Adapted from Kunze et al. [6].

Kilning is the final step of the malting process. During kilning hot air is applied to the germinated barley (known as green malt) [6]. This is done to stop germination, dry the barley grains to approx. 5% moisture, and maintain the enzymatic potential of the grains for further processing [2]. Kilning has an impact on the flavour and colour compounds of the malt. Depending on the brewing process it is destined for, some malts may be roasted in drums, imparting the flavour and colour compounds of the Maillard reaction [5,6]. The green malt is dried using several steps with high temperatures being applied slowly. After pre drying to a moisture content of approx. 12%, the green malt is slowly heated to higher temperatures to continue reducing the moisture of the grain to 4–5% [6]. Kilning regimes cause some loss of enzymatic activity, because high temperatures cause changes to the protein structure [6,21,26]. Kilned malt, unlike green malt, is brittle and fragile and is much more stable in storage due to its lower microbial count [27].

The kilned malt is then cooled and cleaned, removing rootlets and dust. The brittle and fragile nature of the kilned malt makes rootlet and dust removal much easier [5]. Kilned malt is then stored in silos before use for the benefit of the brewing process [6,28].

3. Formation and Processing of Barley Rootlets

Barley rootlets are regarded as the most valuable by-product for the malting industry. Other by-products of malting include malt dust, barley dust and small/broken barley grains, but are of lesser value and quantity than barley rootlets. In the U.K., more than 50 tonnes of these malting co-products are produced annually [29]. Rootlets are known as seminal roots because they develop from the embryo of the barley grain, according to terminology outlined by Hackett [30].

The growth of rootlets is initiated by water entering the embryo. Water enters mainly via the micropyle region of the grain (Figure 1) [31]. The embryo swells as a result of the uptake of water, respiration activities start, and formation of new tissues begins [5]. The rootlets are formed by utilising the nitrogenous material (amino acids and peptides) which becomes available in the endosperm and taken up by the embryo [5,20]. The rootlet first emerges as a white "chit", a yellowish coleorhiza or root sheath [5,20,32]. The "chit" breaks through the testa and pericarp layers of the grain and appears between the valves of the husk, extending from the base of the grain. The chit splits as germination progresses, forming rootlets which grow in length and form a cluster (Figure 2). Rootlets can become matted and entangled with each other during germination, causing crowding and non-uniform germination to occur if parameters such as grain turning are not controlled. The rootlets grow to approximately 5–8 mm in length and 0.3–0.4 mm in thickness [5].

Rootlets are separated from the malt because they give bitter flavours to beer [12]. They may also be a source of nitrosamines when stored under unfavourable conditions and are hydroscopic, which can also pose issues during malt storage [4,24]. Rootlets may be sourced directly from the kiln, during the deculming process, in a combined format of rootlets from the kiln and deculming process or in a pelletised format which also contains other malting by-products. Some of these processes will be discussed further below.

The removal of rootlets from the kilned malt is often referred to as the deculming process [32]. Historically, the deculming process involved crushing the kilned malt by walking on it while the malt was still on the kiln and shovelling the crushed kilned malt against a sieve/wire screen. The kilned malt was then brushed, and the broken rootlets fell through the screen while the kilned malt slid to the bottom and collected in piles [5]. This highly labour-intensive job was mechanised in the 20th century. In contemporary practice, two different types of machines are used: a malt deculming machine or a deculming screw [5,6,32]. In the deculming screw (Figure 3), the kilned malt moves along angled beaters within a perforated walled, U-shaped trough. The rootlets break off, fall through the perforated walls, and are collected [5]. The deculming machine is a pneumatic device. The kilned malt enters an air stream and is forced into a vertical cylindrical vessel. The impact on entering the vessel breaks off the remaining rootlets. The deculmed malt is heavier and falls through the air stream to the bottom of the cylinder, past a separation cone, and is withdrawn. The rootlets and dust collected from the air stream pass through one or two cyclones and is then collected [5]. Rootlets may be collected in the kilns, because they can fall to the bottom of the heating chamber in the kilning vessel. This occurs most often when very high temperatures are applied during kilning. The rootlets that are collected from the heating chamber in the kiln are of lesser nutritional value due to the exposure to high heat [6]. As a result, these darker rootlets may be kept separate from the less intensely heated rootlets. The kilns must therefore be regularly cleaned to collect the small fine particles which fall from the malt, which may cause fire hazards [5].

In previous times, the rootlets and dust were bagged separately in moisture-proof bags and sold as animal feed. In some cases, although rarely, they were burned for heat or composted [5]. However, the bulk density of rootlets as a loose material are low, implying that low amounts utilise large volumes of space, so more commonly, the rootlets are pelletised into a blend. The blend includes other malting by-products, such as barley dust, malt dust and floating barley grains from steeping [5,29]. The rootlets are transferred to a pelletising unit pneumatically. The co-product blends are wetted, mixed and pelletised. The pellets may be produced by compressing and forcing the mixture through a die. The emerging material is then cut to size by rotating blades [5]. Various technologies may be employed

for the pelletising process, and is subject to the malting plant practice. The composition of the end pellet can vary, however the pellet on dry matter basis is approximately 24.5% crude protein, 2.9% oil, 40% NDF (neutral detergent fibre-mainly insoluble fibre [33]), 5.5% starch, and 13% sugar [34]. Pelletising the rootlets increases their bulk density from 224.3 kg/m^3 to between 561–641 kg/m^3 depending on moisture contents and pellet sizes [5,29]. Converting the rootlets to pellets also makes transport and storage much easier for farmers and the animal feed industry.

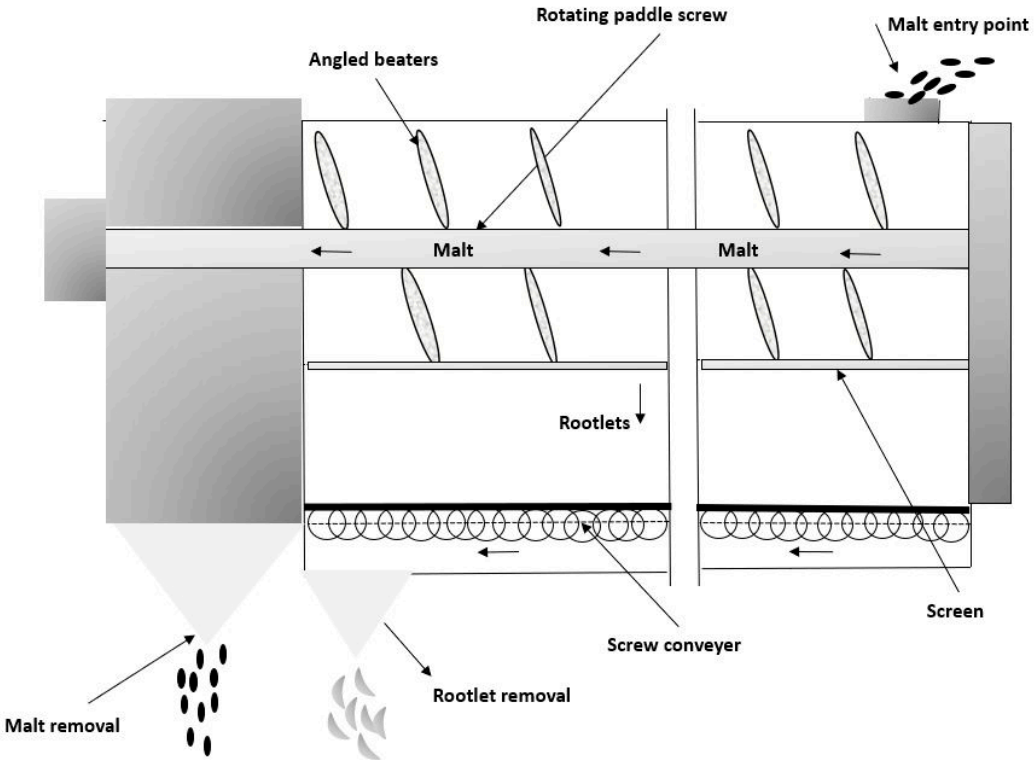

Figure 3. Longitudinal section of deculming screw. Adapted from Kunze et al. [6].

These pellets are a valuable source of nutrition for animals; however, one may question if there is potential for these pellets to be consumed by humans. Malting plants are certified to FEMAS (Feed Materials Assurance Scheme) which is based on the HACCP principles, and by-products are produced with Good Manufacturing Practice [29]. However, there is the potential to have higher levels of mycotoxins in these pellets than the parent malt due to the higher level of husk and outer layers incorporated into the by-product pellets. Mycotoxins are toxic substances that are produced as secondary metabolites of certain moulds (fungi) [35]. These layers have the highest risk of mycotoxin contamination. This is already of concern for some animals such as weaner piglets, which are as sensitive to mycotoxin exposure as humans [29]. Therefore, these pellets may have limited usage in human nutrition currently; however, if the pellets were produced containing only malt rootlets and quality assured, they may have potential as food ingredients in the future.

4. Rootlet Composition and Quality

The composition and quality of barley rootlets is important when considering their nutritive value and potential applications. The composition of the barley rootlets (Table 1) can vary, and often depends on barley variety and malting practices (e.g., germination time, kilning temperatures) [5,13].

Protein constitutes 22.6–38.7% of the composition (Table 1), which makes barley rootlets an excellent source of protein. The protein content in barley rootlets exceeds the level of protein found in the barley grain itself and malted barley [7], as well as other cereals such as wheat which has an approx. protein content of 11–14% [7,36]. The proteins in rootlets are primarily glutelins, followed

by globulins, albumins and prolamins [37]. Analysis of the barley rootlet proteome reveals that the proteins in barley rootlets are more diverse and enriched due to its anatomical complexity and the various processes which occur there for growth [8]. Table 2 illustrates the quantity of amino acids present in barley rootlets reported across various studies. Glutamic acid and aspartic acid are present in the highest amounts. The essential amino acids isoleucine, phenylalanine, lysine and leucine also quantify a significant proportion of the amino acids. The presence of lysine in rootlets is of interest, because it is widely known that lysine is a limiting amino acid in many plant-based cereals. Salama A.R.A et al. [14] reported that 5.29 g/16 g N of lysine was present in barley rootlets, which was slightly lower than the barley malt sprouts (7.12 g/16 g N), acrospires (6.14 g/16 g N) and husks (7.58 g/16 g N). However, with a content of 5.29 g/16 g N, lysine would not be considered a limiting amino acid in barley rootlet protein according to WHO recommendations for lysine intake in adult diets [38]. It was noted by Salama A.R.A et al. [14] that the limiting amino acid in rootlets were the sulphur-containing amino acids, methionine and cysteine. The protein profile of rootlets could potentially complement the amino acid profiles of proteins from cereal based staple foods, such as wheat bread, which contains low levels of lysine but sufficient amounts of sulphur-containing amino acids [39]. The rootlets also have an IVPD (in vitro protein digestibility) value of approx. 81–83.29% [13,14], similar to the IVPD of acrospires and malt sprout mixture [14] and an NPU (net protein utilisation) which supports normal growth in rats [40]. Such parameters combined provide an indication of the quality of the protein in barley rootlets, highlighting its potential in human nutrition.

Fibre levels are difficult to quantify because various analytical methods exist to measure fibre and are used in different studies [33]. Table 1 refers to both crude fibre and total fibre. Crude fibre determination, developed by Einhoff in 1806, is one of the oldest methods to determine fibre based on acid and alkali digestion [33], hence older studies report crude fibre values. Crude fibre values are still used in the animal feed industry today, however their usage in human nutrition is limited because the measurement obtains a lower fibre value [41]. This is because they do not measure all the polysaccharides present in the plant cell walls that are indigestible for humans and only measure a percentage of the levels of cellulose, hemicellulose, and lignin [33,42]. Total fibre values take into account the insoluble and soluble fibre present [43] which gives a better representation of the fibre content. Therefore, crude fibre and total fibre values are not comparable; however, the values still give an indication of the amount of fibre present. Table 1 shows that fibre represents a substantial amount of the composition of rootlets. Fibre in barley rootlets is higher than the fibre in both the barley grain and malted barley [7]. Waters et al. [7] report the most detailed and accurate representation of the level of fibre in barley rootlets. Insoluble fibre comprises 91.19% of the total fibre composition. Arabinoxylans, composed of a xylose backbone with arabinose substitutions with ferulic acid esterified to arabinose [44], comprise about one third of the fibre present in barley rootlets [7]. Arabinoxylans have the potential to cross-link via di-ferulic acid bridges, which can pose a challenge in food applications, particularly cereal based applications [45]. Other fibres which have been identified in barley rootlets include cellulose [37,46] and lignin [47]. High fibre foods are becoming increasingly popular due to the health benefits associated with them [48], and the high fibre content of barley rootlets makes them a potential ingredient as a fibre fortifier in the future.

Starch contents have been reported in the range of 2.6–26.5% (Table 1). The amount of starch found in barley rootlets is much lower than the level of starch found in the barley grain [7]. Sugar levels (monosaccharides, disaccharides, reducing and non-reducing) have been reported in the range of 3.4–13.6% [7,14,37,40]. Glucose and fructose are the main monosaccharides present in barley rootlets, with minor levels of sucrose, maltose and maltotriose also present [7]. The rootlets also have much higher levels of glucose and fructose present than the barley grain and malted barley [7].

Fat levels in barley malt rootlets have been reported in the range of 1.5–4.4% (Table 1). Barley rootlets are lower in saturated fat than wheat flour [7]. Table 3 illustrates the fatty acid profile of the barley rootlets. Variations in the level of fatty acids may be attributed to differences in barley variety. Linoleic and linolenic acid are the dominant fatty acids present, followed by palmitic acid.

Table 1. Chemical composition of malt barley rootlets.

Component	Hashitani Y. [37]	Hegazi et al. [40]	Salama A.R.A et al. [14]	INRA-CIRAD-AFZ [47]	Aggelopoulos et al. [58]	Waters et al. [7]	Begea et al. [46]	Chiş et al. [59]
Protein	23.9	25.0	31.9	22.6	31.1	36.75	20.34	38.7
Fat	3.6	1.8	n.m.	1.8	4.4	1.7	1.9	2.1
Ash	3.4	8.0	8.7	5.9	6.8	2.8	3.78	8.4
Moisture (%)	10.2	8.5	12.6	10.2	12.9	n.m.	8.6	8.2
Carbohydrates	n.m.	n.m.	n.m.	n.m.	n.m.	60	n.m.	50.9
Total Fibre	n.d.	n.m.	n.m.	n.m.	n.m.	43.0	n.m.	n.m.
Crude fibre	20.5	9.7	10.7	13.9	n.m.	n.m.	n.m.	n.m.
Starch	n.m.	7.0	26.5	16.5	n.m.	2.6	n.m.	n.m.
Arabinoxylans (% T.F.)	n.m.	n.m.	n.m.	n.m.	n.m.	14.4	n.m.	n.m.
Polyphenols	n.m.	n.m.	0.35	n.m.	n.m.	0.0102	n.m.	n.m.
Phytic Acid	n.m.	n.m.	0.018	n.m.	n.m.	n.m.	n.m.	n.m.

All values are expressed in g per 100 g based on dry matter unless stated otherwise. % T.F.: percent of total fibre. n.m.: not measured.

Table 2. Essential and non-essential amino acids present in barley malt rootlets.

	Robbins et al. [13] (g/100g AA Rec)	Hegazi et al. [40] (mg/g N)	Salama A.R.A et al. [14] (g/16g N)	Waters et al. [7] (g/100g Protein)
Essential Amino Acids				
Threonine	3.9	298	3.82	0.055
Methionine	2.0	101	n.m.	0.107
Tryptophan	n.m.	122	2.51	0.022
Phenylalanine	3.6	101	3.84	0.875
Isoleucine	3.9	n.m.	3.40	1.055
Leucine	5.8	n.m.	5.43	1.455
Lysine	5.5	244	5.29	n.m.
Non-Essential Amino Acids				
Aspartic Acid	6.3	382	12.62	2.617
Glutamic Acid	13.1	596	11.32	3.025
Asparagine	n.m.	n.m.	n.m.	0.430
Serine	n.m.	306	3.9	0.882
Glutamine	n.m.	n.m.	n.m.	n.d.
Histidine	2.2	260	6.16	7.589
Glycine	4.3	216	4.05	0.470
Arginine	5.2	493	4.78	1.117
Alanine	5.2	200	11.31	1.198
γ-Aminobutryic Acid	n.m.	n.m.	n.m.	7.302
Tyrosine	2.3	295	1.21	0.617
Valine	5.5	268	6.09	1.334
Proline	5.9	110	6.72	n.m.
Cystine	0.4	112	n.m.	n.m.

n.d.: not detected. n.m.: not measured. AA Rec: amino acid recovered.

The ash content in barley rootlets ranges from 2.8–8.6% (Table 1). This is higher than the ash level present in the barley grain, malted barley, acrospire and husk [7,14]. The levels of micronutrients reported varied, which may be attributed to barley variety, however levels of calcium, potassium and phosphorus remained consistent as the highest micronutrients reported in barley rootlets [7,14,37]. Polyphenols and phytic acid have also been reported in barley rootlet composition (Table 1). Polyphenols are compounds which contain at least one phenol unit and originate as secondary metabolites of plants [49]. Phytic acid, also known as myo-inositol hexaphosphoric acid, is the principal storage form of phosphorus in plants [50]. It is widely accepted that both polyphenols and phytic acid have antioxidant properties in humans, however it is also known that they have antinutritional effects with respect to mineral bioavailability. Polyphenols and phytate bind to minerals, making them less bio-available for absorption by humans and monogastric animals. However, in the case of barley rootlets, this may not pose a huge threat because polyphenol and phytic acid levels are relatively low (Table 1).

The quality of the barley rootlets can vary depending on the moisture content, storage, and processing of the rootlets. Moisture contents of rootlets have been reported in the range of 8.2–12.9%. The water contents of the barley rootlets are low post-kilning; however, barley rootlets are hygroscopic [5], meaning such moisture contents can fluctuate and are subject to change. In general, lower moisture contents lead to less microbial contamination but if uncontrolled storage conditions prevail, favouring water uptake, spoilage will occur. Production of mycotoxins in brewing by-products

has previously been highlighted as an area for concern. Cavaglieri et al. [51] found that *Fusarium verticilliodes* and *Aspergillus flavus* were the predominant microbes present in barley rootlets, with very little microbial diversity found. Although mycotoxin contamination has been found in all stages of the malting and brewing process, particular emphasis has been made on the level of mycotoxins present in barley rootlets [52]. Rootlets support the growth of Ochratoxin A, Aflatoxin B_1 [53] and Fumonisin B_1 [51] producing-fungi which are known to be harmful to human and animal health. In addition, rootlets have also been found to support the growth of deoxynivalenol and zearalenone producing-fungi [52]. Studies from Mastanjević et al. [54] and Krstanović et al. [55] also report significant levels of deoxynivalenol present in barley rootlets from malting. Ribeiro et al. [53], found changes in mycotoxin production with changing water activity, temperature, and storage time, indicating that such parameters need to be controlled to limit mycotoxin production on barley rootlets. Regular monitoring during storage may be necessary to consider rootlets as high-quality food ingredients. There is also potential for nitrosamines (carcinogenic compounds [56]) to accumulate in barley rootlets [5]. Under some conditions, such as interactions between the basic nitrogenous components of the rootlets and oxides of the nitrogen present in the kiln gases, formation of nitrosamines can occur [5]. Although the introduction of low levels of sulphur dioxide at the beginning of kilning and indirect heating of kilns has significantly reduced the production of nitrosamines [57], it may still be a parameter to consider when assessing rootlet quality and suitability for their use in food.

Table 3. Fatty acid profile of barley rootlets.

Components	Waters et al. [6]	Chiş et al. [59]
Fat %	1.7	1.9
Saturates	24.12	33.40
Monounsaturated fatty acids	8.39	14.15
Polyunsaturated fatty acids	69.47	70.20
Fatty Acids Present		
Caproic	0.02	n.m.
Caprylic	0.03	n.m.
Capric	0.15	0.31
Lauric	0.11	0.69
Myristic	0.65	n.m.
Pentadecanoic	0.30	0.42
Palmitic	14.81	30.50
Palmitoleic	0.26	0.26
Heptadecanoic	0.10	0.03
Stearic	1.40	1.45
Elaidic	0.09	0.09
Oleic	4.95	12.13
Cis-Vaccenic	1.15	n.m.
Linoleic	34.63	35.61
Linolenic	32.60	32.64
Arachidic	0.79	n.d.
Eicosenoic	0.79	n.m.
Eicosadienoic	0.26	n.m.
Heneicosanoic	0.06	n.m.

Table 3. *Cont.*

Components	Waters et al. [6]	Chiş et al. [59]
Arachidonic	n.d.	0.79
Behenic	1.12	n.m.
Docosenoic	0.16	0.38
Erucic	0.38	n.m.
Docosadienoic	0.12	n.m.
Tricosanoic	0.19	n.m.
Docosatetraenoic	0.61	n.m.
Lignoceric	0.82	n.m.
Docosapentaenoic DPA	0.09	n.m.
Docosahexaenoic DHA	1.16	1.16
Nervonic	0.70	n.m.
Obtusilic	n.m.	0.14
Vaccenic	n.m.	1.15

Based on % total fat. n.d.: not detected. n.m.: not measured.

5. Rootlet Applications

Barley rootlets are an underused by-product. Their potential lies within their excellent composition and availability in high amounts. Various patents (Table 4) are available which employ barley rootlets within their inventions. Studies from literature which use barley rootlets as raw materials are somewhat limited. They have primarily been used as animal feed, however; there has also been efforts to incorporate barley rootlets into food products, extract and utilise their enzymes and antioxidants, as substrates in fermentations (Figure 4), and also as a raw material in biochar production. Results from the studies are promising, which shines a positive light on the potential and diversity of barley rootlets for future applications.

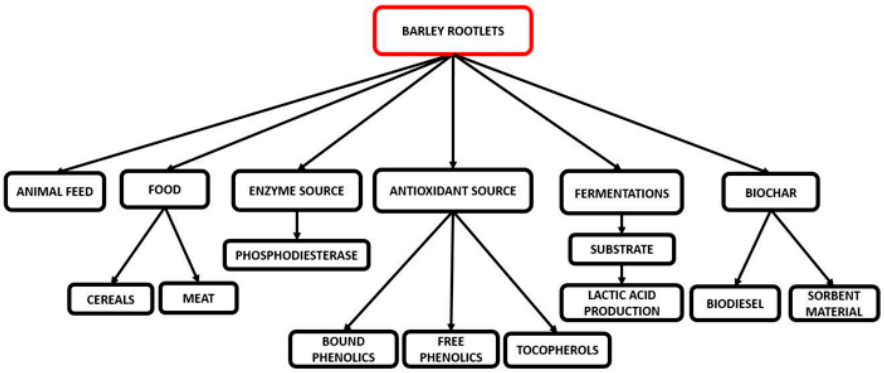

Figure 4. Summary of applications of barley rootlets.

5.1. Animal Feed

The primary application for barley rootlets is as animal feed, because it is a valuable source of nutrients. Barley rootlets are incorporated as feed for both monogastric and ruminant animals and may be implemented as compound feeds or within feed mixtures. When compared to brewers' spent grain, another by-product of the brewing industry, rootlets have a higher rumen nitrogen degradability and lower nitrogen intestinal digestibility [60]. This implies that the rootlets have a lower proportion of rumen by-pass nitrogen (amount of nitrogen which escapes degradation by ruminant microbes and

available to be metabolised by the animal in the small intestine, more commonly referred to as "by-pass protein"). These parameters are important to consider when choosing feed for ruminant animals.

Alterations in the nutritional profile of animal food products depending on the feed the animals are consuming is known to occur, and barley rootlets can have a similar effect. Hashish and Abd El-Samee [61] found alterations in the fat profile in the yolk of eggs from laying hens with barley malt rootlets included in the hens diet. Incorporation of rootlets in the hen feed reduced the level of cholesterol and low-density lipoproteins in the egg yolk, with increasing levels of the rootlets in the diet having a greater impact in reducing these parameters. The enhanced fat profile of the egg yolk is associated with the favourable fat and fibre composition of the barley rootlets [61]. This effect may be true for other animal food products, however further investigations are required to confirm this.

Although barley rootlets are a valuable source of nutrition for animals, there is some caution advised with their usage in equine feeds because rootlets contain the protein hordenine [29,37]. Hordenine has been viewed in some countries as potentially an illegal drug in horse racing. The protein is classified as a naturally occurring prohibited substance (NOPS), and in some countries horses may be disqualified from racing if found in the urine [62]. Hordenine has been shown to induce a pharmacological effect in relation to respiratory function in horses [63]. It increases systolic and diastolic blood pressure and peripheral blood flow volume. Such effects are short lived and found after administration of high doses (2 mg/kg Body Weight) through IV injection [63]. Although there is no evidence for these effects from hordenine consumption in feed [63], barley rootlets and other feed materials containing hordenine should be avoided in equine diets.

5.2. Food Applications

Barley rootlets have a desirable nutritional profile, being high in protein and fibre. Such fractions are highly sought after for incorporation in the human diet, particularly fibre, due to the health benefits associated with it [64]. Utilisation of barley rootlets as a food ingredient has been shown to enhance the nutritional profile of breads, described as flattened breads and pan breads [65]. Increased usage of barley rootlets in food applications may be a cost-effective way to improve the nutritional profile of these products.

Salama A.R.A et al. [14], highlighted the technological properties of barley rootlets. To determine a suitable application for an ingredient, knowledge of the functional properties is needed. Barley rootlets had favourable outcomes in comparison to the acrospires, husks and malt sprouts (mixture of rootlets, husk and acrospires). The study revealed that rootlets had the highest water and oil absorption capacities, as well as the highest emulsification capacity [14]. The barley rootlets had the lowest foaming capacity, but the greatest foam stability in comparison to the husk, acrospire and malt sprouts [14].

Barley rootlets have been incorporated into bread, biscuit, and butcher sausage formulations. Waters et al. [7], examined the effect of substituting milled barley rootlets and fermented milled barley rootlets in wheat bread formulations at 5, 10, 15 and 20% addition. Barley rootlets were made into a flour using a mill feeder and a laboratory mill. The rootlets were fermented by *Lactobacillus plantarum* FST 1.7 to prepare a rootlet sourdough. Waters et al. [7] postulated that the replacement of wheat flour with rootlets in bread formulations could improve the nutritional properties of the bread by enhancing the amino acid profile, increasing fibre levels, reducing saturated fat content, and lowering sodium intake coming from the flour. Increased addition of fermented and unfermented rootlets to bread formulations generally decreased the bread volume, increased hardness, and produced a darker-coloured bread. However, at 5% addition of the fermented rootlets, specific volume and hardness of the substituted breads were not statistically different from the wheat bakers flour control. Rootlet and unfermented rootlet breads with up to 5% inclusion were preferred by the sensory panel, however up to 10% was accepted. Inclusion of rootlets at 10% would likely enhance the overall protein and fibre contents in the bread. Increased intake of fibre improves human health, due to the health promoting benefits associated [64]. Many people around the world do not take in sufficient fibre in the

diet, and increasing fibre in a staple food product such as wheat bread may improve the overall fibre intake for a human. With regards to effects on protein, there is potential for improvements in protein quality in wheat bread with rootlet substitution. Rootlet inclusion could create a better balance in the amino acid profile in wheat bread, as seen with the substitution of other plant based ingredients [39], however further analysis would be needed to confirm this. Inclusion of barley rootlets/fermented rootlets in bread appears to be an option up to 10% addition, and the use of fermented rootlets at a low level of inclusion could enhance bread characteristics [7]. Chiş et al. [59] studied the addition of barley rootlets up to 25% inclusion in biscuit formulations, with emphasis on the volatiles of the rootlets which may affect flavour perception. Increasing levels of barley rootlet addition caused a darker colour to occur as well as increasing intensities of odour, flavour, and taste. Panellists found an intense 'whiskey' or 'alcohol' note with barley rootlet addition, as well as citrus, pine, and mint notes [59]. The study outlined that inclusion of barley rootlets in biscuit formulations up to 15% was acceptable, because over this value an unpleasant aldehydic taste was perceived [59]. The results obtained from these studies were similar to those observed by Salama A.A. et al. [65] with the incorporation of rootlets into bread and biscuits. Salama A.A et al. [65] reported enhancements to the nutritional profile of the bread at 5% addition of rootlets (approx. 1% increase in fibre and approx. 1% increase in protein contents). Additionally, barley rootlets were examined for their usage in sausage formulations as an extender/binder [65]. Successes were observed organoleptically with the inclusion of barley rootlets up to 10%. Incorporation of barley rootlets decreased cooking losses and the authors postulated a reduction in production costs with rootlet inclusion. In addition, the fibre content of the sausages was enhanced (1.18–3.25% Dry Basis (D.B)); however, there was a marked decrease in the relative protein content (65.9–61.7% D.B) and moisture levels (65.2–63.7% D.M) [65]. The reduction in protein content may be associated more to the decrease in the amount of meat used in the formulation, rather than the effects of the rootlet inclusion.

Overall, the incorporation of barley rootlets into food applications up to a certain level has had promising outcomes. Their inclusion in food may have a maximum point, because higher levels of inclusion resulted in various off-flavours in bread and biscuits [7,59]. Although food studies are limited, the analysis available shows that barley rootlets have potential as a fibre fortifier and could possibly improve the protein profiles of foods and reduce production costs when used as extenders in sausage formulations, while also having potential as a fermented ingredient. This may encourage their usage in other food applications in the future. Additionally, the use of rootlets as a food ingredient may encourage industry evolution. Brewing and malting industries could shift focus to creating food ingredients from rootlets rather than producing them as an inherent by-product.

5.3. Enzyme Applications

Rootlets of pale malts are particularly rich in enzymes due to the reduced heat exposure during kilning [6]. Evidence from literature suggests that rootlets contain a variety of different enzymes, some of which include: invertase, superoxide dismutase, nucleases (RNase and DNase), 5′-phosphodiesterase, phosphotransferase and phosphomonoesterase [66–70]. 5′-phosphodiesterase (5′PDE) has been the predominant enzyme isolated and utilised in applications from barley rootlets. It has been found in appreciable amounts in the barley rootlets and in the malted barley grain [71,72]. The enzyme has been used commercially to hydrolyse RNA to make 5′-nucleotides. These 5′-nucleotides can be utilised as flavour enhancers (5′GMP and 5′IMP specifically) that have an umami-like taste, and are also used in the production of pharmaceuticals [73–75]. After the discovery of the flavour nucleotides (5′GMP and 5′IMP) and the synergistic flavour effects with monosodium-L-glutamate (MSG) [76], the flavour nucleotides have been produced as seasonings (mixed with MSG) for use in savoury foods such as soups and broths [77].

Processes for the extraction of 5′PDE from barley rootlets vary, with some patents (Table 4) also developed. The process generally requires a purification step, because various other undesired enzymes

such as phosphatases, 5′-nucleotidase and nucleosidases may also be present in barley rootlets [71,78] that may produce unwanted products or inhibit 5′-nucleotide yield.

Hua and Huang [78] isolated 5′PDE from barley rootlets to form 5′-nucleotides using water extraction, gel filtration and freeze drying. Various parameters were found to affect the extraction rate of 5′PDE from barley rootlets. These included barley rootlet size, pH, temperature, volume of the extraction solvent, and extraction time. The optimum conditions for extraction of 5′PDE were pH 7, 20 °C and 7 h. The optimum solvent (water) and rootlet ratio for extraction was 16:1, along with a rootlet size larger than 120 mesh size. Hua and Huang [78] isolated two types of 5′PDE enzyme (termed 5′PDE (a) and 5′PDE (b)) of different optimum temperatures and pH (70 and 65 °C, 5 and 6, respectively). The study found that the 5′PDE enzymes showed excellent stability to heat (70 °C) over time (420 min). The purified enzymes contained fewer peaks vs the raw enzyme extract upon HPLC analysis. This indicates that the authors' purification step was successful in removing some of the undesired enzymes which could affect 5′nucleotide yield, however it was acknowledged that not all may have been removed [78]. Like Hua and Huang, Beluhan et al. [9] also used a purification step. The purification step involved thermal treatment and acetone precipitation, with the intention to reduce levels of phosphomonoesterase (PME). Beluhan et al. [9] found at least two 5′PDE isoenzymes which also differed in their optimum temperatures (55 and 70 °C).The variances in optimum temperature of the 5′PDE found between Hua and Huang [78] and Beluhan et al. [9] could potentially be linked to differences in extraction and purification methods, however this could also be linked with possible co-extraction of PME. Later studies by Beluhan et al. [79] reported an optimum temperature of 55 °C for PME and 70 °C for 5′PDE. Additionally, Belhuhan et al. [79] found that a thermal treatment step could be a key factor in the purification of 5′PDE enzyme preparations; PME activity was significantly reduced after heat treatment. Beluhan et al. [9] highlighted the excellent storage stability of the 5′PDE in barley rootlets, with enzyme activity remaining almost constant for 90 days when stored at −18 °C. Hua and Huang, Beluhan et al. [79] and Beluhan et al. [9] were all in agreement with the excellent thermostability exhibited by 5′PDE preparations. Benaiges et al. [80,81] used a two-step purification process which included an acetone purification step and DEAE-Sepha-rose chromatography for the isolation of 5′PDE from barley rootlets. This process was also successful in producing 5′-nucleotides. Laufer and Gutcho [71] found green malt rootlets, after oat rootlets, were the most effective in converting RNA to 5′-nucleotides in comparison to the rootlets, stems and kernels of other cereals and legumes tested (rye, oat, soy beans, mung beans, wheat, rice). Green malt rootlets are likely to perform better with regards to enzymatic activity rather than kilned rootlets, due to the reduced exposure to heat and risk of denatured enzymes. Further investigations were carried out into commercially available malt sprouts, which contain rootlets, to explore the 5′PDE activity in these. Laufer and Gutcho [71] found the addition of low levels of Zn^{2+} before a heat treatment of 72 °C for 5 min on washed malt sprouts was the best method for large scale production of 5′-nucleotides from RNA. Such observations are slightly conflicting with reports from Beluhan et al. [9,79], who noted an increase in 5′PDE activity with Mg^{2+} and slight to moderate inhibition with metal ions (Zn^{2+}). Laufer and Gutcho also showed that the heat applied to malt rootlets reduced the microbial load, which reduced the potential of microbial enzymes to participate in the RNA hydrolysis which may inhibit 5′-nucleotide production. A method to separate the flavour enhancing 5′nucleotides (5′GMP and 5′IMP) from the products of the RNA hydrolysis was also reported [71]. Sombutyanuchit et al. [82] used barley rootlets as a source of 5′PDE to produce 5′nucleotides from brewers' yeast with specific emphasis on the production of the flavour nucleotide, 5′GMP. The study concluded that significant levels of 5′GMP could be produced from a heat-treated extract (65 °C for 30 min or 70 °C for 7 min) containing 5′PDE sourced from barley rootlets and hydrolysed for 8–14 h. However, levels obtained for 5′GMP were 50% lower than commercial nucleotide extracts; the author related this more to the RNA source rather than a reduced activity of the enzyme [82]. Sombutyanuchit et al. [82] outlined the commercial nucleotide extracts were prepared using specially selected "high RNA" baker's yeast (*S. cerevisae*) as the RNA source, which under standard autolysed procedure baker's yeast extracts have higher levels of guanine and 5′GMP.

The author also highlighted that RNA levels are closely related to specific growth rate of the yeast, and brewers' yeast (*S. uvarum*) grows at a slower rate under low temperatures in lager production thus a lower level of RNA will be present [82]. Thus, the reduction in RNA present during autolysis, which can often be linked with its source, may be a contributing factor to increasing or inhibiting the overall yield of 5'GMP.

Overall, barley rootlets prove to be a viable source of 5'PDE and can be used to produce 5'-nucleotides. This may be of interest for industry. The process requires purification steps and pre-treatments such as heat to maximise the 5'-nucleotide output and eliminate unwanted enzymes. Compared with other sources such as snake venom [83,84] and bovine intestine [85], rootlets could be more economical for use by industry, however further investigation into the cost effectiveness of using rootlets as an enzyme source for 5'PDE would need to be examined to confirm this.

5.4. Antioxidant Source

Antioxidants are one of the main ingredients used to protect the quality of a food by preventing the oxidation of lipids which is deleterious for food quality [86]. They are also utilised in the cosmetic industry. In addition, antioxidants play an important role in the human diet and have a positive effect in controlling various diseases [87]. Antioxidants can be from natural or synthetic sources, with natural sources being more appealing to the consumer. Barley rootlets are potentially a plentiful source of natural antioxidants which may be utilised in food and cosmetics. Various levels of antioxidant compounds have been reported [7,10,14,88]. Variations in the levels reported may be linked to barley variety and malting practices. The term "antioxidant activity" and "antioxidant power" are used throughout this section. The terms appear to be interchangeable, however "antioxidant activity" is the more commonly used term in relation to the properties of an antioxidant when describing a compound's capability to reduce or inhibit the process of oxidation [89].

Bonnely et al. [90] investigated three different extracts from barley rootlets which contained rootlet oil, free phenolic compounds and bound phenolic compounds (bound to lignin and arabinoxylans). The rootlet oil had a low level of tocopherols (α-tocopherol and γ-tocopherols) with little antioxidant activity. "Tocopherols" may also be referred to as vitamin E. Vitamin E has four tocopherol isomers existing in nature, namely α-tocopherol, β-tocopherol, γ-tocopherol and δ-tocopherol [91]. The bound phenolic extract (containing compounds such as trans-ferulic acid, cis-ferulic acid, cis-p-coumaric acid, trans-p-coumaric and hydroxycinnamic acid) had an appreciable antioxidant power but dry matter yield was low (2%). Bonnely et al. [90] deemed that these extracts were of little interest commercially due to the low quantity present versus the cost and complexity of the extraction. However, the extraction of free antioxidant compounds (containing 52% proteins, 33% sugars and 5.5% reducing compounds) from barley malt rootlets was promising, due to the antioxidant properties and yield associated with it. The free antioxidant extract was also found to have a synergistic effect with α-tocopherol in relation to antioxidant power. No loss in antioxidant activity was noted when the free antioxidant extract from barley rootlets was used to substitute part of an α-tocopherol mixture when compared with α-tocopherol alone [90]. Peyrat-Maillard et al. [92] produced two extracts, "a free rootlets extract" and "bound rootlets extract", and investigated the effect of vitamin E and vitamin C (also referred to as ascorbic acid) on antioxidant power. This was done to determine the antagonistic or synergistic effects of vitamin C and vitamin E on rootlet extract antioxidant power. The free rootlets extract was comprised of the free oxidoreduction agents, while the bound rootlets extract contained phenolic compounds which were previously attached to arabinoxylans and lignin. The study revealed the main phenolic compounds present were trans-p-coumaric acid and trans-ferulic acid, with a higher concentration of these found in the bound rootlets extract than the free rootlets extract. Like Bonnely et al. [90], Maillard et al. [92] found a positive synergistic effect with the malt rootlet extracts and α-tocopherol in relation to antioxidant power, however a negative effect was noted with regards to the malt rootlet extracts and ascorbic acid [92]. The authors outlined that ascorbic acid was a more efficient antioxidant than the rootlets extract, and the presence of the rootlet extracts, both bound and free, hindered the

antioxidant power of vitamin C. The authors also highlighted that further investigation would be needed to explain this. The synergistic effect observed with the bound rootlets extracts and α-tocopherol was not linear, implying that higher doses did not further enhance the synergistic effects, but was linear with the free rootlets extract. The synergistic effects noted was suggested to be due to two mechanisms: (1) the malt rootlets extracts preventing the oxidation of α-tocopherol; or (2) the malt extracts aiding in the regeneration of α-tocopherol in its radical form [92]. An optimised method for extraction of the antioxidant compounds in barley malt rootlets has also been investigated, using a statistical model known as response surface methodology (RSM) [93]. Three independent variables were found to affect antioxidant activity of barley rootlets, which included solvent composition (% v/w), temperature of extraction (°C) and extraction time (min). Meng et al. [93] found the extraction parameters for optimal total antioxidant activity were: 50% ethanol (v/w) solvent and an extraction temperature of 84 °C for 22 min. The predicted antioxidant activity value was 9.49 µmol TE, which was agreeable with the experimental value of 9.79 µmol TE [93]. Cheng et al. [94] determined the optimum conditions for the extraction of the alkali soluble components of barley malt roots (the term used in the study when referring to the malting by-product, likely implying rootlets) and determined its composition. The study concluded that 60 min, 40 °C, pH 9 and a solvent to raw material ratio of 25:1 were the optimal conditions necessary for the highest extraction yield of 29.2%. The composition of the extract was 33.72% protein, 0.1% polyphenols and 0.33% flavonoids [94]. Studies conducted on the barley rootlet proteome by Mahalingam [8], provide evidence for the presence of various antioxidant compounds in barley rootlets. Such compounds include ascorbic acid and glutathione. Analysis of the phenylpropanoid pathway enzymes during the study suggested that barley malt rootlets may also be a source of coumarin, cinnamaldehyde, sinapic acid and sinapyl alcohol [8]. The presence of such compounds present may be of interest in the future, and maypotentially be used in food and pharmaceutical applications [95–97].

The effect of pre-treatments on the antioxidant compounds in barley rootlets has also been analysed. Pre-treatments of the barley rootlets, such as steaming, roasting, autoclaving, microwaving and enzyme treatment, has been investigated in relation to their effect on the antioxidant potential of the phenolic extracts from barley rootlets [10]. Budaraju et al. [10] investigated the effect of these pre-treatments on the free phenolic compound extracts and bound phenolic extracts. The use of pre-treatments generally enhanced the extraction yield and antioxidant activity of the extracts, in comparison to the untreated samples [10]. Autoclaving had the greatest effect on total extraction yield and increasing total phenolic content of the extracts. The increase in total phenolic content was due to the enhancements observed in the free phenolic extract rather than the bound phenolic extract. Dudjak et al. [88] observed an increase in polyphenol content in barley roots upon treatment of the growing barley plant with cadmium in the growth medium. A 10.3% increase in polyphenol content was observed in the barley roots upon the addition of cadmium. However, such treatment had a greater impact on enhancing the polyphenol content in barley shoots (+16.7%) and barley leaf blades (+35.2%) than the barley roots. Treatment with cadmium could be applicable to barley rootlets for increased polyphenol content, however further investigation must be carried out to confirm this.

The studies outlined above indicate that barley rootlets are a potential source of natural antioxidants. They indicate more encouraging results for the potential of free phenolic extracts rather than the bound phenolic extracts. Thus, rootlets may be an abundant source of naturally occurring antioxidants which could be capitalised in the food and/or related industries and may contribute towards clean labelling of products.

5.5. Growth Medium for Fermentation

Barley rootlets can support the growth of micro-organisms, which makes it a potential substrate for microbial cultivation and fermentations. They have been employed as a substrate for lactic acid production as well as a growth and storage medium for lactic acid bacteria. Lactic acid bacteria have a prominent role in food and biotechnology industries as starter cultures for food production and

as probiotic production. Lactic acid is a product of the lactic acid bacteria fermentation and is in the second tier of the 12 most promising value-added building blocks utilised in the production of numerous useful and specialty chemicals [98]. Incorporation of by-products streams as substrates in microbial fermentations to replace costly raw materials and reduce production costs has become increasingly desirable. Barley rootlets have a very low cost associated with them and are produced in high volumes each year, which makes them an attractive substrate for utilisation in these applications. It is important to note, some studies throughout this section do not specify the grain source from which the rootlets originate. However, it is fair to assume the studies that do not specify the grain source could originate from barley and have been included in this review. Malt sprouts is the more common term used in this section, because studies used such terminology.

Cejas et al. [99] investigated the use of barley malt sprouts and barley malt sprouts supplemented with 20% *w/v* fructo-oligosaccharides (MS FOS) as a substrate for the growth of two lactobacillus species, namely, *Lactobacillus salivarius* and *Lactobacillus plantarum*. The results from the malt sprout media in relation to microbe lag times, change in pH and acidification rates were comparable to the MRS control. Twenty percent FOS addition enhanced the growth of *Lactobacillus salivarius* even more than the traditional MRS medium. The authors also found no loss in the culturability of the bacteria stored in malt sprout media after freeze drying and 60 days storage at 4 °C. This was attributed to the FOS which was present which likely had a protective role [99]. Laitila et al. [100] produced a malt sprout medium using a malt sprout extract instead of water in the preparation of the medium for growth of a *Lactobacillus plantarum* strain. The malt sprout extract was a liquid extract which consisted of malt sprouts that had been soaked in water, autoclaved, centrifuged and filtered. This extract was also supplemented with glucose and yeast extract. MRS agar was used as a control for the experiment. Results obtained indicated that the malt sprout extract medium supported the growth of the strain and could replace the MRS medium without affecting the cell count or the strains antimicrobial activity. The cost of the malt sprout extract medium was estimated at 20% of the cost for the MRS medium, which would considerably reduce production costs [100].

Radosavljević et al. [101] used malt rootlets as a carrier for the immobilisation of *Lactobacillus rhamnosus* and found high cell viability during batch fermentations with the immobilised cells as well as a lactic acid yield of 93.3%. A brewery by-products mixture (brewers' spent grain and malt rootlets hydrolysate, brewers' yeast, soy lecithin) was utilised as the substrate for the fermentation. Continuing from this study, Radosavljević et al. [102] determined the optimum levels of brewers' yeast and soy lecithin necessary for optimised lactic acid production with the brewers' spent grain and malt rootlets hydrolysate as the substrate. Both studies highlight the suitability of malt rootlets for utilisation in the growth of lactic acid bacteria and lactic acid production.

Investigation into barley rootlets as nitrogen source replacements in substrates for fermentation and bio stimulants has been conducted, with some patents available in this area (Table 4). Liu et al. [103] used malt sprouts as a nitrogen source during lactic acid fermentation and concluded that the malt sprouts could be used as an alternative nitrogen source at a concentration of 16 g/L in conjunction with corn steep liquor at a concentration of 12 g/L in the growth medium. Similar results were obtained by Hujanen and Linko [104], who also found that a barley malt sprout extract was capable of replacing most of the expensive nitrogen source for the fermentation without compromising on the level of lactic acid produced. Results from Göksungur and Güvenç [105] correlated with these findings; their study showed that malt sprouts were the most suitable alternative nitrogen sources after yeast extract.

Production of a bio-based concentrate from barley malt rootlets for utilisation as a stimulant in biotechnological processes for the vinegar industry at pilot scale has also been investigated [46]. A dark brown, viscous concentrate was produced, containing 51.3% dry matter (d.m.), 5.29% (d.m.) protein, 2.38% (d.m.) ash, 17.15% (d.m.) carbohydrates and 0.96% (d.m.) starch. The optimal conditions to produce this extract was 60 °C for 60 min using water as the extraction solvent, in a solvent to rootlet ratio of 8:1 [46]. The extract produced was intended to be used in the vinegar industry to increase

the substrate concentration for the yeast fermentation that produces the ethanol. This also highlights another pathway for the potential of barley rootlets in the future.

Results from the studies employing barley rootlets as substrates in fermentations and lactic acid production are encouraging. The utilisation of a low-cost material in fermentations such as barley rootlets can reduce production costs [100], and from an economical point of view, this may provoke further investigation of barley rootlets for use in this application and in others.

5.6. Biochar Production

In more recent years, rootlets originating from the malting process, also called malt spent rootlets, have been used in biochar production. Biochar is produced by heating organic matter under oxygen limiting conditions and relatively low temperatures [106]. It may be utilised as an energy source, as an addition to soils for its fertiliser and carbon sequestrant properties, and as an absorbing agent in a range of applications [107]. Examination of rootlet biochar using microscopy techniques indicate that rootlets maintain their shape post biochar production and contain mineral deposits covering the external surface of the rootlets [108]. Various studies employ malt spent rootlet biochar in their investigations, however this review highlights the use of rootlet biochar as a possible sorbent material and as a catalyst in the biodiesel production process. Although the majority of the studies discussed in this section do not state the exact grain the malt rootlets come from post-malting (similar to Section 5.5), it is likely that some of the rootlets used for biochar production originated from the barley grain, hence their inclusion.

Rootlet biochar has been investigated as a potential sorbent for various types of water pollutants such as uranium, chlorine, chloroform, chromium, and methylene blue, with encouraging results observed [109–114]. Grilla et al. [108] used rootlet biochar as a platform to generate sulphate and hydroxyl radicals as well as an electron transfer mediator while exploring advanced oxidation processes to reduce the presence of trimethropin in water matrices. Rootlet biochar has also been used to activate sodium persulphate, which is needed in the oxidation and removal of sulfamethoxazole, an antibiotic microcontaminant which can be present in water supplies [115]. Manariotis et al. [116] and Valili et al. [117] found malt spent rootlet biochar had excellent sorption capacities for phenanthrene and mercury. Additionally, increases in the sorption capacity of rootlet biochar for phenanthrene and mercury was noted with varying pyrolysis temperatures [116,117]. Boutsika et al. [118], Anagnostopoulos et al. [119], and Boutsika et al. [120] also found promising results in relation to the sorption capacity of mercury from aquatic solutions using malt spent rootlets biochar. These studies showed that a range of factors are involved in optimising sorption capacities of the biochar produced from rootlets. However, its use as a sorbent material in aquatic solutions or water treatment applications must also be monitored. Investigation into the toxicological effect of leachate from rootlet biochar by Tsouloufa et al. [121] revealed that washing of the biochar made from malt spent rootlets is a crucial step in the process to avoid any adverse effects. The use of rootlet biochar as a catalyst in the transesterification reaction in biodiesel production has also been successful [122]. Ntaflou and Vakros [123] found that pre-treatments with NaOH of malt spent rootlet biochar enhanced transesterification activity of the biochar, showing activity similar to that of a homogenous catalyst, by increasing the basicity of the biochar. Similarly, Tsavatopoulou et al. [124] also had success in using rootlet biochar as the catalyst during transesterification, with the untreated biochar giving better conversion rates than H_2SO_4-treated biochar.

Malt rootlet biochar appears to have a promising future. The high sorption capacity of the biochar for pollutants highlights it as a potential option as a sorbent material, which may be useful in water treatment regimes. However, the potential leachate from the rootlet biochar in aquatic environments is something which must be monitored, and strict monitoring of this should be considered. Additionally, the use of rootlet biochar in biodiesel production as a heterogenous catalyst in the transesterification process may enhance the sustainability of the process. Heterogenous catalysts are viewed as more environmentally friendly catalysts because they can be easily separated and potentially reused in the process [125].

Table 4. Patents which utilise barley rootlets.

Barley Rootlet Patents			
Google Patent Number	**Title**	**Area of Usage**	**Summary**
US20070148317A1 [126]	Functional component-enriched barley malt rootlets and process for producing same	Food/cosmetic/medicinal ingredient	Process for the extraction of functional components from rootlets of barley which can be utilised as a raw material in food, cosmetic and medicinal formulations
US9326542B2 [127]	Process for producing food and beverage products from malt sprouts	Food and beverage ingredient	Technology for utilising malt sprouts of a specific particle size as a raw material in food or beverages
US5034325A [128]	5′Phosphodiesterase enzyme preparation and method for its production	Enzyme preparation	An extraction method to obtain 5′phosphodiesterase from barley malt sprouts which is stable in storage
US3304238A [129]	Enzymatic material and method of preparing same	Enzyme preparation	Preparation of an aqueous enzyme medium from barley (and other grains) rootlets and stems capable of producing mainly 5′nucleotides
US3459637A [130]	Enzyme digestion of nucleic acids	Enzymatic production of 5′nucleotides	Method for enzymatically digesting RNA to primarily form 5′nucleotides using the aqueous extract of plant rootlets and stems (including barley)
US2925345A [131]	Preparation of an antioxidant from rootlets	Antioxidant extract	Method to limit auto-oxidation in a fatty material which involves the mixing of pulverised rootlets with the fatty material
US2694011A [132]	Poultry and swine feeds containing rootlets of germinated barley	Animal feed	Utilisation of barley rootlets within animal feeds for poultry and swine
US4613507A [133]	Malt-like flavour from cereal grain root cultures	Food and beverage flavour ingredient	Method of creating malt-like flavour ingredient from roots of grains (including barley) which can be used in food and beverage formulations
WO2019238928A1 [134]	Process for preparing a cereal based beverage with malt and malt rootlets	Beverage ingredient	Utilisation of barley rootlets in wort to obtain a malt-based beverage
US20200178580A1 [135]	Malt sprouts extracts and their uses	Extract	Use of malt sprouts as raw materials in extract production for various uses
WO2018104531A1 [136]	Compositions and methods for stimulating plant growth	Extract	Incorporation of malt sprouts in extract preparation and use as a bio stimulant

6. Conclusions

Increased focus has been placed on the recycling of food processing by-products, such as barley rootlets, for applications in food and other industries, to enhance sustainability. Barley rootlets are produced in large volumes each year as a by-product of the malting, brewing and distilling industry, but their primary use to date has been as animal feed. However, there is evidence to suggest a promising potential for barley rootlets to be used in food products and fermentations due to their nutritive value, but also as sources of enzymes, antioxidants and in biochar production.

The use of barley rootlets as a nutrient-rich food ingredient may be of great interest in the future, with its high fibre content and interesting protein quality. Although studies are limited, successes have mainly been observed in their ability to enhance the nutritive value of cereal-based products, but increased attention and knowledge of their potential may provoke more investigations into their use in other food products. However, a key element which needs to be considered and addressed is the quality and safety of the barley rootlets, because evidence suggests that mycotoxins are prevalent. A system to regulate the quality of rootlets will be necessary to monitor this and ensure that a safe food ingredient is produced. A need for this has been stressed previously in relation to the consumption of rootlets and other brewing by-products in animals [11]. Thermal food processing and controlled storage conditions of the barley rootlets may be an option to counteract this challenge.

Nevertheless, barley rootlets have untapped potential, and their applications may not be limited to those considered in this review. Increased awareness of their potential may spark future investigations into barley rootlets and open new pathways for their exploitation.

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