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Raw Material Influence on the Formation of Light Induced Off-Flavors in Cereal Based Beverages

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Caminante, son tus huellas
el camino y nada más;
Caminante, no hay camino,
se hace camino al andar.

Antonio Machado

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It is common to conclude a thesis with an acknowledgement letter. Just like a book's prologue, it is something that is written at the end and placed at the beginning (and some say that neither the introduction nor conclusion are read). In this case however, gratitude is something that I've tried to show (or express) throughout the realization process of this project...., serve this letter simply as a ratification clause.

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Content

Summary

Flavor stability has always been of particular interest in the brewing industry. Besides the well-known beer oxidation process caused by oxygen, the off-flavor known as “sunstruck flavor” may occur if beer is exposed to light without protection. Brown bottles and cans prevent the formation of the sunstruck flavor; however, a prestigious poll states that people prefer to drink beer filled in white glass bottles. Compositional ways to prevent the formation of sunstruck flavor, for example, reduced isomerized hop extracts, are available on the market. However, the growing trend towards natural products (clean label), the higher costs of protected bottles and reduced isomerized products as well as brewing in accordance to the German Purity Law motivate the search for other natural products.

Cereal-based beverages contain substances capable of preventing the formation of sunstruck flavor. Polyphenols, such as catechin, epicatechin, and quercitin as well as the amino acid tryptophan have been described in the literature as sunstruck flavor inhibitors, thus preventing its formation. However, this type of beverage also contains substances capable of promoting the formation of sunstruck flavor, sunstruck flavor initiators which are riboflavin, cysteine, methionine, phenylalanine and glutathione. Therefore, albumin and glutenin fractions, probable sources of those amino acids, are also considered to promote the formation of sunstruck flavor.

Although many ways of preventing of sunstruck flavor have been described, none of these deals with the idea of decreasing or increasing the levels of sunstruck flavor initiators or inhibitors respectively. Whereas the cereal used in the brewing process serves as a source of all sunstruck flavor initiators and inhibitors, the hop product only provides polyphenols. Profound knowledge about the content of initiators and inhibitors in different raw materials, cereals and malts other than barley as well as hop products, is needed to specify the possible natural ways of reducing sunstruck flavor in cereal-based beverages. Additionally, the impact of the malting and brewing process on the content of initiators and inhibitors is important in order to determine the potential of the raw materials employed.

The content of sunstruck flavor initiators and inhibitors as well as the changes during the brewing process are analyzed stepwise and in detail in this work in order to establish the necessary background knowledge to reduce the formation of sunstruck

flavor in cereal-based beverages. First different commercially available hop products were analyzed for their single and total polyphenol content, alpha-acid content as well as their potential of developing sunstruck flavor in beer. The results revealed an enrichment or depletion of polyphenols and bittering acids during the hop product production. The lowest sunstruck flavor perception in beer was found employing either tannin extract or spent hop 45.

Complementary to the analysis of hop products, the content of sunstruck flavor initiators and inhibitors during the malting process in different cereals (spelt and triticale) was investigated. An increase of the content of initiators cysteine, methionine, phenylalanine and those peptides resulting from the degradation of the proteins during malting was confirmed, having, therefore, a higher potential of develop sunstruck flavor. Also, the majority of the proteins found in the albumin fraction increased, and the proteins in the glutenin fraction decreased during malting. Finally, the influence of different malting parameters on the content of sunstruck flavor initiators and inhibitors was analyzed. Although the lowest content of sunstruck flavor initiators was found under lower germination temperatures, shorter germination times and lower degrees of steeping (under modified malts) concurrently, the content of sunstruck flavor inhibitors is also low under those conditions. For this reason, it can be concluded that in order to reduce the formation of sunstruck flavor a compromise should be found using under-modified malt (with low content of initiators and inhibitors) together with a rich polyphenol hop product (with no humulone content).

This work evaluates the potential of different raw materials, malting and brewing process on modifying the content of sunstruck flavor initiators and inhibitors. Furthermore, the results achieved by this work provide a valuable basis for the design of light stable cereal-based beverages.

Zusammenfassung

Die Aromastabilität des Bieres ist von jeher von besonderem Interesse in der Brauindustrie. Neben dem wohlbekannten, durch Sauerstoff verursachten, Oxidationsprozess im Bier kann das Fehlaroma Lichtgeschmack auftreten, falls Bier ungeschützt dem Licht ausgesetzt wird. Braunglasflaschen und Dosen verhindern die Entstehung von Lichtgeschmack. Indes ergaben Repräsentativ-Umfragen, dass die Befragten es bevorzugen, Bier aus Weißglasflaschen zu trinken. Eine weitere Möglichkeit zur Vermeidung der Bildung von Lichtgeschmack im Bier bietet die Veränderung der Inhaltsstoffzusammensetzung. Zum Beispiel sind Reduzierte Iso-α-Säure-Extrakte auf dem Markt erhältlich. Allerdings bestärken der steigende Trend zur Verwendung von natürlichen Produkten (clean label), die höheren Preise für UV-geschützte Flaschen und reduzierende isomerisierte Produkte sowie das Brauen gemäß dem deutschen Reinheitsgebots die Suche nach natürlichen Alternativen.

Getreide basierende Getränke enthalten Substanzen, die die Fähigkeit besitzen, der Bildung von Lichtgeschmack vorzubeugen. Die Polyphenole Catechin, Epicatechin, und Quercitin sowie die Aminosäure Tryptophan werden in der Literatur als Lichtgeschmackinhibitoren beschrieben. Jedoch enthalten diese Getränke auch Inhaltsstoffe, die die Bildung von Lichtgeschmack fördern können. Diese Substanzen sind Riboflavin, Cystein, Methionin, Phenylalanin und Glutathion. Da die Albumin- und Glutenin-Fraktionen die mutmaßlichen Quellen dieser Aminosäuren darstellen werden sie ebenso als Lichtgeschmack fördernd beschrieben.

Obwohl viele verschiedene Ansätze zur Vermeidung von Lichtgeschmack beschrieben sind, beschäftigt sich keiner mit der Verminderung der Initiatoren beziehungsweise der Erhöhung der Inhibitoren. Während Getreide alle bekannten Lichtgeschmackinitiatoren und –inhibitoren in den Brauprozess einbringen, beinhalten die eingesetzten Hopfenprodukten einzig Polyphenole.

Es wird ein umfassendes Wissen über den Gehalt an Initiatoren und Inhibitoren in Getreiden, Malzen und Hopfenprodukten benötigt. Damit können die möglichen natürlichen Wege zur Verminderung von Lichtgeschmack in Getränken auf Getreidebasis aufgedeckt werden. Des Weiteren ist es wichtig den Einfluss des Mälzungs- und Brauprozesses auf den Gehalt an Initiatoren und Inhibitoren zu betrachten um das Potential eingesetzter Rohstoffe zu erfassen.

In dieser Arbeit werden die Gehalte an Lichtgeschmackinitiatoren und –inhibitoren in Braurohstoffen sowie deren Veränderungen im Mälzungs- und Brauprozess im Detail analysiert. Dadurch wird das benötigte umfassende Hintergrundwissen zur Verminderung der Lichtgeschmackbildung in Getränken auf Getreidebasis geschaffen. Es wurden verschiedene kommerziell erhältliche Hopfenprodukte auf ihre einzelnen Polyphenole, den Gesamtpolyphenolgehalt, den alpha-Säuren Gehalt sowie deren Potential zur Bildung von Lichtgeschmack in Bier untersucht. Die Ergebnisse zeigten auf, dass im Herstellungsprozess unterschiedlicher Hopfenprodukte eine An- bzw. Abreicherung von Bittersäuren und Polyphenolen stattfindet. Die geringste sensorische Lichtgeschmackwahrnehmung im Bier wurde bei dem Einsatz von entweder Tannin Extrakt oder Hopfentreber 45 erzielt.

In Ergänzung zur Untersuchung der Hopfenprodukte wurde der Gehalt an Lichtgeschmackinitiatoren und –inhibitoren während des Mälzungsprozesses der Getreidearten Dinkel und Triticale analysiert. Es konnte bestätigt werden, dass während der Mälzung der Gehalt an den Initiatoren Cystein, Methionin, Phenylalanin und anderer aus dem Proteinabbau resultierender Peptide erhöht wird. Somit steigt das Potential zur Bildung von Lichtgeschmack durch den Mälzungsprozess. Ebenso führt der Mälzungsprozess zu einem Anstieg an Proteinen der Albuminfraktion während der Proteingehalt der Gluteninfraktion abnimmt. Abschließend wurde der Einfluss der unterschiedlichen Mälzungsparameter auf den Gehalt an Lichtgeschmackinitiatoren und –inhibitoren untersucht. Der niedrigste Gehalt an Lichtgeschmackinitiatoren wurde mit niedrigen Keimtemperaturen, kürzeren Keimzeiten und niedrigeren Weichgraden (unterlöstes Malz) erzielt. Dies führte allerdings gleichzeitig zu einem niedrigen Gehalt an Lichtgeschmackinhibitoren. Daraus ergibt sich, dass der Einsatz von unterlöstem Malz in Verbindung mit einem polyphenolreichen Hopfenprodukt (keine Humulone) die Bildung von Lichtgeschmack vermindert.

Die vorgelegte Arbeit zeigt das Potential eingesetzter Rohstoffe, der Mälzung und des Brauprozesses zur Beeinflussung der Gehalte an Lichtgeschmackinitiatoren und –inhibitoren auf. Des Weiteren dient sie als Grundlage zur Entwicklung von lichtstabilen Getränken auf Getreidebasis.

1. Introduction

1.1. Sunstruck Flavor

1.1.1. First Sights

Flavor stability has always been of particular interest in the brewing industry. Besides the well-known beer oxidation process caused by oxygen, the off-flavor known as “sunstruck flavor” may occur if beer is exposed to light without protection. This off-flavor, detected as early as 1875 by Lintner [1], is described as having meaty, sulfury and skunky-foxy notes.

All fermented non-distilled beverages suffer from light-induced development of off-flavors and deterioration of the product [2]. The development of a specific off-flavor during exposure to light has been also described in wine [3, 4] and dairy products [5-7], being described as “skunky”, cooked cabbage and onion-garlic in wine and burnt-feather in dairy products.

1.1.2. 3-Methyl-2-butene-1-thiol as an Aroma Active Compound

Trying to explain the formation process of the sunstruck flavor, Gray *et al.* [8] discovered that cysteine and sulfites increase the tendency toward the development of a "sunstruck" flavor. They determined that the flavor is due to a sort of mercaptan because while mercaptan is scarcely detectable in fresh beer, it develops parallel with the occurrence of the sunstruck flavor [9]. Kuroiwa and Hashimoto [9] and Kuroiwa *et al.* [10] suggested the aroma active compound 3-methyl-2-butene-1-thiol as the key compound responsible for sunstruck flavor. They have shown that photolysis with visible light (up to 500 nm) of solutions containing a mixture of riboflavin, isohumulones, ascorbic acid, and sulfur-containing amino acids produces 3-methylbut-2-ene-1-thiol, the compound believed to be largely responsible for the offending flavor and odor [11].

Not until 1978, 3-methyl-2-butene-1-thiol was identified by Gunst and Verzele using the head space Gas Chromatography analysis and Flame Photometry Detection [12]. They described the photolysis of iso-α-acids in the presence of a thiol donor leading to 3-methyl-2-butenethiol [12]. 3-Methyl-2-buten-1-thiol, with an aroma threshold of 0.2–0.4 ng/L in water [12] and of 4–35 ng/L [13] in beer, is considered to be one of the most important aroma active compounds. Under exposure to sunlight 0.1–1 µg/L of the thiol can be generated [12,14,15]. It should be noted that the light-struck flavor

is developed from iso-α-acids and is clearly detectable (odor and taste) without noticeable degradation of the iso-α-acids due to their low threshold level [16]. A simple calculation shows that, in a beer having a concentration of 20 ppm iso-α-acids, formation of 1 ppt 3-methyl-2-butene-1-thiol corresponds to a yield of only 10^{-5} % [16]. Sakuma [15] proposed that when beer is exposed to sunlight, isohumulones decompose to the 3-methyl-2-butenyl radical, and sulfur-containing amino acids and protein decompose to the SH radical in riboflavin-photosynthesized reactions. Both radicals then combine to form 3-methyl-2-butene-1-thiol [15].

1.1.3. 3-Methyl-2-butene-1-thiol Initiators

The Japanese researchers Kuroiwa *et al.* [10] described the effective wave length as being in the range of about 500 to 350 nm. Although, problematic to the iso-α-acids is their pronounced light sensitivity [17], isohumulones show absorption bands with a maximum of around 250–255 nm (shoulder around 270–280 nm) [18, 19] and are fully transparent at those wavelengths, and riboflavin is the only light-absorbing species [18, 20]. Therefore, photo-reactivity can only occur via the intervention of riboflavin [21]. Moreover, it has been found that the concentration of 3-methyl-2-butene-1-thiol increases linearly with an increase in riboflavin concentration [19, 22].

Riboflavin, present in several hundreds of micrograms per liter beer [23], shows maximum absorption at 375 nm and at 445 nm [24], and under visible-light irradiation, it may be excited to the first excited state and then undergo intersystem crossing, ISC, to a triplet-excited state (3RF*) [25]. According to Muller [26], the light-induced loss of color of riboflavin in solution is due to the photochemical reduction of the riboflavin [22]. The excited riboflavin is able to extract electrons from electron-rich substances such as proteins that may also be in solution [22, 26]. However, while many reviews have focused on riboflavin to the exclusion of other flavins, it is important to note that flavin compounds in beer consist of free riboflavin, flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD) and flavoproteins. Since they all undergo similar degradation by light, it is conceivable that any or all of them may take part in the formation of the sunstruck character [27].

Both sulfur containing amino acids, cysteine [3, 15, 27-29] and methionine [3, 5, 13, 15, 29] were also identified in the formation of the sunstruck flavor. Sulfur-containing amino acids and, by extension, also sulfur-containing polypeptides and proteins are prone to undergo photo-oxidation caused by visible light in the presence of riboflavin

or other flavin derivatives [21] and serve as a sulfur source for the development of the sunstruck flavor, even accelerating its formation.

Kuroiwa *et al.* [10] experimented with beer proteins dialyzed and fractionated according to its solubility and indicated that the donor molecules are of both high and low molecular weight; thus proteins, polypeptides and free amino acids may all participate in the sunstruck reaction to some degree [27]. They also studied the evolution of hydrogen sulfide (a sunstruck flavor indicator) from these fractions in the presence of riboflavin after exposure to sunlight and found that the alcohol-soluble fraction (gliadin) was not responsible for the evolution of hydrogen sulfide, but the water (albumin) and alkali (glutenin) soluble fractions were. Templar stated that 3-methyl-2-butene-1-thiol is formed from H_2S only when the medium is highly reduced [27]. For example, Sakuma *et al.* [15] proved that, when high concentrations (>50 mg/L) of ascorbic acid were present, H_2S in addition to iso-α-acids and riboflavin contribute to 3-methyl-2-butene-1-thiol formation. Therefore, in commercial beers the reduction of H_2S to form the SH radical may be less significant than the decomposition of sulfur-containing amino acids or proteins [27].

Amino acids such as cysteine do not directly absorb visible light, but, clearly, triple-excited flavins may interact with sulfur-containing amino acids (and derivatives) via electron abstraction [30], thus furnishing the corresponding sulfur-centered radicals [21, 28]. Moreover, the photo-reduced stage of riboflavin has no influence on the photochemical evolution of hydrogen sulfide from sulfur-containing compounds [10].

1.1.4. 3-Methyl-2-butene-1-thiol Reaction Pathway

Kuroiwa *et al.* [10] already proposed that the photochemical sensitization of riboflavin was considered indispensable for the formation of 3-methyl-2-butene-1-thiol from isohumulones and from sulfur-containing amino acids or protein [15]. However, Huvaere *et al.* [18] were the first to determine the reaction products formed on the photo-oxidation of isohumulones and described the complete reaction of the formation of 3-methyl-2-butene-1-thiol.

Riboflavin is excited to a singlet electronic energy state, followed by the transition to a triplet energy state, in which it has a strong affinity for electrons and hydrogen ions [19, 22].

Consequently, sensitized irradiation of iso-α-acids induces an electron transfer from the ionized tricarbonyl group of isohumulones to the riboflavin triplet energy state leading to the formation of reactive triacylmethyl radicals [19]. Stabilization pathways for these species may include inter- or intra-molecular hydrogen abstraction [31]. The activation of the iso-α-acids with UV light leads to bond cleavage by α Norrish Type I reaction, in turn leading to a ketyl-acyl radical pair [32]. Here, the side-chain of isohumulones at carbon 4 of its five-membered ring, namely the 4-methyl-3-pentenoyl group, is first produced photochemically, and then photo-degraded to a minute amount of 3-methyl-2-butenyl radical [9], the main precursor of 3-methyl-2-butene-1-thiol in the presence of a suitable sulfur source [19, 33]. Thiol-containing substrates such as sulfur-rich proteins derived from barley (*Hordeum vulgare* L.) malt or low molecular-weight sulfur-containing compounds, including cysteine, originally present in the cereal-based beverage and in the malted barley [4, 34], lead to the formation of sulfhydryl radicals [19, 28]. Figure 1.1 shows the formation pathway of 3-methyl-2-butene-1-thiol due to the photo-oxidation of isohumulones by riboflavin.

The three iso-α-acid analogues have been described to have different UV absorption profiles, and hence extinction coefficients; these differences are reported to be minimal at 270 nm [35-38]. However, trans-isohumulones have been reported to be less stable than cis-isohumulones [39, 40]. It has been demonstrated that trans-isohumulones are readily degraded during beer-aging by oxidative and photochemical processes, while cis-isohumulones seem to better resist oxidative degradation [32, 40-42].

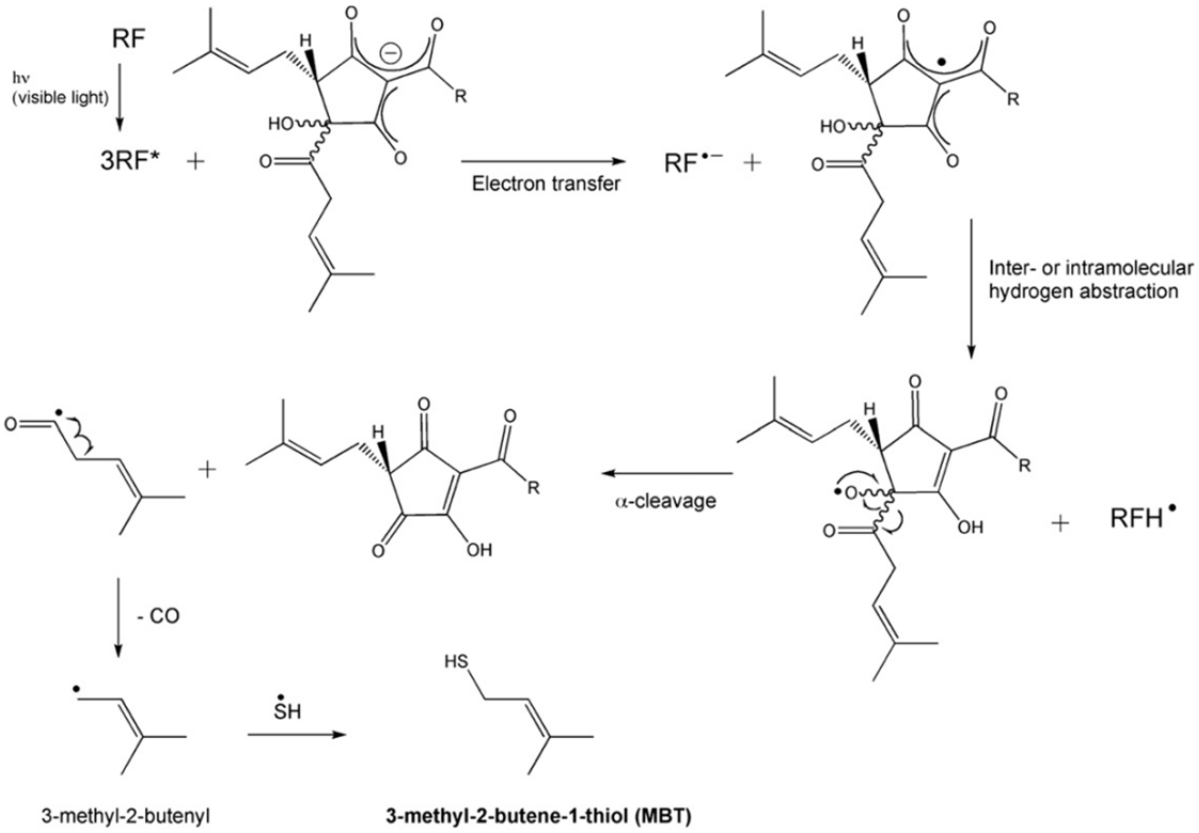

Figure 1.1: Photo-oxidation of isohumulones by riboflavin [19]. RF: Riboflavin, 3RF*: Riboflavin triplet energy state

1.1.5. Other Aroma Compounds Responsible for the Sunstruck Flavor

The results of Gunst and Verzele [12] showed that after synthesis and appropriate dilution of 3-methyl-2-butene-1-thiol the smell was similar but not identical to beer exposed to light. Therefore, it was assumed that also other compounds are responsible for the overall aroma impression of the sunstruck flavor in beer. The flavor of sunstruck beer does not arise solely from 3-methyl-2-buten-1-thiol, although it is the major impact character [27]. This fact was approved later on by other authors who identified more aroma active compounds involved in the perception of the sunstruck flavor in beer. Haboucha and Masschelein [43] and Kattein *et al.* [14] stated that the increase of methanethiol, during exposure to light, contributed to this flavor difference observed. Methional also contributes to the flavor [27, 44].

Komarek *et al.* [13] analyzed pilsner and pale ale beer before and after exposure to sun-light using Aroma Extract Dilution Analysis (AEDA). Besides 3-methyl-2-buten-1-thiol, other substances such as 3-(methylthio)-propanal, phenylacetaldehyde, 2-

methylpropanal, 3-methylbutanal, methanethiol, and an unknown compound with a sulfury odor note were detected in the beers exposed to light. Moreover, a recent study [45] described in illuminated beer an increased content of these substances as well as of methanthiol. These authors also identified methionine and phenylalanine as precursors of 3-(methylthio)-propanal and phenylacetaldehyde, respectively. Also Wainwright *et al.* [46] described 3-(methylthio)-propanal as an intermediate in the formation of methanethiol from methionine; however, the reaction pathway is not known. These results show that the perception of sunstruck flavor after exposure of beer to sunlight is due to a combination of many aroma active compounds and not only 3-methyl-2-butene-1-thiol.

1.1.6. Sunstruck Flavor Inhibitors

Beer contains substances capable of preventing - to some extent - the degradation of riboflavin [22]. Polyphenols are described by many authors [3, 6, 13, 22, 33, 47-50] as inhibitors of the formation of the sunstruck flavor. Phenolic compounds found in beer include phenolic acids, flavonoids, proanthocyanidins, tannins, and amino phenolic compounds [51]. Charpentier and Maujean [3] stated that the formation of sulfur compounds is slower in the presence of tannins, and their reactions depend on the concentration and structure of tannins however, no quantification of the compounds was conducted. Years later, Cardoso *et al.* [33] analyzed the influence of single phenols on the formation of the light-induced off-flavor. Their results suggested that these compounds quench both the singlet- and triplet-excited states of riboflavin responsible for the lightstruck flavor development with rates close to the diffusion control. Also Pozdrik *et al.* [22] found that the polyphenols catechin and epicatechin preserve the riboflavin absorbance in light-exposed model beers by quenching its excited energy state. Alternatively, these authors also describe the possibility that isohumulone may extract electrons from the polyphenols to regain stability rather than to degrade to more stable minor products. Finally, polyphenols might extract electrons from photo-reduced riboflavin, possibly returning the riboflavin to its original state before exposure to light [22]. Also the reduction of the sunstruck flavor by the addition of quercetin has been proven [45]. Although the interaction among phenols and singlet-excited riboflavin occurs with a rate constant close to the diffusion limit, a content of phenolic compounds of >0.3 M is necessary to quench 90 % of the riboflavin in the singlet-excited state, and consequently, to hinder triplet-excited riboflavin generation [33].

The efficient deactivation by polyphenols makes these compounds of importance as a protectors against light-induced oxidation [6]. However, polyphenols have also been described as catalyzing the transformation of amino acids to their corresponding Strecker aldehydes, methional and phenylacetaldehyde [13, 48] which may provide an aged flavor. AEDA, carried out by Komarek *et al.* [13], revealed an increase in the Flavor Dilution-factors of the Strecker aldehydes, methional and phenylacetaldehyd in both "sunstruck" beer samples [13]. The polyphenols acting as antioxidants and in the presence of oxygen may be oxidized and can act as dicarbonyl compounds required for the Strecker degradation [13]. Nevertheless, the mechanism supporting Strecker aldehyde formation induced by light is still unclear [47].

Sakuma *et al.* [15] stated that an increase in the beer color greatly suppressed the formation of sunstruck flavor. Dark beer contains more polyphenols, whereas the polyphenol content of lager beer is intermediate [52] . Moreover, according to Gray *et al.* [8], in darker beers, the caramel coloring absorbs the light before it can exert its full photochemical effect on the sulfur bodies.

Goldsmith *et al.* [49] also demonstrated that tryptophan is able to quench the triplet state and inhibit the formation of the light-struck character in beer and in model beers. As afore-mentioned, tryptophan at similar concentrations in beer and model beer will protect riboflavin from photo-degradation [22].

1.1.7. Protection of Beer Against Light

The formation of oxidation flavor is suppressed when beer is bottled under good conditions, i.e., kept free of oxygen and not exposed to sunlight. Whereas, sunstruck flavor develops when beer is bottled under good conditions, with the bottles containing a minimum amount of oxygen but exposed to sunlight [10] without protection. Figure 1.2 shows the electromagnetic spectrum with light highlighted. Riboflavin upon photo activation by light in the wavelength region between 350 nm and 500 nm becomes energetically excited in a triplet state (denoted 3RF'), which may subsequently oxidize available isohumulones [53]. This range is within the blue, violet, and near ultraviolet regions of the spectrum.

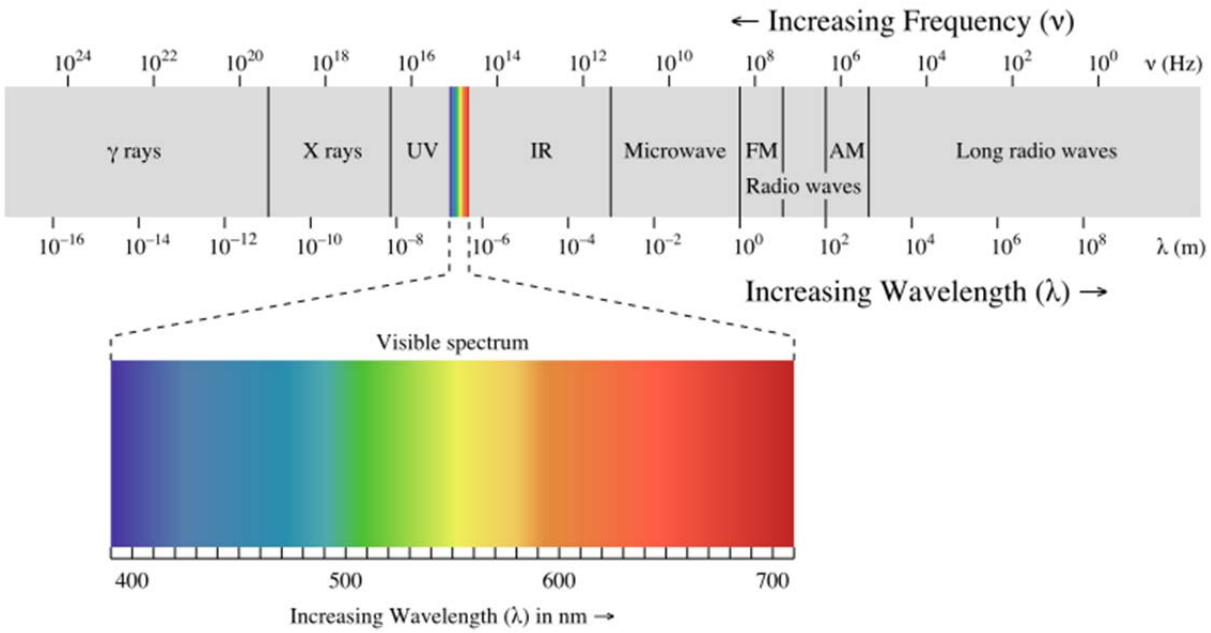

Figure 1.2: Electromagnetic spectrum with light highlighted [54]

Ways of preventing 3-methyl-2-butene-1-thiol formation may be divided into physical and compositional options. Physical solutions are essentially ways of preventing light interacting with the product [55]. Sturgeon [56] measured the light transmitted through different colors of beer bottle packaging and found that clear glass does not protect the beer in any way from photochemistry, brown glass filters the largest amount of high energy light, and in a green or blue bottle some of the light is absorbed, although the protection is not as great as with the brown bottle. Figure 1.3 shows the emission spectra from the sun filtered with blue glass, green glass, and brown glass compared to that of the sun and a standard tungsten light bulb. Brown glass cuts off light around 500 nm, hence, beer in a brown bottle seems to be protected [21]. On the other hand, green glass has a cut-off around 400 nm, indicating that the most energetic part of the visible spectrum, namely blue light, may penetrate through a green glass, hence, protection against sunstruck flavor is compromised [21].

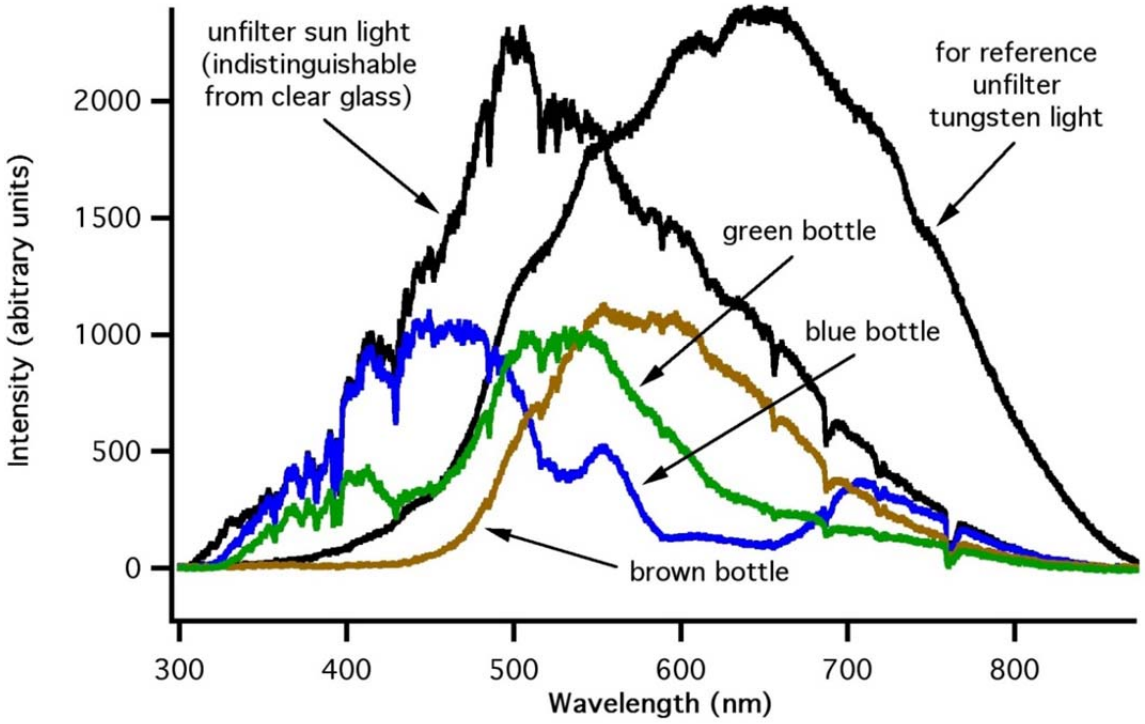

Figure 1.3: Emission spectra from the sun (black line left), standard tungsten light bulb (black line right), and the sun filtered with blue glass (blue line), green glass (green line), and brown glass (brown line) [56]

Due to the cut-offs of light of brown bottles around 500 nm, some authors [8,13, 56, 57] have defined brown bottles as the best protection against the formation of sunstruck flavor. Also packaging in cans minimizes light ingress [55]. However, a prestigious rational psychology poll states that people prefer to drink beer filled in white glass bottles [58]. Therefore, breweries first started to fill beer into transparent bottles for marketing reasons [59]. The use of a sunscreen on the bottle helps to prevent skunking of beer [60]. Nevertheless, this kind of bottle normally has higher acquisition expenses.

Compositional solutions to prevent 3-methyl-2-butene-1-thiol formation have been described. According to Mitter [59], there are mainly two possibilities for minimizing taste impairment resulting from UV light shining on transparent bottles. Apart from storing beer in light-proof containers or immediate consumption, the photosensitivity can be circumvented by quenching of the excited triplet state of the isohumulones

and/or riboflavin or by the use of chemically modified isohumulones, whereby formation of the sunstruck flavor is prohibited [17].

The chemical modification of isohumulones is complex, cumbersome, time consuming, and obviously, expensive [61]. By the targeted disruption of double bonds with hydrogen, reduced downstream products are produced from the already isomerized products. There are three types of reduced iso-alpha-acids: the rhohydro-iso-alpha-acids exhibit a twofold, the tetrahydro-iso-alpha acids a fourfold and the hexahydro-iso-alpha acids a six-fold hydrogenation [62]. The chemical modification has the effect that here no separation of side-chains can occur, which is ultimately the cause of the light taste in beer [63]. Figure 1.4 shows the structures and production steps of the reduced iso-alpha-acids: rhohydro-iso-alpha-acids, tetrahydro-iso-alpha acids, and hexahydro-iso-alpha acids.

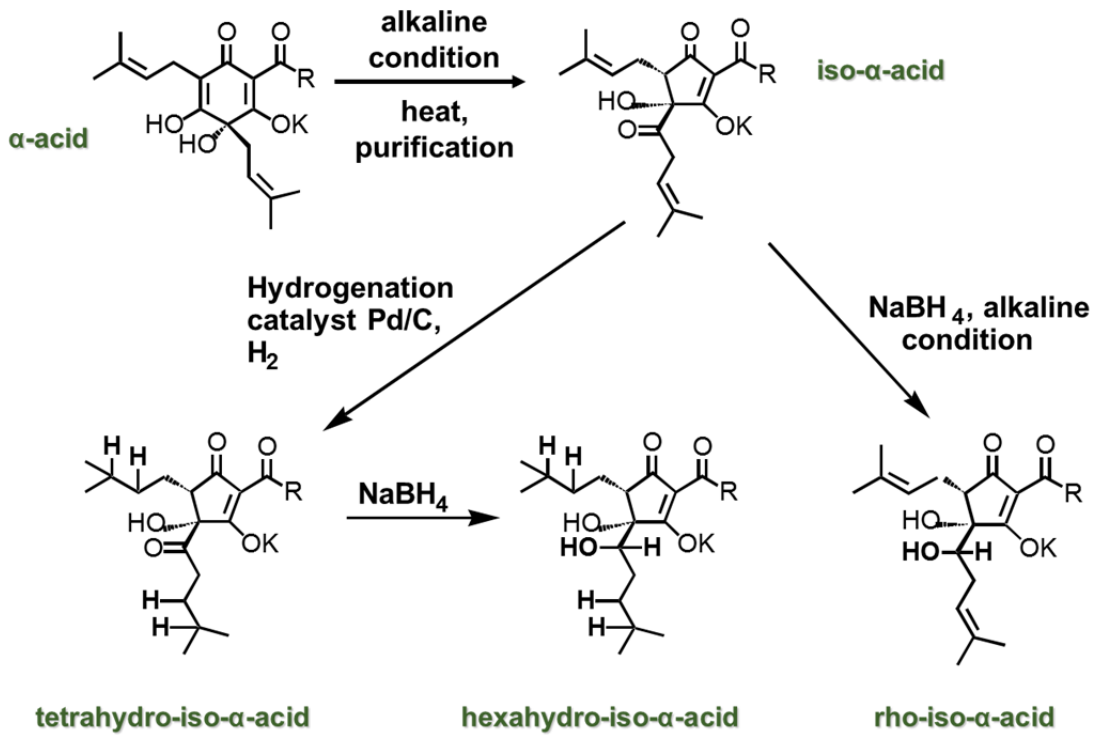

Figure 1.4: Structures and production steps of the reduced iso-alpha-acids: rhohydro-iso-alpha-acids, tetrahydro-iso-alpha acids, and hexahydro-iso-alpha acids [64]

Although reduced iso-alpha-acids are normally described to be light stable, contradictory studies have shown that tetrahydro-isohumulones and/or rhohydro-isohumulones are also oxidized by triplet-excited riboflavin [39]. 3-methyl-2-butene-

1-thiol cannot be formed by the decomposition of tetrahydro-isohumulones because the double bond is lacking [21]. However, Huvaere and De Keukeleire [24] stated that the irradiation of beers containing tetrahydro-isohumulones furnishes an obnoxious off-flavor, which evidently is not due to 3-methyl-2-buten-1-thiol, but most likely due to other sulfur-containing compounds. On the contrary, Burns *et al.* [11] showed that dihydro-iso-a-acids have light resistance but tetrahydro-iso-alpha-acids are as photo-reactive as the iso-alpha-acids; however, 3-methylbut-2-ene-1-thiol (ªskunkyº thiol) cannot be formed from these compounds subsequent to photo-cleavage.

The addition of non-natural reduced forms of iso-alpha-acids is not allowed in beers for the German market due to the “Reinheitsgebot” stating that only natural hop compounds may be used in the brewing process [65]. Therefore, alternatives for reducing the appearance of sunstruck flavor are of great interest for the brewing industry.

Other compositional ways of reducing the formation of 3-methyl-2-butene-1-thiol are, for example, the use of flavoproteins or riboflavin-binding proteins which have been described to be able to bind riboflavin [66-68]. Laane *et al.* [68] investigated how riboflavin-binding proteins are able to reduce the amount of riboflavin-induced sunstruck flavor formation in a model beer system and stated that these selective adsorbents of riboflavin might help to limit sunstruck flavor formation in beer. Moreover, in 2011 a patent [69] described N-heterocyclic substances to be advantageous as additives in beverages and foodstuffs in order to protect these against light induced flavor changes. These authors stated that N-heterocyclic substances are capable of absorbing ultraviolet light without being decomposed into undesirable off-flavor generating substances, thus being used to inhibit decomposition or reaction of light sensitive substances as a result of UV-induced excitation.

Irwin *et al.* [70] created a patent where the treatment of wort with an effective amount of an absorbent clay to absorb the riboflavin reduced its amount in less than about 0.2 ppm, and the resulting hopped malt beverage had enhanced stability to light. The clay employed as the absorbent of the invention was a hydrated aluminum silicate or a hydrated aluminum-magnesium silicate. Also, Palamand [61] believed that the addition of 1,8 epoxy compounds to the malt beverage prevents the formation of the 3-methyl-2-butene-1-thiol, either by reacting with the iso-pentenyl fragment or by

protecting the isohexenoyl side-chain from fragmenting or blocking the sulfhydryl group by reacting with the iso-pentenyl fragment.

Although many ways to prevent the formation of sunstruck flavor have been described, none of these treats with the idea of decreasing the levels of sunstruck flavor initiators or increasing the content of sunstruck flavor inhibitors. The studies described before deal mainly with the idea of decreasing the content of riboflavin present in beer or protecting the beer from the light. To be able to reduce the presence of sunstruck flavor initiators or to increase the presence of sunstruck flavor inhibitors, it is important to clarify their origin and their way through the malting and brewing process to the final beer.

1.2. Origin of the Substances Involved in the Formation of Sunstruck Flavor

Summarizing the information found in the literature, many substances play an important role in the formation of the sunstruck flavor. The light-induced degradation reaction of isohumulones occurs in the presence of riboflavin, phenylalanine, and the sulfur-containing amino acids and polypeptides such as cysteine, methionine, and glutathione. Moreover, the albumin and glutenin fraction of barley has been described to promote the formation of the sunstruck flavor, probably serving as a source of the previously mentioned amino acids. These substances can, therefore, be referred as sunstruck flavor initiators and are of great importance while trying to reduce the formation of sunstruck flavor in beer.

Riboflavin is present in beer at concentrations between 0.3 and 1.3 mg/L [19, 68], being in bottom fermented beers lower than in top-fermented beers [27]. Possible sources of riboflavin in beer are the malt and yeast [19, 23]. Barley contains riboflavin (1.2–1.5 mg/kg) [27], and the concentration increases during malting, especially at higher germination temperatures [2, 71]. The increase of riboflavin content in different cereals during germination has been determined by many authors [72-77], it can grow up to 1.5-fold content of barley; a concentration from 1.0 to 3.7 mg/kg malt d.m. [74, 77]. This vitamin is located in the epithelium of seedlings and the aleurone layer of the barley grain, and together with other vitamins is involved in enzyme synthesis [78].

Riboflavin is extracted quantitatively into wort and survives wort boiling and is produced by yeast with the production rate being proportional to yeast growth [2, 79]. However, the riboflavin content of beer is relatively high because riboflavin comes from malt, and the yeast absorbs hardly any riboflavin from wort during fermentation [74]. During the fermentation of the wort, yeast (*Saccharomyces cerevisiae*) produces 50–100 µg of riboflavin/L d.m., which is retained within the cells [23, 80]. The maximum biomass production is 4-6 g of yeast cells/L, of which 1 g/L is inactive cells; 10–20 % of the inactive cells leak [81], which may cause release of riboflavin into the beer [23]. From this data follows that the maximum contribution of yeast to the riboflavin content of beer is 50 nM [23]. On the other hand, part of riboflavin is destroyed during the stationary phase of the yeast as a result of enzymic breakdown [2].

It is believed that reducing the amount of riboflavin to a relatively low level prior to hopping inhibits the formation of sulfur compounds imparting a “skunky” favor and, hence, is responsible for the enhanced light stability [70]. It is also advantageous to use a yeast that is substantially free of riboflavin or at least is riboflavin-deficient [70].

Beer contains ~500 mg/L of proteinaceous material including a variety of polypeptides [82]. Up to one percent of protein bodies derived from malt are present in beer, in which sulfur is a characteristic element, and may also contain various soluble sulfur compounds resulting from the metabolism of the yeast [8]. Barley use for malting has ideally a protein content of between 9–11 % d.m. The amino acids cysteine, methionine, and phenylalanine, described as sunstruck flavor initiators, are present in barley in quantities of between 150–270 mg/100 g d.m., 140–210 mg/100 g d.m. and 520–630 mg/100 g d.m., respectively [83].The most important factors for the protein composition in finished beer are barley cultivars, and the level of protein modification during malting [82]. Variation of the malting parameters, for example, longer germination times, exerts an influence on the degradation of proteins, normally increasing the content of free amino acids [73]. During kilning, amino acids may increase initially but finally decline in amount [72].

Glutathione is a tripeptide characterized by an N-terminal glutamyl moiety, a central cysteine residue, and a C-terminal glycine (L-γ-glutamyl-L-cysteinylglycine). Due to the presence of an active thiol group in the form of a cysteine residue, it can also be described as a sunstruck flavor initiator. It has long been known that reduced

glutathione (GSH) is rapidly formed in the first stages of the germination of seeds [84, 85] and plays an important role in antioxidant protection mechanisms in the plant cell [86]. Non-dormant grain embryos are able to release glutathione and cysteine when the grains are soaked [84, 87]. Belderok [84] also reported a correlation between the total amount of glutathione and cysteine in embryos after 24 h wetting and the percentage of grain germinated in three days. However, these authors [84, 87] agree that the content of glutathione decreases to further increase during the germination of barley.

Substances known to reduce or minimize the formation of sunstruck flavor are here named as sunstruck flavor inhibitors and are comprised of polyphenols, such as catechin, epicatechin, and quercitin, and the amino acid tryptophan.

Polyphenols can be subdivided into three main sub-classes, the flavonoids, phenolic acids, and the stilbenoids [88]. Among others, beer contains phenolic compounds of the flavonoid group such as catechin, epicatechin and quercitin, [52]. Total flavonoid content for lager beer has been estimated at between 13.4 and 52.6 µg/mL [52, 89].The great variations in phenolic profiles for different beers are caused by the differences in raw material and the brewing process [19, 51, 52]. In wort and beer, up to 20–30 % of the polyphenols present are derived from hop and 70–80 from malt, despite the fact that malt is added in much larger amounts (nearly 100 times more) [52, 90, 91]. However, some flavonoids, catechin and epicatechin, arise equally from malt and hops [92] and their levels in the beer are in the ranges of 0.03-4.00 mg/L and 0.02–0.73 mg/L, respectively [19, 51]. The polyphenols in hop cones consist of diverse classes of which proanthocyanidins, monomeric flavanols, flavonol glycosides, and prenylated flavonoids are the major ones, and hydroxybenzoic acids, hydroxycinnamic acids, and flavonols are minor classes [93]. Depending on the hop variety, its geographic origin and freshness, the harvesting procedure, and the manner in which the dried hop cone is packaged, total polyphenols account for 3-6 % (w/w) [52, 90, 94]. In cereals, polyphenols are deposited in the husk, the pericarp and the aleurone layer of barley [52, 77, 95-97]. The polyphenol content of barley depends on the variety and increases during malting [98]. Also, the extraction of phenolics from malt into the wort is highly dependent on the malt to water ratios [52].

During the brewing process, mashing increases the concentrations of hydroxycinnamic acid derivate as they are released from previously non-extractable

combinations [52]. Moreover, polymerization of phenolics and formation of polyphenols can occur during wort boiling, and possibly also during fermentation and storage of beer [52, 99].

Polyphenols have an influence on the final beer quality. Whereas some authors point to an increase of mouth-feel due to proanthocyanidins [93, 100], others associate a harsh astringent flavor, and thus, a detriment in the mouth-feel [101]. They contribute to astringency and color, [102] serve as browning substrates, participate in chill haze formation and are responsible for overall beer stability [91, 103].

The raw material employed in the beer or cereal-based beverage production processes are the main source of sunstruck flavor initiators and inhibitors. Investigation and analysis of the content of sunstruck flavor initiators and inhibitors present in different raw materials is necessary to ponder the different raw materials employed in the production of malted cereal-based beverages.

1.3. Content of Sunstruck Flavor Initiators and Inhibitors in Cereals

The initiators and inhibitors of the aroma compounds 3-methyl-2-buten-1-thiol, methanethiol, methional, 3-(methylthio)-propanal, phenylacetaldehyde, 2-methylpropanal, 3-methylbutanal can be found in the cereal or malt used for the malting and beer production. The use of other cereals other than barley and of the hop products for the cereal-based beverage production offer the possibility of changing the content of the sunstruck flavor initiators and inhibitors, and thereby of being able to produce a new light stable cereal-based beverage that can be filled into a white bottle. However, the cereal use has to have also a good brewing quality and performance. Spelt (*Triticum spelta* L.) and triticale (× *Triticosecale Wittmack*) have been investigated upon their use in the cereal-based beverage production [75, 104-118]. Both cereals show good processability and offer a good possibility for the production of new cereal-based beverages. Moreover, the content of sunstruck flavor initiators and inhibitors differs with that of barley. Table 1.1 shows the content of initiators and inhibitors in spelt, triticale and barley. The lower content of riboflavin, cysteine, methionine and phenylalanine in spelt than in barley could reduce the formation of sunstruck flavor. Moreover, although the content of riboflavin and methionine is higher in triticale than in barley, phenylalanine is found in lower quantities than in barley, and tryptophan and polyphenols are found in higher concentrations, the latter being almost twice as high as in barley.

Table 1.1: Content of sunstruck flavor initiators and inhibitors in spelt, triticale and barley.

	Substance	Units	Spelt	Triticale	Barley
Sunstruck Flavor Initiators	Riboflavin	µg/100 g	173 [a]	320 [b]	180 [b]
	Cysteine	mg/100 g	210 [a]	200 [b]	220 [b]
	Methionine	mg/100 g	170 [a]	180 [b]	180 [b]
	Phenylalanine	mg/100 g	480 [a]	510 [b]	590 [b]
	Glutathione	nmol/g d.m.	-	-	210 [d]
Sunstruck Flavor Inhibitors	Tannins	g/kg	1.96 [c]	3.48 [c]	1.9–2.6 [f]
	Tryptophan	mg/100 g	150 [a]	157 [e]	150 [b]
Values obtained from [a]: [119] [b]: [83] [c]: [120] [d]:[121] [e]:[122] [f]: [123]					

Moreover, the albumin and glutenin protein fractions are known to increase the sunstruck flavor formation probably serving as a source of the amino acids involved. Table **1.2** shows the composition of the Osborne fraction of spelt, triticale, and barley. Although the data about the composition of the Osborne fractions in cereals is sometimes contradictory, the literature shows a higher proportion of the glutenin fraction in spelt and in barley than in triticale. The composition of the albumin+globulin fraction is relatively similar between cereals.

Table 1.2: Composition of the Osborne fractions of spelt, triticale, and barley

	Albumin	Globulin	Prolamin/Gliadin	Glutenin
	%	%	%	%
Spelt	17–27 [a b]		29–34 [a b]	52–55 [a]
			62–68 [c]	13–17 [c]
Triticale	12–19 [d e]		24-33 [f g]	9–17 [f g]
	28–32 [f]			
Barley	3–11 [h i]	10–20 [h]	35–50 [h i]	30–45 [i]
Values obtained from [a]: [124] [b]: [125] [c]: [126] [d]: [127] [e]: [128] [f]: [129] [g]: [130] [h]:[131] [i]: [82]				

The lower content of some sunstruck flavor initiators and the higher content of some sunstruck flavor inhibitors found in spelt and triticale compared to barley together with

a good brewing performance, define both cereals as a good alternative to barley for the production of light stable cereal-based beverages.

1.3.1. Spelt

In the last few years the interest in hulled wheat has increased due to the low-input techniques used for its management, its outstanding stress resistance, and quality performance [132, 133]. Spelt is an interesting (hexaploid) crop for marginal regions where environmental factors prevent the cultivation of wheat (*Triticum aestivum* L.) [134]. Spelt is well adapted to cool and wet climatic conditions of higher altitudes, and is, therefore, described as a 'robust' cereal [135, 136]. The protecting hull allows the spelt grain to be resistant against pollutants and insects without using a pesticide [137]. Furthermore, spelt grain could give a high yield with a low fertilizing rate and shows a better mineral intake than common wheat [138]. Although spelt is genetically closely related to wheat, it has been demonstrated that there is a clear divergence between spelt and common wheat based on DNA or protein variability [119]. Nowadays, spelt is still considered as an alternative crop compared with widely cultivated modern winter wheat [139].

Spelt is probably originated from the mutation of wheat because no wild form of spelt is known [140]. This domesticated hulled wheat was one of the major feed and food grains in ancient Europe, extensively grown, and a staple in the everyday diet, but its diffusion has progressively diminished throughout the centuries.

Uses of Spelt

Spelt has applications in many areas of the food industry. This hulled bread cereal is intended both for animal feeding and human consumption [139], showing potential in a variety of end applications, including bread, pasta, and breakfast [119]. Several cultivars have been examined for the potential in the baking industry [134, 141-150]. Spelt has also been attached importance due to its uses for unconventional foods, therapeutic properties attributed to their derivatives, and their potential as a source of genes for breeding hull-less wheats [151, 152].

There are many applications of spelt in the baking industry but not many in the beverage industry. Although spelt has promising aptitudes for beer and cereal-based beverage production, it is still considered as a niche product. Only few research projects [75, 118] have been carried out on the capability of spelt for this purpose.

Spelt is processed for the production of spelt beer in Germany [140], but seldom exceeding 50 % of the used malt, according to the purity law. This provides new approaches in the malting and brewing process which require further investigation. The normal commercial requirement of barley and wheat is a maximum of 11.5 g/100 g [95] and 12.5 g/100 g d.m. [153] protein in the d.m. respectively, which is slightly lower than that of spelt. Therefore, the high protein content of spelt may cause difficulties in the brewing process such as lautern detriment, haze and poor flavor stability. To avoid this problem, spelt protein content should be controlled during the process or it could be used for opaque beers with a need for stable turbidity. For these reasons, spelt has potential in the beverage industry for the production of new alternative cereal-based beverages.

Composition of Spelt

The nutritive value of spelt grain is high, compared with other cereals, and it contains all the basic components which are necessary for human beings such as saccharides, proteins, lipids, vitamins and minerals [147, 154]. The principal storage carbohydrate in spelt is starch. According to Abdel-Aal *et al.* [155], the starch content of the spelt grain varies from 60.9 to 65.8 g/100 g d.m. These values are slightly higher than those of barley (*Hordeum vulgare* L.), 50–63 g/100 g [95], and within range with those of wheat, 60–68 g/100 g [156], which represents an advantage for the brewing process.

Spelt contains more dietary fiber, between 12.9–13.8 g/100 g d.m., than wheat, between 9.8–13.2 g/100 g [148], and barley, 9.8 g/100 g d.m. [83] but β-glucan is just present at low levels [157] (good from a brewing point of view). However, consumption of whole-grain or bran products of spelt and other wheat species would also reduce caloric density and possibly intake, and therefore, the incidence of diseases of the lower digestive tract. But the intake of other components in the diet would be needed to supply soluble dietary fiber to reduce blood lipids [119].

Generally, spelt may contain higher levels of protein than barley and wheat, ranging between 11.0 and 18.3 % d.m. [124, 158]. However, there is a substantial variation among cultivars [148, 158, 159]. Bognar and Kellermann [154] and Abdel-Aal *et al.* [155] attribute this difference to the larger aleurone layer present in spelt. Moreover, no important differences are found in the amino acid composition between spelt and

wheat [160], which means that these products are not suitable for people with celiac disease [147, 161]. Campbell [133] supports that persons suffering from certain allergies to wheat, have reported to be able to tolerate products made from spelt. Therefore, it could be said that persons with wheat allergies, who are able to tolerate spelt, may suffer from something other than gluten intolerance [133]. However, there is no other supporting scientific or medical evidence, and therefore, more research should be conducted into determining the allergenicity of spelt.

Data about the distribution of Osborne fractions in spelt are contradictory. Table 1.2 shows the distribution of the Osborne fraction of spelt compared to other cereals. Koenig *et al.* [126] stated that gliadins are the most abundant proteins in spelt (61.5–67.6 %) followed by the albumin/globulin fraction (19.9–22.8 %) and the glutenin fraction (12.5–17.4 %). Belitz *et al.* [124] determined gutenins as the most abundant proteins of spelt (54.6–49.4 %) followed by gliadins (28.6–30.6 %), and then the albumin/globulin fraction (16.5–20 %). Although considerable work has been performed in the investigation of the gliadin and glutenin, little is known about the proteomic profiles during the grain development stages.

The investigation of spelt proteins has been used for several applications. By analyzing the composition of protein fractions, some authors investigated the origins of European spelt [162] and its genetic diversity [132], analyzed its baking quality attributes [149, 160], attempted to differentiate between spelt and wheat to classify and endorse new varieties and cross breeds or for cultivar legal protection [126, 163-166], or tried to evaluate the influence of the crossing between these two cereals [167].

1.3.2. Triticale

Triticale (*x Triticosecale Wittmack*), the first man-made cereal, is an intergenic hybrid between female wheat and male rye (*Secale cereale L.*). Triticale belongs to the family of sweet grass (poaceae) and the tribe Triticeae. The grade of ploidy is tetraploid, hexaploid or octoploid depending on the parental breed. The hexaploid triticale is the most common form, usually with the genomic formation AABBRR. In 1875 the first intended hybrid was made by A. S. Wilson in Scotland [129]. After decades of research the first profitable cultivar was created in 1969 [168].

Triticale was designed to combine the productivity, uniformity and superior grain quality of wheat with the hardness, disease resistance and better nutritional quality of rye [127, 169]. The main characteristics of the early triticales were good disease resistance, shriveled grain, minor grain yield, high content of proteins, disproportionate size and pre-harvest sprouting. Achievements in breeding improved the negative factors and allow for a real alternative to other crops, especially wheat [170]. Nowadays, this cereal is also capable of producing much higher agricultural yields and biomass than other cereals and has better tolerance to many diseases and pests than its parental species [112] which thereby reduces the necessity of chemical protection against such harmful agents [170].

Triticale offers several advantages over other cereal grains, such as better-quality protein in terms of amino acid composition and digestibility, higher content of soluble dietary fiber and minerals and a pleasing, nutty, mild-rye flavor [171]. All the characteristics of triticale lie somewhere between wheat and rye. The body is a bit bigger, the profile is longer and the color is slightly darker than wheat.

Uses of Triticale

The current uses of triticale are largely determined by its chemical composition [172]. McGoverin *et al.* [172] reviewed the uses of triticale. The main use of triticale is for animal feeding, especially poultry, monogastrics and ruminants. It is an alternative to other feeding grains and protein sources such as soybean [173]. It has a high content of proteins, high starch digestibility and is often used in diets for chickens as the only cereal component or is added to compound diets [170, 174, 175]. The unsuitable nature of triticale for baking, bread-making specifically, was acknowledged early, and subsequently, efforts were made to improve triticale for baking [172]. Triticale flours produce weak doughs due to a low gluten content, inferior gluten strength and high levels of α-amylase activity [176, 177]. However, Lorenz *et al.* [178] demonstrated the possibility of producing triticale bread of very acceptable quality by making only minor modifications in the mixing procedure. In contrast, the ease with which triticale produces high levels of α-amylase [176] and high proteolytic activity, in combination with the low gelatinization temperatures (59–65°), has its positive side; it allows triticale to perform well in malting and brewing. Intense research has been done on the use of triticale as an adjunct for brewing [111, 113-115] and for its suitability in the malting and brewing process [104-110, 112, 116, 117].

Triticale malt is described, in general, to have higher malt losses, but also higher malt extracts, higher diastatic power, and higher α- and β-amylase activity than barley malt [106, 112, 175]. Triticale malt has been also described to have high nitrogenous content, high proteolytic activity [106, 107]. Blanchflower and Briggs [110] defined pentosans and proteins as the main constituents contributing to the high viscosity and turbidity of triticale wort, respectively. Due to these characteristics and to its composition, triticale serves as a good cereal for the production of new alternative cereal-based beverages.

Composition of Triticale

The starch content of triticale is similar to wheat and rye. The protein content of whole-grain triticale is often higher than that of wheat [179]. Some authors [106, 180] report the protein content of triticale to be between 8.0 and 22.5 %. This fact is apparently due to the combination of the protein fractions from wheat and rye [181-183]. The gluten content of triticale is lower and of inferior quality (weak and less elastic) than that of wheat [170]. In addition, some triticale lines display high levels of amylolytic and proteolytic activities in their unmalted form [115, 184, 185].

Chen and Bushuk [130, 186, 187] studied the proteins and amino acid composition of triticale and its parents, and showed that both solubility characteristics and amino acid composition of triticale proteins are, in general, intermediate between those of its parent species. However, Lei and Reeck [121] indicated that the electrophoretic patterns of triticale proteins are generally similar, but not identical, to those of the corresponding combined protein fractions of its parental species.

Table 1.2 shows the distribution of the Osborne fraction of triticale compared to other cereals. The triticale albumin fraction is defined to be larger than that of wheat; an important fact, since many proteins in the water-soluble fraction exhibit biological activity such as enzymes and exogenous enzyme inhibitors [182]. Some authors [186, 188] have reported an albumin fraction between 17 % and 26.4 % in triticale. Chen and Bushuk [186] quantified the globulin fraction in triticale as 6.5 %, lower than that of rye (10.7 %), but higher than that of wheat (4.7 %). However, the globulin fraction is normally quantified together with the albumin fraction. Jonnala *et al.* [127] and Naeem *et al.* [128] analyzed triticale cultivars and determined an albumin and globulin fraction to be between 11.7 and 19.0 %. These results do not agree with

those of Varughese *et al.* [129] who summarized the results of various studies and found the albumin and globulin fraction to range between 27.9 and 32.2 %. Although there is a discrepancy, all authors agree that this cereal's albumin and globulin fraction is larger than that of wheat. Taking into consideration the high presence of the albumin fraction with respect to the total protein content of triticale, it can be said that the globulin fraction is the smallest Osborne fraction. The gliadin and glutenin fractions of triticale have been quantified as about 24.4-33.3 % and 9.4-17.3 % respectively, lower than that of wheat [129, 186].

2. Aim

Flavor stability has always been of particular interest in the brewing industry. Besides the well-known beer oxidation process caused by oxygen, the off-flavor known as “sunstruck flavor” may occur if beer is exposed to light without protection. The growing trend towards natural products (clean label) and the higher costs of protected bottles and reduced isomerized products as well as brewing in accordance to the German Purity Law motivate the search for other natural hop products which may help towards isohumulone photo-degradation.

Ways of preventing 3-methyl-2-butene-1-thiol formation may be divided into physical and compositional options. The best physical solution is the use of brown bottles and cans; however, a prestigious rational psychology poll states that people prefer to drink beer filled in white glass bottles. The other physical solution is the use of sunscreen on the bottles; however, this implies higher acquisition expenses.

Compositional solutions to prevent 3-methyl-2-butene-1-thiol formation have been described. The most common solution is the use of chemically modified isohumulones such as rhohydro-iso-alpha-acids, tetrahydro-iso-alpha acids, and hexahydro-iso-alpha acids. However, addition of non-natural reduce forms of iso-alpha-acids is not allowed in beers for the German market due to the “Reinheitsgebot”. To date, other compositional solutions to reduce the formation of the sunstruck flavor have been patented, among other, the use of flavoproteins or riboflavin-binding proteins, N-heterocyclic substances, absorbent clay, and 1,8 epoxy compounds. However, these substances are also prohibited by the “Reinheitsgebot”.

Beer contains substances capable of preventing the degradation of riboflavin. Although many ways to prevent the formation of sunstruck flavor have been described, none of these deals with the idea of decreasing the levels of sunstruck flavor initiators or increasing the content of sunstruck flavor inhibitors. Possible natural solutions are, for example, the circumvention of the photosensitivity by quenching the excited triplet state of the isohumulones and/or riboflavin. However, no natural ways of reducing the formation of sunstruck flavor formation have been described. Knowledge about the content of sunstruck flavor initiators and inhibitors in different raw materials, cereals as well as hop products, is needed to specify the

possible natural ways of reducing the formation of sunstruck flavor in beer or other cereal-based beverages.

Additionally, the impact of the malting and brewing process on the content of the substances involved in the formation of the sunstruck flavor is important to determine the potential of the raw materials employed in the brewing process. In conclusion, only through a detailed understanding of the behavior of the substances involved in the formation of the sunstruck flavor will it be possible to achieve a high light stability together with high quality products.

Therefore, the aim of this work is to analyze the origin and content of sunstruck flavor initiators and inhibitors in different cereals and hop products as well as the change in their content during the manufacturing process. To achieve this comprehensive knowledge, this work is structured in three main parts:

1. Screening of the content of polyphenols in hop products and their effect on sunstruck flavor formation in beer.
2. Detailed analysis of the content of amino acids and protein fractions in different cereals and the technological impact of the malting process,
3. Influence of different malting regimes on the content of sunstruck flavor initiators and inhibitors in different cereals and,

A firm understanding of these interaction mechanisms will enable the use of alternative raw materials and malting regimes through the reduction of sunstruck flavor initiators and increase of sunstruck flavor inhibitors without decreasing the product quality.

3. Results

3.1. Paper Summary

CHAPTER A USE OF POLYPHENOL-RICH HOP PRODUCTS TO REDUCE SUNSTRUCK FLAVOR IN BEER

PAGES

Reproduced, by permission, from Munoz-Insa, A., Gastl, M., and Becker, T. 2015. Use of polyphenol-rich hop products to reduce sunstruck flavor in beer. J Am Soc Brew Chem. 73(3):228-235.

Polyphenols are known to be sunstruck flavor inhibitors. Polyphenols such as catechin, epicatechin, and quercitin have been described to protect beer against the formation of sunstruck flavor by quenching both the singlet- and triplet-excited states of riboflavin, giving electrons to isohumulone to regain stability rather than to degrade to more stable minor products or extracting electrons from photo-reduced riboflavin returning it to its original state before exposure to light.

Hops are an important source of polyphenols and humulone in beer. During the extraction procedure of some products, the polyphenol content is discarded or increased. However, the content of the single polyphenols in the different hop products has not been extensively examined. An increase in the contents of catechin, quercetin, and epicatechin in the final beer should reduce the formation of the sunstruck flavor. The aim of this study was to examine the single and total polyphenol content of different hop products and specify a suitable hop product to be tested for the reduction of sunstruck flavor. This study shows the possibilities of different products according to their polyphenol composition. Also, the appropriate process parameters using tannin extract to be used for the reduction of sunstruck flavor perception in beer were described.

CHAPTER B INFLUENCE OF MALTING ON THE PROTEIN COMPOSITION OF SPELT AND TRITICALE

SECTION 1 INFLUENCE OF MALTING ON THE PROTEIN COMPOSITION OF SPELT (*TRITICUM SPELTA* L.) "FRANKENKORN"

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Reproduced, by permission, from Munoz-Insa, A., Gastl, M., and Becker, T. 2016. Influence of malting on the protein composition of spelt (Triticum spelta L.) 'Frankenkorn'. Cereal Chem. 93:1-9.

In the last few years the interest towards hulled wheat has increased due to the low-input techniques used for its management, its outstanding stress resistance, and its quality performance. Spelt has applications in many areas of the food industry, in the majority of cases as an ingredient for the baking industry. However, although spelt has also promising aptitudes for beer and beverage production, almost no investigation about its protein modifications during malting has been conducted. One of the most important physical–chemical changes during malting is the degradation of the proteinaceous matrix and their conversion into soluble peptides and amino acids. The most important factors for the protein composition, as origin in finished beer are barley cultivars and the level of protein modification during malting. Beer contains ~500 mg/L of proteinaceous material including a variety of polypeptides among others, cysteine, methionine, and phenylalanine, described as sunstruck flavor initiators. The aim of this project is to investigate spelt protein changes during and caused by malting. This paper contributes to the understanding of the protein modifications and metabolic changes during the malting process of spelt and facilitates the determination of the potential of this cereal for the production of cereal-based products.

CHAPTER B INFLUENCE OF MALTING ON THE PROTEIN COMPOSITION OF SPELT AND TRITICALE

SECTION 2 INFLUENCE OF MALTING ON THE PROTEIN COMPOSITION OF TRITICALE (*X WITTMACK*) "TRIGOLD"

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Reproduced, by permission, from Munoz-Insa, A., Gastl, M., and Becker, T. 2016. Influence of malting on the protein composition of triticale (× Triticosecale Wittmack) 'Trigold'. Cereal Chem. 93:10-19.

Triticale (x *Triticosecale Wittmack*), the first man-made cereal, is an intergenic hybrid between female wheat (*Triticum aestivum* L.) and male rye (*Secale cereale* L.). Triticale was designed to combine the productivity, uniformity and superior grain quality of wheat with the hardness, disease resistance and better nutritional quality of rye. Triticale is a promising cereal for malting and brewing due to its high levels of α-amylase and proteolytic activity and low gelatinization temperatures. However, almost no investigation about its protein modifications during malting has been conducted. One of the most important physical–chemical changes during malting is the degradation of the proteinaceous matrix and their conversion into soluble peptides and amino acids. The most important factors for the protein composition, as origin in finished beer are barley cultivars and the level of protein modification during malting. Beer contains ~500 mg/L of proteinaceous material including a variety of polypeptides among others, cysteine, methionine, and phenylalanine, described as sunstruck flavor initiators. The aim of this project is to investigate triticale protein changes during and due to malting. This paper contributes to the understanding of the protein modifications and metabolic changes during the malting process of spelt and facilitates the determination of the potential of this cereal for the production of cereal-based products.

CHAPTER C VARIATION OF SUNSTRUCK FLAVOR-RELATED SUBSTANCES IN MALTED BARLEY, TRITICALE AND SPELT

PAGES

Reproduced, by permission, from Munoz-Insa, A., Gastl, M., and Becker, T. 2016. Variation of sunstruck flavor-related substances in malted barley, triticale and spelt. Eur Food Res Technol. Vol. 242, Issue 1, 11-23.

Riboflavin, phenylalanine, cysteine, methionine and the sulfur-containing peptide glutathione have been described as sunstruck flavor initiators, promoting the formation of the sunstruck flavor. Moreover, tryptophan and polyphenols are known to reduce the formation of the sunstruck character in beer (inhibitors). The initiators and inhibitors of sunstruck flavor originate in the raw material used, and their content can be influenced during manufacturing. During germination, proteins are degraded and the content of free amino acids increases. Also, the polyphenol and riboflavin content is known to increase during germination. The aim of this paper was to define the influence of the malting process parameters on the content of initiators and inhibitors involved in the formation of sunstruck flavor in barley, spelt and triticale. Moreover, the influence of malting process on the content of these substances as well as the differences between the raw materials were defined. With the knowledge gained, the potential of barley, spelt, and triticale for their use in the development of light stable malted cereal-based beverages was determined.

3.2. Use of polyphenol-rich products to reduce sunstruck flavor in beer

Use of Polyphenol-Rich Hop Products to Reduce Sunstruck Flavor in Beer

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ABSTRACT

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Sunstruck flavor is a widely known off-flavor in beer described as having meaty, sulfury, and skunky-foxy notes. It occurs when beer is exposed to sunlight during storage owing to photodecomposition of isohumulone in the presence of riboflavin. Polyphenols, tryptophan, ascorbic acid, and tryptophol are known to be off-flavor inhibitors. Hops are an important source of polyphenols and humulone. The aim of this study was to specify a suitable hop product and the process parameters to reduce the sunstruck flavor perception in beer. A selection of six hop products was analyzed for their content of total and single polyphenols as well as their bittering acid content. Moreover, brewing trials were carried out with single hop product batches. Because of the high total and single polyphenol content and low sunstruck flavor perception in beer, the tannin extract was selected for further trials. First, the time for hop addition and any added concentration during boiling were optimized to maximize the poly phenol transfer rate to the wort. Then, depending on these results, beers with different tannin extract concentrations and iso extract were pro duced. After exposure to light, the tasting results concluded that the higher the tannin extract level the lower the sunstruck perception. Furthermore, the effect on beer standard attributes resulting from the addition of the tannin extract was also evaluated.

Keywords: Sunstruck flavor, Polyphenols, Hop products, Tannin extract

Flavor stability has always been of particular interest in the brewing industry. Besides the well-known beer oxidation process caused by oxygen, the off-flavor known as sunstruck flavor may occur if beer is exposed to light without protection. This off-flavor, detected as early as 1875 by Lintner (35), is described as having meaty, sulfury, and skunky-foxy notes.

Sunstruck flavor occurs when beer bottled in translucent or green bottles without UV protection is subjected to light. Some authors (12,20,31) have defined brown bottles as the best protection against the formation of sunstruck flavor. However, a prestigious rational psychology poll states that people prefer to drink beer filled in clear glass bottles (Salaction Public Relations, Hamburg, Germany). The use of sunscreen on the bottle helps to prevent skunking of beer (36). Nevertheless, this kind of bottle normally has higher acquisition expenses. Because of the abovementioned sensitivity of beer components to the presence of light, bottling beer or other beverages in clear glass bottles is considered problematic.

The literature (33,34) describes the aroma-active compound 3-methyl-2-buten-1-thiol (MBT), of all odorless precursors, as the key compound responsible for sunstruck flavor. This substance, with an aroma threshold of 0.2–0.4 ng/L in water (21) and of 4–35 ng/L in beer (31), is considered to be one of the most important aroma-active compounds. However, after synthesis and appropriate dilution of MBT, Gunst and Verzele (21) stated that although similar, the smell was not identical to beer exposed to light. Therefore, it was assumed that other substances also are responsible for the overall aroma impression of sunstruck flavor.

Komarek et al. (31) analyzed pilsner and pale ale beer before and after exposure to sunlight by means of aroma extract dilution analysis. Besides MBT, other substances such as 3-(methylthio)-propanal, phenylacetaldehyde, 2-methylpropanal, 3-methylbutanal, and an unknown compound with a sulfury odor note were detected in the beers exposed to light.

By exposing model solutions containing isohumulones, riboflavin, and cysteine to light, Kuroiwa (33,34) suggested isohumulone as the main substrate to initiate the formation of MBT. Under these conditions, riboflavin is the only light-absorbing species (26,37). Therefore, photoreactivity can only occur via the intervention of riboflavin (16). Huvaere et al. (26) were able to determine the reaction products formed on the photo-oxidation of isohumulones and described the complete reaction. Hence, the photochemical sensitization of riboflavin is considered indispensable for the formation of MBT from isohumulones (42).

On exposure to visible light, riboflavin is excited to a singlet electronic energy state, followed by the transition to a triplet energy state, in which it has a strong affinity for electrons and hydrogen ions (13,41). Consequently, sensitized irradiation of iso-α-acids induces an electron transfer from the ionized tricarbonyl group of isohumulones to the triplet energy state, leading to the formation of the reactive triacylmethyl radicals. Stabilization pathways form a 3-methylbutyl-2-enyl radical, the main precursor of MBT in the presence of a suitable sulfur source (13,14).

Both sulfur-containing amino acids, cysteine (15,25,27,42,45) and methionine (15,27,31,39,42), were also identified in the formation of the sunstruck flavor. These amino acids serve as a sulfur source for the development of the sunstruck flavor, even accelerating its formation. On the other hand, polyphenols are described as inhibitors of the formation of the sunstruck flavor (5,10,14,15,19,22,31,32,41). Cardoso et al. (14) analyzed the influence of single phenols on the formation of the light-induced off-flavor. Their results suggested that these compounds quench both the singlet- and triplet-excited states of riboflavin responsible for the lightstruck flavor development, with rates close to the diffusion control. Pozdrik et al. (41) found that the polyphenols (+)-catechin and (–)-epicatechin preserve the riboflavin absorbance in light-exposed model beers by quenching its excited energy state. Alternatively, these authors also describe the possibility that isohumulone may extract electrons from the polyphenols to regain stability rather than to degrade to more stable minor products. Finally, polyphenols might extract electrons from photoreduced riboflavin, possibly returning the riboflavin to its original state before exposure to light (41). Also, the reduction of the sunstruck flavor by the addition of quercetin has been proven (6).

Polyphenols, on the contrary, have been described as catalyzing the transformation of amino acids to their corresponding Strecker aldehydes, methional and phenylacetaldehyde (10,31), which may provide an aged flavor. Nevertheless, the mechanism supporting Strecker aldehyde formation induced by light is still unclear (32).

Beer contains substances capable of preventing, to some extent, the degradation of riboflavin (41). The efficient deactivation by polyphenols makes this compound of importance as a protector

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against light-induced oxidation (5). The hop industry already offers reduced isomerized products such as rho- and hexa-iso-extracts, which protect beer from developing sunstruck characters (23). However, the growing trend toward natural products (clean label) and the higher costs of reduced isomerized products as well as brewing in accordance with the German purity law motivate the search for other natural hop products that may help toward isohumulone photodegradation. The selection of appropriate raw materials may contribute to the reduction or increase of polyphenol content, thereby reducing the sunstruck flavor.

The aim of this paper is to find a suitable hop product to reduce sunstruck flavor in beer by increasing the content of inhibiting polyphenols. During the extraction procedure of some products, the polyphenol content is discarded or increased. For example, tannin extract (TE) and polyphenol-enriched extract (PEE) are products both with an extremely high concentration of polyphenols. Other products such as pellets type 90 (P90) hardly suffer any loss of polyphenols during the manufacturing process and have around the same polyphenol concentration as hop cones. With this purpose, an increase in the contents of catechin, quercetin, and epicatechin in the final beer should reduce the formation of sunstruck flavor.

However, the increase of polyphenols in the final beer may also influence the final quality. Hence, it is also the aim of this project to find appropriate parameters for hop addition and to define the differences in the quality and sensorial characteristics of the final beers as a result of the higher presence of polyphenols.

EXPERIMENTAL

Materials

Commercially available hop products iso-extract 31% (IE) (Hallertauer Herkules), spent hop CO_2 extract ($SHCO_2E$) (Hallertauer Tradition), spent hop 45 (SH45) (Hallertauer Tradition), P90 (Hallertauer Tradition), and TE (Hallertauer Perle), and a commercially available PEE (Hallertauer Tradition) from the 2010 crop were provided by Hopsteiner (Simon H. Steiner, Mainburg, Germany). For the screening of the hop products and the determination of the boiling time and hop product concentration, unhopped Weyermann Bavarian pilsner extract (Weyermann, Bamberg, Germany) was used. For the brewing trials 100% pilsner malt (Weyermann) was used. The yeast employed was bottom-fermenting yeast (*Saccharomyces cerevisiae*) strain 34/78.

Screening of Hop Products

Hop products were analyzed on the contents of total and single polyphenols, bittering acid content, and sunstruck flavor perception in beer after exposure to light.

The single polyphenols determined were catechin, epicatechin, kaempferol glycoside, quercetin glycoside, iso-xanthohumol, xanthohumol, co-multifidol glycoside, kaempferol, and quercetin. The polyphenols were completely extracted by mixing 1.5 g of milled pellets ($SHCO_2E$, SH45, or P90) or 100 mg of extract (IE, TE, and PEE) with 30 mL of methanol for 10 min in an ultrasonic bath and then separated by decantation. The supernatant was filtered with a folded filter into a 100-mL volumetric flask, and then it was made up to the final volume. Before injection the solution was diluted 1:10 with methanol.

Determination of the content of single polyphenols was performed with an UltiMate 3000 ultra HPLC (Dionex, Idstein, Germany) coupled to an API 4000 QTRAP linear ion trap quadrupole mass spectrometer (AB Sciex, Darmstadt, Germany). The separation was performed in a Luna C18 column of 150 × 2 mm, 5 µm particle size, 100 Å pore size (Phenomenex, Aschaffenburg, Germany). The chromatographic conditions were flow rate of 0.3 mL/min and sample injection volume of 5 µL. The mobile phase consisted of 5 m*M* NH_4OAc (pH 5.0) in acetonitrile/water (95/5) as eluent A and 5 m*M* NH_4OAc (pH 5.0) in water as eluent B. A six-point external calibrating curve was used to quantify the single polyphenols.

The total polyphenol content was determined by mixing the corresponding product in water to a concentration of 10 g/L. The solutions were boiled for 60 min and filtered with a folded filter, and then the polyphenol content was determined according to MEBAK II Method 2.16.1 (28). The bittering acid content was likewise determined according to EBC Method 7.5 (17). Every sample was prepared and analyzed in triplicate.

Sensory trials to prove the sunstruck flavor perception were carried out by dissolving the same amount of the single hop products with a standard unhopped beer brewed with the unhopped Weyermann Bavarian pilsner extract. The extract was diluted with boiling distilled water to an original gravity of 12°P and fermented with an initial cell count of 5×10^6 cells of bottom-fermenting yeast. Fermentation was done for 5 days at 12°C and for 3 days at 16°C (3 bar). After 10 days at 0°C (0.5 bar), the unhopped beer was filtered (Seitz-KS80, Pall Seitz Schenk, Bad Kreuznach, Germany) before bottling. A control sample, without hop product addition, was included in the trial. For sunstruck flavor formation, the beers were bottled into clear glass bottles and flushed with pure nitrogen to exclude any oxygen influence. To induce sunstruck flavor formation the samples were standardized and exposed to light under two Osram daylight bulbs (18 W; 20 cm distance) for 3 days.

After illumination, the sensory evaluation of the beers was performed with a trained sensory panel of 10 persons. The sensory panel scored the smell intensity of the following descriptors rated on a six-point intensity scale from 0 to 3 (0 is no perception, and 3 is the highest perception): malty, fruity, meaty, sulfury, flowery-honey, potato-like, and wort-like. The descriptors malty, fruity, and flowery-honey are typical for a beer without the sunstruck flavor, and the descriptors meaty, sulfury, and potato-like are typical for a beer with the sunstruck flavor. The panel was trained with different dilution steps of beer exposed for 2 days to light to ensure maximal sunstruck flavor formation and with pure substance spiking to beer for the perception of the single descriptors. A beer exposed to light for 2 days received the highest score of the sunstruck flavor descriptors (meaty, sulfury, and potato-like) and the lowest for malty, fruity, and flowery-honey. Prior to data collection, the panelists trained for 2 weeks using the treatments in question. A consensus for the definitions and discussion were required to obtain unanimity in the use of the descriptors. Using the results of this evaluation, the most promising hop product (high polyphenol content and good sensory characteristics) was chosen for further trials.

Hop Product Concentration and Boiling Time of TE

For the determination of the appropriate boiling time and the product level of TE, standardized wort was produced with unhopped Weyermann Bavarian pilsner extract. The wort extract was diluted with boiling distilled water to an original gravity of 12°P. Every sample was analyzed in triplicate.

First, TE was added in six different concentrations to the boiling wort. The concentrations were 75, 150, 300, 600, 1,200, and 2,400 mg/L. Also, a control sample without TE was included. The seven samples were boiled for 60 min and filtered with a folded filter, and at the end the polyphenol concentration was determined according to MEBAK II Method 2.16.1 (28). The TE concentration was chosen so that the polyphenol content was maximized.

Subsequently, the boiling time was analyzed by boiling six samples with a concentration of 1,200 mg of TE/L (the selected concentration) for 30, 40, 50, 60, 70, and 80 min. After filtration with a folded filter, the polyphenol content of the samples was

determined according to MEBAK II Method 2.16.1 (28). The boiling time was chosen so that the polyphenol content was maximized.

Brewing Trials

The brewing trials were conducted in a 60 L pilot-scale brewing plant. Four beers with the same amount of IE but different TE were brewed. Every sample was brewed and analyzed in triplicate. The wort was brewed with a grist load consisting of 100% pilsner malt (Weyermann) with the following infusion mashing method: 62°C (30 min), 72°C (30 min), and 76°C (5 min). Following lautering, the wort was homogenized and divided into four aliquots (the four beers) and each one boiled for 60 min (selected boiling time). Both hop products were added at the beginning of boiling. The total addition of IE was calculated for 25 bitterness units in the beer. TE concentrations were 0 mg/L for Beer 0, 600 mg/L for Beer 1, 1,200 mg/L for Beer 2, and 2,400 mg/L for Beer 3.

After separation of the hot break and subsequent wort cooling, fermentation occurred in cylindroconical fermentation tanks. The initial cell count was 5×10^6 cells of bottom-fermenting yeast (*S. cerevisiae*). Fermentation was done for 5 days at 12°C and 3 days at 16°C (3 bar). Before filtration (Seitz-KS8, Pall Seitz Schenk) and bottling the beer was stored for at least 10 days at 0°C (0.5 bar).

The beers were bottled in brown glass bottles for quality analysis and for beer sensory examination (no exposure to light samples) and in clear glass bottles for sunstruck flavor sensory evaluation (after standardized exposure to light). The polyphenol content (MEBAK II Method 2.16.1), original gravity and alcohol (MEBAK II Method 2.9.6.3), iso-α-acids (MEBAK II Method 2.17.1), apparent rest extract (MEBAK II Method 2.9.6.3), limit of attenuation (MEBAK II Method 2.8.2), pH (MEBAK II Method 2.14), color (MEBAK II Method 2.12.2), haze stability (MEBAK II Method 2.14.2.1), foam (MEBAK II Method 2.18.2), and beer aroma compounds (MEBAK II Method 2.23.6) of each sample were analyzed according to MEBAK methods (28). The samples were also sensory evaluated according to Deutsche Landwirtschafts-Gesellschaft (DLG) standards as explained by MEBAK Method 4.5.2.1.1 (29) and also after exposure to light as described previously.

RESULTS AND DISCUSSION

Polyphenols (especially catechin, epicatechin, and quercetin) are known to protect beer against the formation of sunstruck flavor. Most of the polyphenols present in beer originate from malt; however, the hop product employed influences the final polyphenol content. Thus, choosing an appropriate (polyphenol-rich) hop product offers the possibility to enhance the polyphenol content. However, the polyphenol composition of the different hop products is normally unknown. The aim was to find suitable hop products to reduce the sunstruck flavor perception in beer by increasing the polyphenol content and with little, if any, impact on the sensory and analytical beer quality.

Screening of Hop Products

A selection of hop products was evaluated for total and single polyphenol and bittering acid contents. Also, the sensorial characteristics of hop products in beer after exposure to light were evaluated. The different hop products were IE as the reference sample, $SHCO_2E$, SH45, P90, TE, and PEE. This product panel (except IE used as the reference) was selected to provide a high source of polyphenols with a different composition but with little to no contribution to the iso-humulone content.

Table I shows the results obtained after the polyphenol (water-free), bittering acid, and single polyphenol contents determination. PEE showed the highest polyphenol content and IE (reference sample) the lowest. $SHCO_2E$, SH45, TE, and PEE have low levels of bittering acids compared with those of P90 and IE. SH45 and P90 have similar polyphenol content, but the bittering acid content of P90 is higher than that of SH45. Also, TE has relatively high polyphenol and low bittering acid contents.

The products either originate from different hop plant sections or are manufactured in a different manner that influences the polyphenol profile. As Table I shows, P90 has the highest catechin content followed by SH45 and TE. TE presented the highest epicatechin content. It is worth noting that quercetin and kaempferol were not detected in any of the samples, being only present as glycosylated structures (quercetin glycoside and kaempferol glycoside). Although PEE presents the highest quercetin glycoside content, neither catechin nor epicatechin nor quercetin was detected.

These results correspond with those found in the literature. SH45 polyphenols, delivered from the leaf fraction of the hop plant, are described as having a high (+)-catechin, procyanidin B3 content (1). The xanthohumol content of SH45 is lower than that of P90 and the rutin content is similar. Moreover, the hop cone polyphenols are mainly flavonols and prenylflavonoids. However, during the ethanol extraction for the production of the PEE and TE, the prenylflavonoids, such as xanthohumol, remain in the polar phase and do not end up in the final product. TE is also known to contain mainly low-molecular-weight polyphenols such as catechin, 2-[(2-methylpropanoyl)-phloroglucinol]1-*O*-beta-D-glucopyranoside, rutin, astragalin, and epigallocatechin gallate.

Beers brewed with the single hop products were standard illuminated and scored by the panel according to the smell intensity

TABLE I
Water-Free Polyphenol Content (mg/g), Bittering Acid Content (%), and Single Polyphenol Content (mg/kg) of the Different Hop Products[a]

Component	IE	$SHCO_2E$	SH45	P90	TE	PEE
Polyphenol	10 ± 3.5	41 ± 1.2	64 ± 2.3	67 ± 1.7	144 ± 5.4	>845 ± 0.0
Bittering acid	31.0	0.2	1.0	6.1	0.5	<0.2
Catechin (C)	n.d.	84 ± 11.0	228 ± 28.2	374 ± 52.7	110 ± 17.8	n.d.
Epicatechin (E)	n.d.	42 ± 5.6	67 ± 11.0	188 ± 33.0	249 ± 25.4	n.d.
Quercetin glycoside (QG)	n.d.	288 ± 37.8	155 ± 19.2	409 ± 55.3	1.588 ± 260.4	7,321 ± 863.9
Quercetin (Q)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ΣC+E+QG+Q	n.d.	414 ± 54.4	449 ± 58.4	971 ± 140.9	1,947 ± 303.6	7,321 ± 863.9
Kaempferol glycoside	n.d.	102 ± 12.6	79 ± 11.1	185 ± 20.1	673 ± 65.6	2,598 ± 314.3
Kaempferol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Iso-xanthohumol	n.d.	256 ± 33.5	17 ± 2.8	100 ± 14.1	159 ± 25.8	2893 ± 341.4
Xanthohumol	n.d.	4,573 ± 599.0	537 ± 54.2	3,202 ± 285.6	1.287 ± 208.4	352 ± 41.5
Co-multifidol glycoside	n.d.	1,534 ± 210.2	361 ± 40.4	1.395 ± 140.9	4,263 ± 380.3	20,801 ± 2123.8

[a] IE – iso-extract 31% (reference sample); $SHCO_2E$ – spent hop CO_2 extract; SH45 – spent hop 45; P90 – pellets type 90; TE – tannin extract; PEE – polyphenol enriched extract; and n.d. = not detectable.

of the descriptors: malty, fruity, meaty, sulfury, flowery-honey, potato-like, and wort-like. Figure 1 displays the sensory results of the beers after exposure to light. The sample with IE (the reference sample) had the highest scores for sunstruck flavor descriptors owing to the high presence of iso-α-acid, which degrades to MBT after exposure to light. $SHCO_2E$ was described as sulfury and P90 as meaty. TE and SH45 presented the lowest off-flavor perception. TE showed slightly higher scores than SH45 for the descriptor flowery-honey. The descriptors malty and fruity were similarly evaluated for both hop products.

The results show no correlation between polyphenol content and total sensory perception of the sunstruck flavor. Although P90 and $SHCO_2E$ have similar polyphenol contents as SH45, both products showed a higher sunstruck flavor perception. For P90, this was probably because of the higher bittering acid content.

Catechin, epicatechin, and quercetin are known (6,41) to preserve riboflavin absorbance in light-exposed model beers by quenching the singlet- and triplet-excited states of riboflavin. In Table I the contents of catechin, epicatechin, quercetin glycoside, and quercetin was added (indicated as ΣC+E+QG+Q). The high total and single polyphenol contents (and low bittering acid content) present in TE and SH45 may help to prevent sunstruck flavor formation, explaining the lower perception in both samples.

Although PEE has the highest polyphenol and ΣC+E+QG+Q content, it was described as having a high meaty impression. Polyphenols have been described by some authors (9,10,31,32,39) as catalyzing the transformation of amino acids to their corresponding Strecker aldehydes; in this case, methional and phenylacetaldehyde (10,31) are of importance. Komarek et al. (31) identified them in beer after exposure to light, and they are known to contribute to a meaty and sulfury off-odor. According to Allen and Parks (2) and Ballance (4) methional is the initial product of the Strecker degradation of methionine. Moreover, methional was demonstrated to be a product of the interaction of methionine and riboflavin in an aqueous system exposed to sunlight (39) and in milk (2). Also Hughes (24) states that methional is known to yield methanethiol on irradiation with light, influencing the subsequent description of sunstruck flavor. The high meaty impression provided by PEE may give insight into a catalyzation of the formation of both Strecker aldehydes contributing to the higher sunstruck flavor impression described by this product.

Regarding the results obtained, the TE has a high polyphenol content (144 mg/kg), high contents of catechin, epicatechin, and quercetin glycoside, a low bittering acid content, and the lowest sunstruck flavor impression. Therefore, this hop product was selected for the following brewing trials.

Hop Product Concentration and Boiling Time of TE

To ensure the presence of polyphenols provided by the TE in the final beer, different concentrations and boiling times were investigated. First, the TE was added in seven different concentrations (0, 75, 150, 300, 600, 1,200, and 2,400 mg/L) to unhopped wort. Table II shows the contribution of TE to the total polyphenol content and the calculated increase in relation to the polyphenol content of unhopped wort. Unhopped wort contained 149 ± 4.3 mg/L of polyphenols and increased to 276 ± 0.9 mg/L by the highest added TE level. This increase represents a content of more than 140 mg/L of polyphenols solely provided by the hop product. Whereas the polyphenol content increased considerably between TE levels of 75 and 300 mg/L, in contrast samples with 300–2,400 mg/L increased only slightly. According to the results, the concentration range of 600–2,400 mg of TE/L was considered for further investigations.

TABLE II
Contribution of Tannin Extract to the Total Polyphenol Content (mg/L) and Increase Relative to Unhopped Wort (%)

Tannin extract doses (mg/L)	Polyphenol content (mg/L)	Δ Polyphenol content to unhopped wort (%)
0	149 ± 4.3	0.0
75	144 ± 1.8	−4.0
150	181 ± 3.4	17.6
300	205 ± 3.5	27.1
600	222 ± 5.4	32.6
1,200	230 ± 0.9	35.1
2,400	276 ± 0.9	45.8

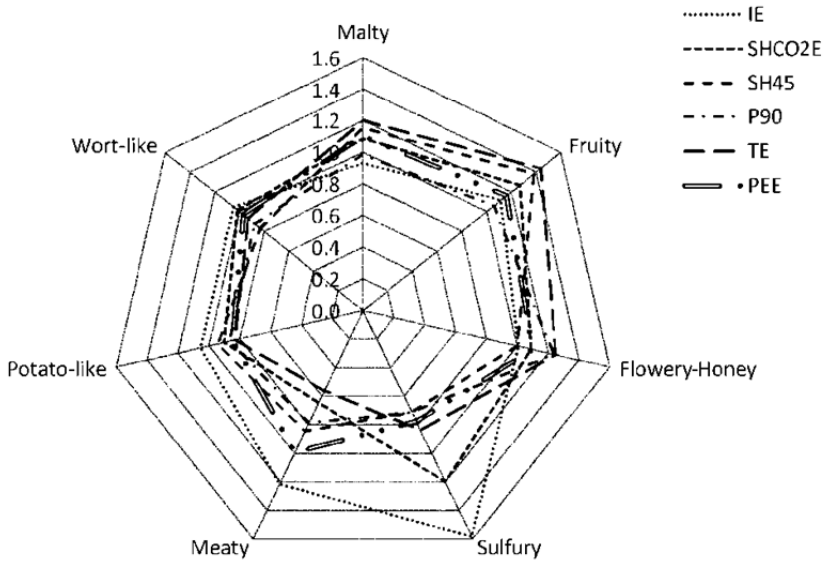

Fig. 1. Sensory results of the hop products dissolved in beer after exposure to light. IE = iso-extract 31% (reference sample); $SHCO_2E$ = spent hop CO_2 extract; SH45 = spent hop 45; P90 = pellets type 90; TE = tannin extract; and PEE = polyphenol enriched extract.

For the determination of an appropriate boiling time, TE (1,200 mg/L) was dissolved in unhopped wort and boiled for 30, 40, 50, 60, 70, and 80 min. Table III shows the total polyphenol content influenced by the boiling time and the increase in relation to unhopped wort. The polyphenol content augmented for boiling times of up to 60 min, reaching a concentration of about 213 mg/L (42.5% higher than unhopped wort). Longer boiling times than 60 min caused a decrease in the content, probably owing to interaction of the polyphenols with the proteins. According to these results, the selected boiling time used was 60 min.

Brewing Trials

To evaluate the influence of TE on the sunstruck flavor formation, four beers with different content of TE (Beer 0 as reference, 0 mg/L; Beer 1, 600 mg/L; Beer 2, 1,200 mg/L; and Beer 3, 2,400 mg/L) were brewed. Aliquots of IE were given to each beer to enable the formation of the sunstruck flavor. To induce the formation of the sunstruck flavor, the beers, in transparent bottles, were exposed to light under standardized conditions and were sensory evaluated. Figure 2 shows the influence on the sensorial descriptors of the added TE content to the beers. Beer 0 (the reference beer) presents a sensorial profile described by sunstruck flavor characteristic descriptors. On the contrary, the higher the concentration of TE added, the lower these three descriptors were weighted. Moreover, the higher the TE content, the higher the score of the descriptors malty, fruity, and flowery-honey (typical for a beer not exposed to light). The most significant difference is represented by the descriptor sulfury between Beer 0 and 3. This descriptor decreases in perception by more than 1 point when increasing the TE content. Although the weighting of the typical beer aroma descriptors (malty, fruity, and flowery-honey) slightly increased, the perception of sunstruck flavor decreased when more TE was added to the beer.

As confirmed in Figure 2, the sunstruck flavor can be reduced by adding the TE during the brewing process. However, it is also important to determine the influence of the TE on the final beer quality (foam, turbidity, original gravity, etc.). Therefore, the beers were analyzed according to the standard quality parameters. Furthermore, the aroma compounds of the final beers were determined, and a sensory evaluation according to the DLG standards was carried out. Table IV shows the results of the chemical-technical analyses.

TABLE III
Total Polyphenol Content (mg/L) by Different Boiling Times and Increase Relative to Unhopped Wort (%)

Boiling time (min)	Polyphenol content (mg/L)	Δ Polyphenol content to unhopped wort (%)
30	160 ± 3.7	7.1
40	174 ± 1.7	16.6
50	181 ± 1.5	20.9
60	213 ± 9.9	42.5
70	206 ± 3.4	38.1
80	184 ± 2.1	22.9

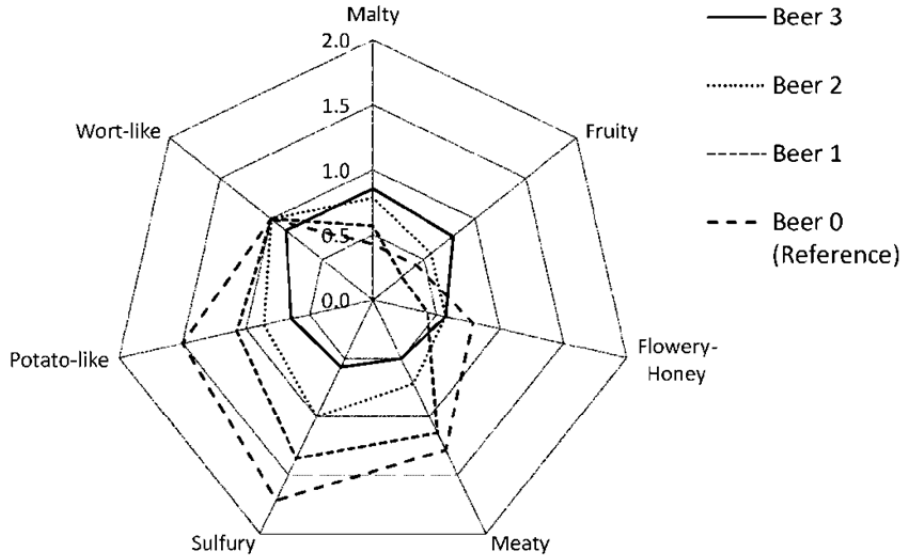

Fig. 2. Sensory evaluation after exposure of the beers with different tannin extract to light.

TABLE IV
Standard Quality Analysis of the Final Beers Brewed with Variation of Tannin Extract Level and the Reference Beer

Parameter	Units	Beer 0 (reference)	Beer 1	Beer 2	Beer 3
Original gravity	w/w %	12.2 ± 0.07	12.1 ± 0.24	12.2 ± 0.03	12.2 ± 0.07
Alcohol	Vol %	5.2 ± 0.02	5.2 ± 0.13	5.2 ± 0.08	5.1 ± 0.14
Total polyphenol content	mg/L	160 ± 12.5	187 ± 7.0	216 ± 10.1	255 ± 8.2
Iso-α-acids	mg/L	24.25 ± 0.15	24.6 ± 0.40	26.55 ± 2.65	27.1 ± 1.30
Apparent rest extract	w/w %	2.4 ± 0.13	2.3 ± 0.01	2.4 ± 0.19	2.7 ± 0.20
Limit of attenuation	%	80.7 ± 0.95	81.3 ± 0.30	80.5 ± 1.50	78.2 ± 1.75
pH		4.8 ± 0.06	4.8 ± 0.01	4.9 ± 0.01	4.9 ± 0.03
Color	EBC	7.9 ± 0.15	8.1 ± 0.05	8.0 ± 0.30	9.4 ± 0.30
Foam NIBEM	sec	287 ± 14.5	277 ± 4.0	266 ± 2.5	279 ± 12.0
Haze stability (0/60°C)	Days	4	4	4	4

The results show an increase in polyphenol content with higher TE levels. An addition of 2,400 mg/L (Beer 3) leads to an increase of about 40% of the polyphenol content with respect to the reference beer. Hop polyphenols are also known to contribute partly to the color of beer, clearly seen in the results of these analyses. The higher the TE level the higher the color level. Gray et al. (20) mentioned that substances responsible for the color of the beer (such as melanoidins or tannins) selectively absorb light before it exerts its full photochemical effect and thus stimulates sunstruck flavor. Also, Kuroiwa et al. (34) considered that colored substances inhibit the evolution of hydrogen sulfide by removing energy absorbed by riboflavin. Later these ideas were confirmed by Sakuma et al. (42) by exposing different brands of beer to light and determining the content of MBT. Their results showed that in dark lager beer this compound was not detected. The increase in the polyphenol content because of the TE may serve as protection against sunstruck flavor not only because of the antioxidative capacity of the polyphenols but also because of the increase in the color of the final sample.

Moreover, there is a slight increase of iso-α-acid concentrations present in the beer. According to the producer, the TE contains <2% of bitter compounds, which may explain the increase of these acids when increasing the TE.

Polyphenols undergo a number of transformations during the brewing process and are able to interact with substances present in beer, thus being able to influence the overall aroma and beer flavor. The beer aroma compounds were quantified, to monitor the impact on the beer flavor provided by the higher polyphenol content. Table V shows the quantities of these compounds present in the four beers.

Beer 3 (highest TE concentration) had a slightly higher concentration of the following compounds: ethyl butyrate, ethyl decanoate, isovaleric acid, hexanoic acid, caprylic acid, decanoic acid, and lauric acid. These compounds are mainly fatty acids and fatty acid esters. According to Boulton and Quain (11), short-chain fatty acids appear via an autolytic mechanism or through ethanol-induced membrane leakage. A possible reason for the increase of fatty acids and fatty acid esters in Beer 3 may be an antimicrobial activity of TE. Some authors (30,38,44) have described the antimicrobial activity of polyphenols from tea, rosemary, or *Artemisa* spp. against yeast. Also, the hop polyphenol xanthohumol has gained considerable interest (7,8) because of its antimicrobial activity (among others). The quantity of polyphenols added to Beer 3 may exert an important antimicrobial activity to the yeast present, inducing autolysis and thereby increasing the content of fatty acids. Another explanation of this increase may be an interaction between polyphenols and odorants. In red wines, although polyphenols are considered a fraction of the nonvolatile matrix, it has been proved that the existence of odorant–polyphenol interactions thus modifies the aroma volatility and perception (40). However, no specific information of the interaction mechanism between fatty acids and fatty acid esters with polyphenols in beer was found.

These compounds are undesirable components because of the negative impact on beer flavor and foam. However, according to Back (3) the concentration of these compounds found in Beer 3 is within the average for lager beer. Also, the measured foam is not influenced owing to the higher amount of these substances.

Moreover, the concentration of nerol decreased with the increasing polyphenol contents in the sample. The monoterpene alcohol nerol originates from hop. According to Takoi et al. (43), nerol can be converted into linalool and α-terpineol by yeast, which may explain the decreased levels found in the results. However, this compound has a higher perception threshold than other monoterpene alcohols and, therefore, might not contribute significantly to the hopped beer flavor (43).

To clarify the influence of TE addition on the total sensory profile and presence of other common off-flavors, the beers were also sensory evaluated according to the DLG scheme. The beers were scored on the following attributes: smell, taste, mouthfeel, carbonation, and bitterness. Table VI shows the results of the sensory evaluation. The results correspond with the conclusions of Gawel (18), who described the monomeric and polymeric flavan-3-ols as inducing both astringent and bitter mouth sensations. The higher the TE content, the lower the score obtained by the descriptor mouthfeel. The detriment in the mouthfeel may be caused by an increased astringency owing to the higher content of polyphenols. The same was found for the attribute bitterness: the higher the TE content, the lower the bitterness score. When the beers were compared according to smell and carbonation, there was no significant difference when adding TE. In addition, the descriptive comments of the samples are summarized here. Beer 0 was described in some cases as having diacetyl off-flavor, which may be the reason that the taste average of Beer 0 was lower than that for the other

TABLE V
Aroma Compounds Concentrations (μg/L) of the Beer Brewed with Increasing Tannin Extract[a]

Compound	Beer 0 (reference)	Beer 1	Beer 2	Beer 3	
1-Hexanol	5 ± 0.5	10 ± 1.0	9 ± 0.5	12 ± 1.0	
1-Heptanol	2 ± 0.5	1 ± 0.0	1 ± 0.0	1 ± 0.0	
1 Octanol	13 ± 0.5	14 ± 0.5	15 ± 0.5	13 ± 0.0	
1-Decanol	7 ± 1.0	1 ± 0.0	7 ± 0.5	1 ± 0.0	
2-Phenylethanol	2,861 ± 0.3	2,825 ± 1.1	2,949 ± 0.2	2,838 ± 0.3	
Isobutyl acetate	28 ± 2.5	29 ± 1.5	26 ± 0.5	32 ± 1.5	
Hexyl acetate	3 ± 0.0	3 ± 0.0	3 ± 0.0	5 ± 0.0	
Heptyl acetate	2 ± 0.0	2 ± 0.0	3 ± 0.0	2 ± 0.0	
Octyl acetate	3 ± 0.0	2 ± 0.0	3 ± 0.0	2 ± 0.0	
Furfuryl acetate	3 ± 0.5	2 ± 0.0	3 ± 0.0	3 ± 0.5	
2-Phenylethyl acetate	695 ± 3.0	654 ± 1.4	667 ± 2.5	643 ± 0.6	
Ethyl butyrate	76 ± 5.5	79 ± 4.0	78 ± 0.5	87 ± 4.0	↑
Ethyl hexanoate	222 ± 1.3	243 ± 3.5	184 ± 1.7	206 ± 0.4	
Ethyl octanoate	255 ± 1.0	248 ± 0.1	226 ± 2.4	264 ± 0.1	
Ethyl decanoate	28 ± 2.0	37 ± 0.5	37 ± 0.5	52 ± 0.5	↑
Isovaleric acid	440 ± 11.1	556 ± 8.0	670 ± 1.0	636 ± 5.2	↑
Hexanoic acid	1,402 ± 0.0	1,307 ± 2.0	1,317 ± 1.8	1,729 ± 0.8	↑
Caprylic acid	4,530 ± 0.6	4,851 ± 0.7	4,793 ± 12.1	5,620 ± 0.8	↑
Nonanoic acid	15 ± 0.5	14 ± 1.5	12 ± 1.0	17 ± 0.5	
Decanoic acid	526 ± 0.9	604 ± 0.4	635 ± 0.5	813 ± 3.2	↑
Lauric acid	56 ± 4.0	62 ± 1.0	64 ± 1.0	117 ± 23.0	↑
γ-Lactone	16 ± 0.5	16 ± 0.5	16 ± 1.0	17 ± 0.5	
Geraniol	4 ± 0.5	7 ± 0.0	1 ± 0.5	2 ± 0.0	
α-Terpineol	2 ± 1.0	3 ± 0.0	3 ± 1.0	3 ± 0.0	
Caryophyllene	0 ± 0.0	0 ± 0.0	0 ± 0.0	1 ± 0.0	
Nerol	62 ± 7.5	37 ± 0.0	37 ± 0.0	31 ± 0.0	↓
Humulene	3 ± 0.5	2 ± 0.5	1 ± 0.0	1 ± 0.0	

[a] Arrows: ↑ indicates concentration increase between Beer 0 and 3, and ↓ indicates concentration decrease between Beer 0 and 3.

TABLE VI
Sensory Evaluation of the Beers with Addition of Tannin Extract According to Deutsche Landwirtschafts-Gesellschaft Standards

Beer	Smell	Taste	Mouthfeel	Carbonation	Bitterness	Total Evaluation	Description
Beer 0	4.0	3.9	4.6	4.1	4.3	4.1	Strong mouthfeel
Beer 1	4.1	4.1	4.2	4.1	4.2	4.1	Fruity, malty
Beer 2	4.0	4.0	4.2	4.1	3.8	4.0	Slightly lagging bitterness
Beer 3	4.2	4.2	4.3	4.2	3.9	4.1	Hoppy, pronounced bitterness, herbaceous

three samples. Beers 2 and 3 were described with a stronger bitterness than Beers 0 and 1; however, Beer 3 may have an extremely pronounced bitterness that may alter the characteristic sensory profile of the beer.

CONCLUSIONS

Polyphenols such as catechin, quercetin, and epicatechin are described as protectors from the formation of sunstruck flavor by quenching the singlet- and triplet-excited states of riboflavin and thus preventing the formation of sunstruck flavor. Compositional analysis and brewing trials of six hop products were carried out to evaluate their ability to prevent the formation of sunstruck flavor through the polyphenol content provided to beer. The ranking of the total polyphenol content of the hop products was IE < $SHCO_2E$ < SH45 < P90 < TE < PEE. Brewing trials were carried out with single hop product batches. The results showed no correlation between the total polyphenol content and the sensory perception of sunstruck flavor. However, samples with high catechin, epicatechin, and quercetin glycoside contents (TE and SH45) presented the lowest off-flavor perception. Besides the high catechin, epicatechin, and quercetin glycoside contents, both hop products were also found to have low bittering acid content. Comparing both samples, TE showed slightly better scores and a higher total polyphenol content and was, therefore, selected for the following brewing trials.

After optimizing the dosing parameters to ensure a high polyphenol transfer rate to the wort, four beers with different TE concentrations (0, 600, 1,200, and 2,400 mg/L) were brewed. The influence of the increased polyphenol content on the reduction of sunstruck flavor perception was evaluated. The results showed that it is possible to reduce the sunstruck flavor perception in final beer by adding TE during boiling due to the higher presence of polyphenols. Although the weighting of the typical beer aroma descriptors slightly increased, the perception of sunstruck flavor decreased when more TE was added to the beer.

Also, the differences in quality and sensorial characteristics of the developed final beers owing to the higher presence of polyphenols were defined. An increase of up to 40% of the polyphenol content in the beers was obtained. Moreover, TE also increased partially the color of the beers (1.5 EBC), which may also contribute to the absorption of light and, therefore, enhance protection against sunstruck flavor.

Fatty acid and fatty acid ester contents in the beer brewed with the highest TE concentration were higher than in the other three beers (see Table V). However, the concentration of these compounds was within the average concentrations of lager beer.

Sensory profiling of the samples (not exposed to light) according to the DLG scheme showed that the sample with the highest TE content was described to have a pronounced pleasant bitterness.

It can be concluded that a concentration of TE between 600 and 1,200 mg/L supports the prevention of sunstruck flavor. A higher concentration may have negative effects on the sensory characteristics of the final beer.

ACKNOWLEDGMENTS

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3.3. Influence of Malting on the Protein Composition of Spelt (*Triticum spelta* L.) Frankenkorn

Influence of Malting on the Protein Composition of Spelt (*Triticum spelta* L.) 'Frankenkorn'

Alicia Munoz-Insa, Martina Gastl,† and Thomas Becker

ABSTRACT

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Although spelt has promising aptitudes for beer and beverage production, almost no investigation about its protein modifications during malting has been conducted. Spelt proteins during the malting process were separated and analyzed, first, according to the isoelectric point and molecular weight (MW) and, second, according to solubility and MW. Moreover, the composition of the free amino acid was determined. Spelt proteins could be divided into a total of 13 zones according to their isoelectric point and MW. Most of the present proteins (90%) had a MW between 35,000 and 60,000 and an isoelectric point between 5.84 and 9.52. The most prominent protein fraction was defined by an isoelectric point between 5.84 and 6.89 and a MW between 35,000 and 55,000. Generally, a protein degradation process during malting was found. The Osborne fractionation revealed an albumin band pattern between 14,000 and 131,700, globulin ranging from 13,400 to 130,500, gliadin from 14,500 to 220,800, and glutenin from 19,800 to 215,300. The free amino acid composition increased in concentration except for asparagine, which decreased during malting. This paper contributes to the understanding of the protein modifications and metabolic changes during the malting process of spelt and facilitates the determination of the potential of this cereal for the production of cereal-based products.

In the last years the interest toward hulled wheat has increased owing to the low-input techniques used for its management, its outstanding stress resistance, and its quality performance (Campbell 1997; An et al. 2005). Spelt has also been attached special importance because of the uses it can be put to for natural, organic, healthy, and special foods, therapeutic properties attributed to their derivatives, and their potential as a source of genes for breeding hull-less wheats (Sharma et al. 1981; Piergiovanni et al. 1996). Nowadays, spelt is still considered an alternative crop compared with widely cultivated modern winter wheat (Rozenberg et al. 2003). Although spelt is genetically closely related to wheat, it has been demonstrated that there is a clear divergence between spelt and common wheat based on DNA and protein variability (Abdel-Aal and Hucl 2005).

Spelt has applications in many areas of the food industry, in the majority of cases as an ingredient for the baking industry. Several cultivars have been examined for the potential in the baking industry (Abdel-Aal et al. 1996, 1997, 1998, 1999; Marconi et al. 1999, 2002; Bonafaccia et al. 2000; Skrabanja et al. 2001; Zanetti et al. 2001; Bojnanska and Francakova 2002; Schober et al. 2006). Although spelt has promising aptitudes for beer and beverage production, it is still considered a niche product. Only a few research projects (Krahl et al. 2008; Munoz-Insa et al. 2012) have been done on the capability of spelt for this purpose.

Generally, spelt may contain higher levels of protein than barley and wheat, ranging between 11.0 and 18.3% dry basis (Belitz et al. 1989; Grela 1996). Bognar and Kellermann (1993) and Abdel-Aal et al. (1995) attribute this difference to the larger aleurone layer present in spelt. Investigation of spelt proteins has been used for several applications. By analyzing the composition of protein fractions, some authors investigated the origins of European spelt (Yan et al. 2003) and its genetic diversity (An et al. 2005), analyzed its baking quality attributes (Smolková et al. 2000; Schober et al. 2006), attempted to differentiate between spelt and wheat to classify and endorse new varieties and cross breeds or for cultivar legal protection (Harsch et al. 1997; Radić et al. 1997; Radić-Miehle et al. 1998; Dvořáček and Čurn 2003; Koenig et al. 2015), or tried to evaluate the influence of the crossing between these two cereals (Schober and Kuhn 2003).

Data about the distribution of Osborne fractions in spelt are contradictory. Koenig et al. (2015) stated that gliadins are the most abundant proteins in spelt (61.5–67.6%), followed by the albumin and globulin fraction (19.9–22.8%) and the glutenin fraction (12.5–17.4%). Belitz et al. (1989) determined glutenins as the most abundant proteins of spelt (54.6–49.4%), followed by gliadins (28.6–30.6%) and then the albumin and globulin fraction (16.5–20%). Although considerable work has been performed in the investigation of the gliadin and glutenin, little is known about the proteomic profiles during the grain development stages.

Malting includes the controlled germination of cereals, in which hydrolytic enzymes are synthesized, and the cell walls, proteins, and starch of the endosperm are largely digested, making the grain more friable (Enari and Sopanen 1986; Bamforth and Barclay 1993; Celus et al. 2006). One of the most important physicochemical changes during malting is the degradation of the proteinaceous matrix (storage proteins) that surrounds the starch granules within the cells of the endosperm and their conversion into soluble peptides and amino acids to provide substrates for the synthesis of proteins in the growing embryo (Briggs 1998; Klose et al. 2009). Amino acids influence the aroma and flavor profile of the final product (e.g., the beer) by participating in different reactions.

The high protein content of spelt may cause difficulties in the brewing process such as lautering detriment, haze, and poor flavor stability. Therefore, understanding the protein modifications and metabolic changes during the malting process of spelt may help to determine the potential of this cereal and cereal products for the development of cereal-based beverages, among others. The aim of this project is to investigate spelt protein changes during and owing to malting. The proteins were analyzed at the different malting stages according to their isoelectric point (pI), molecular weight (MW), solubility, and free amino acid composition.

MATERIALS AND METHODS

Materials. Dehulled spelt (*Triticum spelta* L.) 'Frankenkorn' with a moisture content of 14%, a protein content of 17%, and germination capacity of >95% was procured from Schapfen Mühle (Ulm, Germany). The raw material was grown in 2011 and harvested and dehulled by Schapfen Mühle. Thereafter, the spelt was stored under cool and dry conditions until use.

Spelt was malted according to the optimum malting parameters given by Munoz-Insa et al. (2012) and according to the Mitteleuropäische Brau- und Analysenkommision (MEBAK)

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malting method 2.5.3.1 (Anger 2006): five days of germination, 47% moisture content, and a set germination temperature of 17°C in temperature-controlled chambers with 75% relative humidity.

The steeping water was equilibrated by placing it for 24 h prior to steeping in the climate chambers. Thus, the degree of steeping was determined by weighing the samples and calculating the water content of each one, taking into account the moisture content at the beginning of the malting process. In comparison with common barley, dehulled spelt has a lower ability to absorb water and tends to puff up; therefore, steeping was done progressively.

Samples were taken every 24 h: raw material (RM), first (1D), second (2D), third (3D), fourth (4D), and fifth (5D) day of malting, and the final malt. Samples 1D, 2D, 3D, 4D, and 5D were dried by means of a BETA 2-16 freeze dryer (Martin Christ GmbH, Osterode am Harz, Germany). Sample malt was kilned according to MEBAK method 2.5.3.1 (Anger 2006). All the samples had a water content between 4.5 and 5.0%. After freeze drying or kilning, rootlets and sprouts were removed. Before analyzing, the samples were milled with a disc mill (Bühler, Braunschweig, Germany) set at a fine setting of 0.05 mm.

Characterization of Proteins According to pI and MW. The proteins of every sample were characterized according to pI and MW by using, in combination, both the 3100 OFFGEL electrophoresis system (Agilent Technologies, Santa Clara, CA, U.S.A.) and the Agilent 2100 bioanalyzer. First, the proteins present in the sample were extracted by mixing 40 mg of milled sample with 400 µL of buffer. The buffer used was composed of 2M urea, 15% glycerol, 0.1M Tris HCl, and 0.1M dithiothreitol with a pH of 8.8, and it is normally used for the protein extraction for samples analyzed with the bioanalyzer. The mixture was vortexed briefly and immediately placed in an ultrasonic water bath for 5 min. Afterward, the samples were centrifuged at 14,000 × *g* for 15 min.

The pI-based separations were performed on the 3100 OFFGEL fractionator in combination with the OFFGEL kit at pH 3–10 with a 24-well setup according to the supplier's protocol. The MW-based separations were then performed on the Agilent 2100 bioanalyzer in combination with the Protein 230 kit and the Agilent 2100 Bioanalyzer Expert software. All samples were prepared according to the supplier's protocol. Each run included a ladder comprising reference proteins with MWs of 7,000, 15,000, 28,000, 46,000, 63,000, 95,000, and 150,000 plus an upper marker of 240,000 and a lower marker of 4,500. Each sample contained an internal standard comprising the upper and lower markers to allow for quantification. The results obtained were standardized to the same upper marker and plotted with Matlab version 7.10.0.499, R2010a (MathWorks, Natick, MA, U.S.A.). All samples were analyzed in triplicate ($n = 3$).

Characterization of Proteins According to Solubility—Modified Osborne Fractionation. Osborne fractions were separated as previously described (Faltermaier et al. 2013). The samples were then MW separated by the lab-on-a-chip technique without any further preparation by using the Agilent 2100 bioanalyzer. For this, the Protein 230 kit and the Agilent 2100 Bioanalyzer Expert software were used. All samples were prepared according to the supplier's protocol. Each run included a ladder comprising reference proteins with MWs of 7,000, 15,000, 28,000, 46,000, 63,000, 95,000, and 150,000 plus an upper marker of 240,000 and a lower marker of 4,500. Each sample contained also an internal standard comprising the upper and lower markers to allow for quantification. All samples were analyzed in triplicate ($n = 3$). The fractions were assigned according to the MW.

Composition of Free Amino Acids. Sample preparation for amino acid analysis with HPLC was carried out by mixing 0.15 g of milled sample with 1.5 mL of distilled water. The mixture was stirred for 60 min and 329 × *g* at 65°C and finally centrifuged at 2,900 × *g* for 10 min. The supernatant was diluted 1:5 with distilled water. The amino acids present in the diluted supernatant were quantified by an HPLC (U3000, Dionex, Sunnyvale, CA, U.S.A.) as described by Krömer (2006). Quantification was done by a six-point external calibrating curve for each amino acid. All samples were analyzed in triplicate ($n = 3$).

α-Amylase Activity. The α-amylase activity was measured by using a Megazyme enzyme kit (Megazyme, Bray, Ireland) according to the standard methods of the European Brewery Convention (Eerde 1998). Analytical procedures were carried out in triplicate.

Statistical Analysis. Statistical analysis was performed by one-way ANOVA to test for differences within the malting process. Additionally, unpaired two-way *t* tests were applied to determine differences between the individual malting stages. The software used for both tests was Microsoft Excel 2010 (Microsoft, Seattle, WA, U.S.A.). In all cases, statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Characterization of Proteins According to pI and MW. Spelt proteins were fractionated and analyzed according to their pI and MW during the different stages of the malting process. Figure 1 shows the results of the samples from RM through the malting process until the final malt obtained by the combination of OFFGEL fractionation (separation according to pI) and capillary electrophoresis (separation according to MW). The combination of the OFFGEL fractionation and the bioanalyzer offers an alternative technique to the conventional two-dimensional gel electrophoresis (2D-PAGE). Other authors (Faltermaier et al. 2013) obtained comparable results by analyzing wheat proteins by means of the 2D-PAGE and the combination of the OFFGEL fractionator and the bioanalyzer.

Figure 1 shows simulated "gel-like" images of spelt proteins separated according to the MW (*x* axis) and the pI (*y* axis). This figure clearly reveals a protein degradation process during malting. Most of the proteins found in the samples have a pI between 6 and 7. According to the results, spelt proteins could be divided into several separate zones (named from A to M). Table I shows the different protein zones and their corresponding pI and MW ranges. The MWs of spelt proteins ranged between 13,000 and 236,000. However, most of the proteins were characterized by a MW range from 35,000 to 60,000. The proteins found were mainly localized in zones C to F, which account for about 90% of the total protein concentration.

Figure 2 depicts the protein concentration of zones C to F. Spelt proteins were mainly present in zone C, with a pI range between 5.84 and 6.89 and a MW between 35,000 and 55,000 (Fig. 1). Zone C accounted for 40–50% of the present proteins in all samples. During malting this peak underwent a significant degradation, diminishing by more than 50% of its original concentration. The degradation of this zone mainly took place during the first 24 h of germination. Also, zones A, B, E, F, H, J, and L decreased to some extent during the process. Protein zone D (pI 6.89–8.47, MW 35,000–55,000) showed significant differences during malting; however, no clear tendency may be described.

Modified Osborne Fractionation. To provide fundamental understanding of the degradation of spelt proteins during malting, the proteins were separated according to their solubility in water (albumin), salt solutions (globulin), alcohol (gliadin), or acidic solutions (glutenin). Thereafter, each fraction was separated according to its MW by means of the bioanalyzer. Figures 3–6 show the changes on the protein profile and the peak relative concentration of albumin, globulin, gliadin, and glutenin fractions during malting.

Albumin Fraction. The albumin fraction consists of a complex mixture of structural, metabolic, and storage proteins (Gupta et al. 1991; Shewry and Halford 2002). The MW of the albumin fraction of spelt ranged between 14,000 and 131,700. The electropherograms showed a band pattern with a total of 12 protein peaks characterized with typical bands of MWs 14,000, 15,500, 19,000, 25,100, 29,000, 39,300, 50,700, 59,800, 68,400, 74,500, 98,400, and 131,700. Overlapping peaks were quantified together as Alb-A

(peaks 14,000 and 15,500), Alb-B (peaks 25,100 and 29,000), and Alb-C (peaks 59,800, 68,400, and 74,500).

Figure 3A shows the albumin fraction differences between RM, 3D, 5D, and the final malt. The relative concentration of every peak is depicted in Figure 3B. Peaks Alb-B, 39,300, and 50,700 increased steadily during the malting process up to 5D and then decreased in concentration during kilning. Alb-C showed the same behavior; however, it increased up to 2D and then decreased steadily. Alb-A and peak 19,000 did not show significant changes during malting. Peaks 98,400 and 131,700 were completely degraded after the 1D sample. These results correspond with the findings of Gupta et al. (1991), who demonstrated that some HMW albumins in bread wheat disappeared rapidly during seed germination and early seedling growth. These authors suggested that HMW albumins might serve as nutritional sources in the early growth stages of wheat.

The increase of peak 50,700 between 4D and 5D is worthy of note. It almost quadrupled its concentration, and then it decreased slightly during kilning. Rothstein et al. (1984) determined that α-amylase of wheat consists of two polypeptide bands with an apparent MW of 49,000 and 42,000. The latter is probably a polypeptide formed by the methylation or glycosylation of the first one. Also Muralikrishna and Nirmala (2005) characterized this enzyme with a MW between 42,000 and 54,000 in cereals such as wheat, barley, sorghum, and rice. The α-amylase activity was measured to prove its correlation with the increase in peak 50,700 concentration. The enzyme activity during the malting process is shown in Table II. The increase of α-amylase activity from 4D to 5D, as well as the decrease from 5D to malt, corresponded with the behavior of peak 50,700.

Globulin Fraction. Similarly to albumin, spelt globulin was characterized by a band pattern composed of 12 peaks ranging from 13,400 to 130,500: 13,400, 14,500, 22,700, 23,700, 27,500, 38,600, 46,400, 52,300, 59,400, 67,200, 97,200, and 130,500. Figure 4A shows the protein profiles of the globulin fractions of RM and final malt. The globulin fraction was divided into three large areas: Glo-A (peaks 13,400 and 14,500), Glo-B (peaks 22,700, 23,700, and 27,500), and Glo-C (peaks 38,600, 46,400, 52,300, 59,400, and

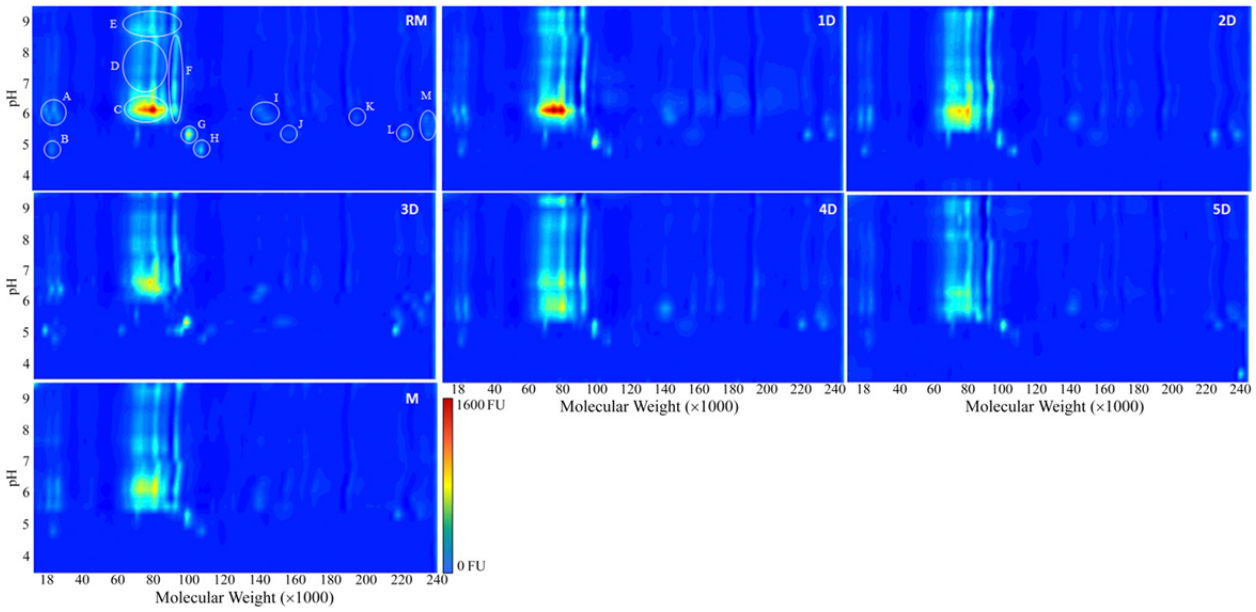

Fig. 1. Two-dimensional separation of spelt proteins as affected by germination. The *y* axis shows separation according to isoelectric point, and the *x* axis represents molecular weight. RM = raw material; D = days of germination; and M = malt.

TABLE I
Spelt Protein Zones and the Corresponding Isoelectric Point (pI), Molecular Weight, and Relative Concentration[y]

Zone	pI	MW (×10³)[z]	Raw Material (FU)	Day 1 (FU)	Day 2 (FU)	Day 3 (FU)	Day 4 (FU)	Day 5 (FU)	Malt (FU)
A	5.84–6.37	13–18	15,647 ± 316.7a	8,709 ± 407.1b	10,602 ± 946.1b	7,812 ± 322.0c	7,340 ± 116.5c	5,509 ± 195.9d	11,901 ± 248.6e
B	4.53–5.06	14–16	2,012 ± 352.6a	1,796 ± 363.9a	2,101 ± 98.6a	1,751 ± 321.6a	1,461 ± 87.8a	1,304 ± 62.6a	1,038 ± 53.7b
C	5.84-6.89	35–55	221,846 ± 339.7a	142,022 ± 485.5b	135,613 ± 140.0c	82,685 ± 1,097.8d	123,046 ± 562.9e	94,573 ± 847.6f	119,009 ± 760.3g
D	6.89-8.47	35–55	56,239 ± 947.3a	67,393 ± 705.6b	62,946 ± 720.5b	64,934 ± 1,245.0b	67,560 ± 980.7b	53,431 ± 1,390.6c	58,243 ± 1,479.5c
E	8.47–9.52	35–60	53,859 ± 920.9a	47,005 ± 1,374.5a	56,344 ± 1,094.1a	34,017 ± 1,744.5b	44,230 ± 948.8b	34,422 ± 517.6b	42,894 ± 648.1b
F	5.84–8.47	55–60	40,130 ± 889.4a	31,524 ± 837.4a	21,503 ± 68.8b	24,034 ± 648.8b	31,098 ± 823.3b	17,349 ± 432.3c	26,268 ± 1,082.5c
G	5.06–5.58	62–68	7,361 ± 226.8a	5,233 ± 78.3b	8,125 ± 111.7c	12,305 ± 398.4d	5,634 ± 66.1e	4,597 ± 191.1f	6,269 ± 120.1g
H	4.79–5.06	67–74	3,356 ± 198.0a	2,378 ± 24.5b	2,463 ± 80.6b	2,256 ± 71.6c	1,254 ± 299.2d	1,021 ± 0.0d	1,823 ± 269.7e
I	5.84–6.11	105–115	8,558 ± 217.1a	3,217 ± 256.1b	7,821 ± 516.0c	3,252 ± 172.2d	5,412 ± 449.8e	2,494 ± 160.7f	2,595 ± 98.7f
J	5.32	125–130	2,038 ± 164.5a	844 ± 192.3b	842 ± 31.6b	1,730 ± 50.2c	952 ± 45.2d	639 ± 120.8e	411 ± 259.6e
K	5.84	170–175	1,161 ± 407.2a	415 ± 33.7a	2,254 ± 741.3b	470 ± 150.3c	2,449 ± 355.1d	0 ± 0.0e	227 ± 68.6f
L	5.32–5.58	210–220	2,533 ± 326.4a	3,080 ± 259.6a	2,118 ± 125.9b	2,035 ± 163.5b	1,891 ± 0.0c	1,667 ± 116.2c	1,493 ± 89.6d
M	5.32–5.84	225–236	3,555 ± 173.7a	3,272 ± 557.1a	4,348 ± 284.5b	4,963 ± 1,124.7b	4,817 ± 344.0b	3,296 ± 485.8c	1,976 ± 911.9c

[y] Days of germination. Mean ± SD. Different letters following numbers indicate significant differences between malting stages. FU = fluorescence units.
[z] Molecular weight in thousands.

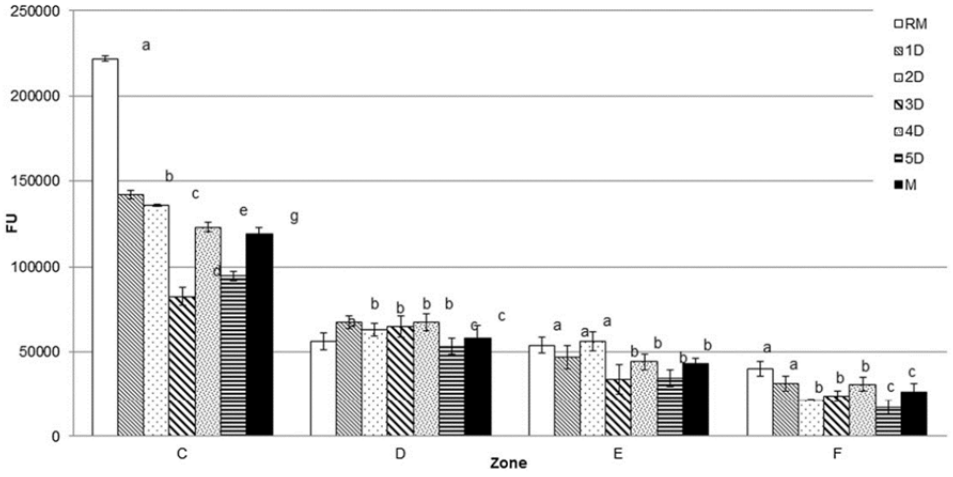

Fig. 2. Fluorescence areas of major protein zones of spelt proteins as affected by germination. FU = fluorescence units; RM = raw material; D = days of germination; and M = malt. Lowercase letters indicate significant differences between malting stages. Zones are defined in Table I.

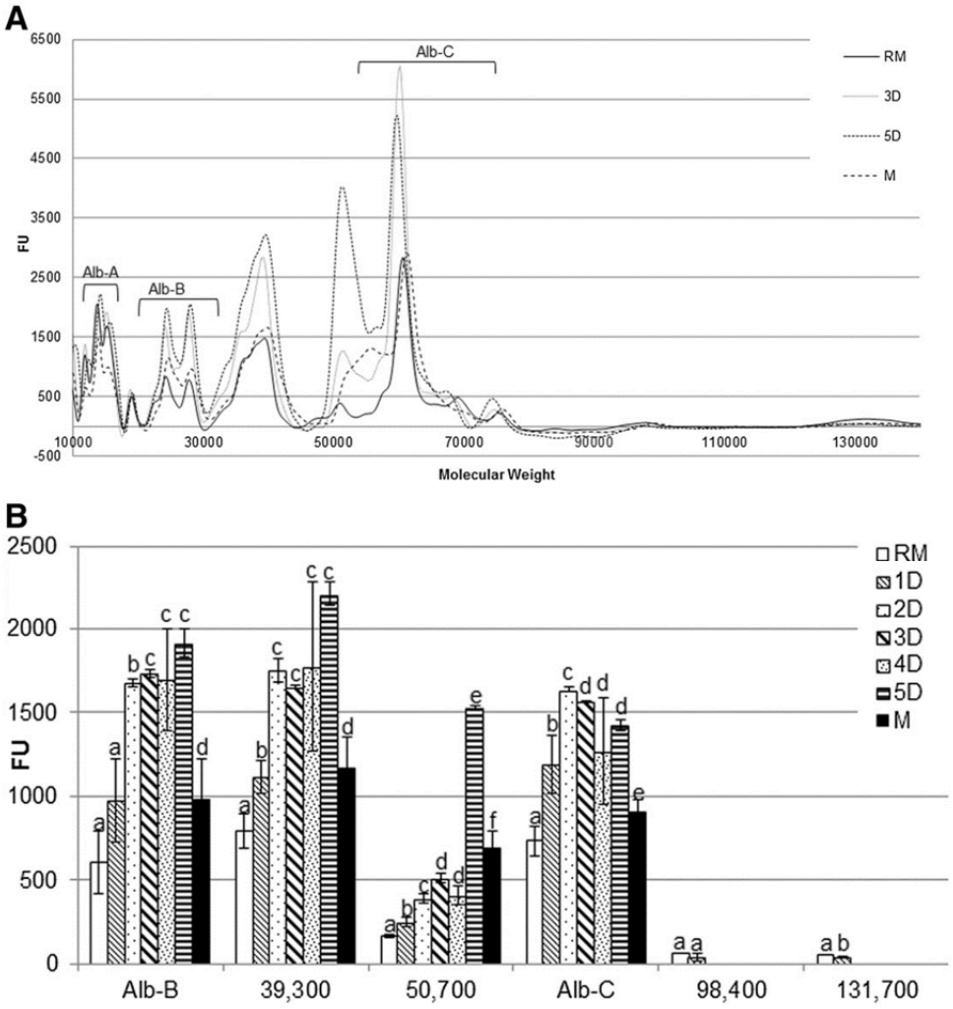

Fig. 3. A, Molecular weight protein profile; and **B,** peak relative concentration of the albumin fraction of spelt affected by germination. FU = fluorescence units; RM = raw material; D = days of germination; and M = malt. Lowercase letters indicate significant differences between malting stages. Peaks not plotted in B did not show significant differences between the malting stages.

67,200). The remaining protein peaks (97,200 and 130,500) represented a low percentage of the globulin fraction. The concentrations of the globulin peaks are shown in Figure 4B. According to the statistical analysis, Glo-A, Glo-B, and Glo-C did not show any significant variation within the malting process. However, peaks 38,600, 59,400, and 67,200 within area Glo-C showed a trend to increase, whereas peaks 46,400, 52,300, and 59,400 decreased (Fig. 4A). Similar to the albumin fraction, peak 97,200 underwent a complete degradation from RM up to 1D. Peak 130,500, although no significant difference was found, showed a tendency to decrease, to some extent, during malting.

Gliadins and glutenins from spelt have been well investigated owing to their use in wheat and spelt differentiation. In contrast, albumin and globulin fractions are analyzed marginally and most of the time collectively as a salt-soluble fraction. The albumin plus globulin fraction is described to have a high metabolic activity (Dvořáček and Čurn 2003). Albumins and globulins in wheat are known to include enzymes and inhibitors of enzymes that regulate the different development stages within the grain (Gao et al. 2009). Celus et al. (2006) analyzed the effects of malting and mashing on barley proteins and stated that the apparent increase in albumins and globulins during malting is the result of the proteolytic breakdown of the disulfide-linked hordeins and the secretion of enzymes in the endosperm. Therefore, it is assumed that the decrease in these fractions is owing to protein hydrolysis needed for embryo germination.

According to Belitz et al. (1989) and Dvoracek et al. (2001), the albumin and globulin fraction distribution ranges between 17.3 and 27.4% of the total protein and depends on the cultivation year. Dvořáček, and Čurn (2003) defined the spelt albumin plus globulin pattern as 24–32 bands with a MW range from 2,000 to 106,000 and with two relatively wide areas in the ranges 23,000–66,000 and 2,000–16,000. These results correspond with the results of the present study, in which a relatively wide protein area was found between 14,000 and 74,000 and between 13,000 and 67,000 for the albumin and globulin fractions, respectively.

Gliadin Fraction. The gliadin fraction of spelt has been extensively analyzed by many authors because of its importance for the differentiation between spelt and wheat. Depending on the authors and the method used, the gliadin fraction represents between 28.6 and 34.1% of the spelt proteins (Belitz et al. 1989; Dvoracek et al. 2001) or between 61.5 and 67.6% (Koenig et al. 2015). Figure 5 shows the protein changes of the gliadin fraction of spelt between

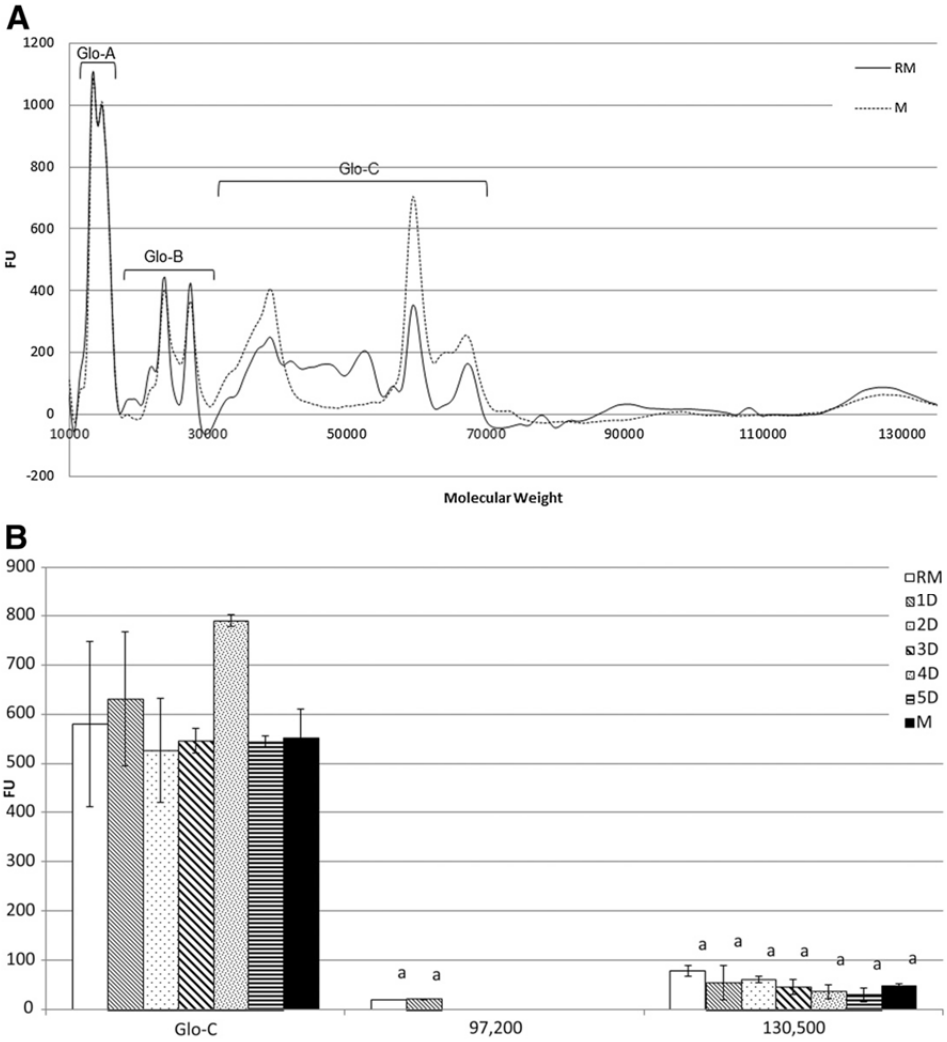

Fig. 4. A, Molecular weight protein profile; and **B,** peak relative concentration of the globulin fraction of spelt affected by germination. FU = fluorescence units; RM = raw material; D = days of germination; and M = malt. Lowercase letters indicate significant differences between malting stages. Peaks not plotted in B did not show significant differences between the malting stages.

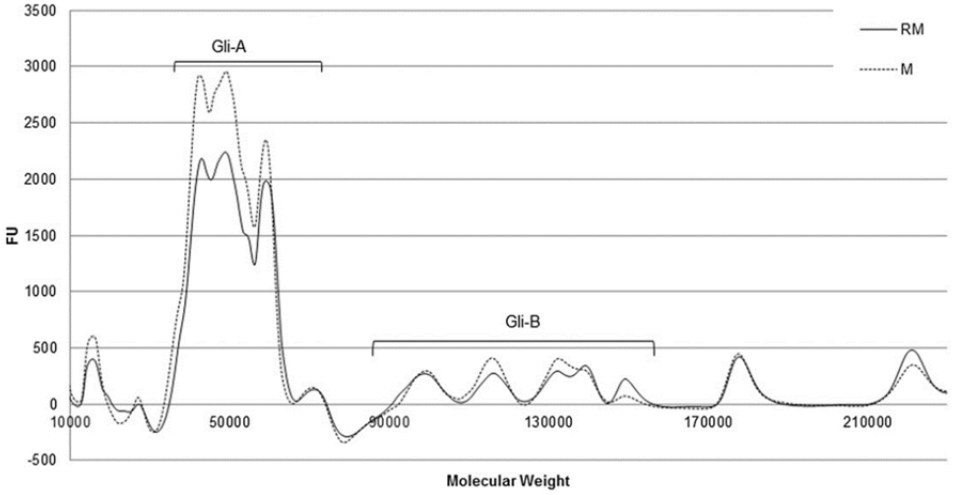

Fig. 5. Molecular weight protein profile of the gliadin fraction of spelt during malting. FU = fluorescence units; RM = raw material; and M = malt.

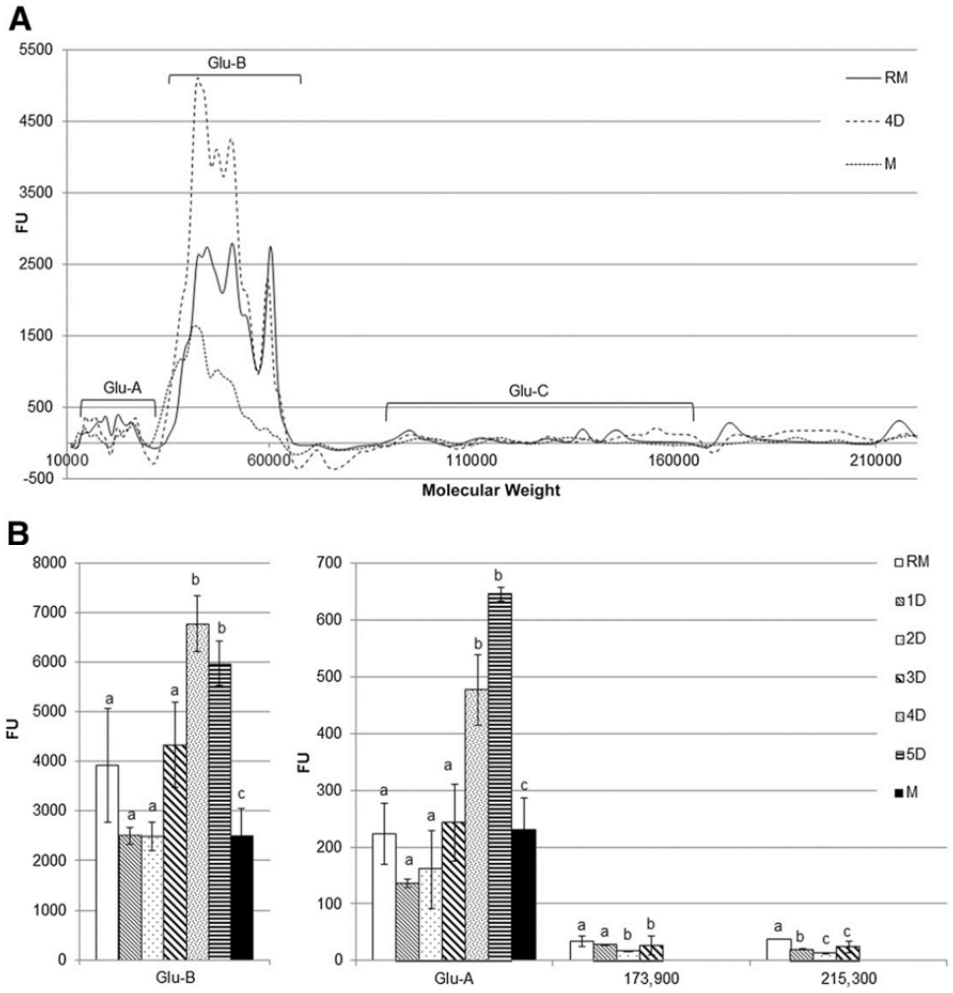

Fig. 6. A, Molecular weight protein profile; and **B,** peak relative concentration of the glutenin fraction of spelt affected by germination. FU = fluorescence units; RM = raw material; D = days of germination; and M = malt. Lowercase letters indicate significant differences between malting stages. Peaks not plotted in B did not show significant differences between the malting stages.

RM and malt. This fraction presented a band pattern with a total of 13 protein peaks characterized by typical bands with MWs of 14,500, 28,400, 42,100, 48,400, 59,000, 70,200, 97,800, 114,100, 131,600, 136,800, 148,800, 178,700, and 220,800. Abdel-Aal et al. (1996) analyzed the gliadin pattern of spelt and estimated a MW between 34,000 and 113,000. Dvořáček, and Čurn (2003) defined this pattern between 3,000 and 115,000 and divided it into three different bands: 3,000–16,000, 24,000–53,000, and 80,000–115,000. The results depicted in Figure 5 show that the majority of the proteins were represented in the following peaks: 42,100, 48,400, 59,000, and 70,200 (Gli-A). Balázs et al. (2012) described a molecular size overestimation of HMW glutenins owing to their highly asymmetric linear structure when using lab-on-a-chip methodology, which may explain the presence of protein peaks in the HMW gliadin fraction.

The statistical analysis of the gliadin peaks did not show any significant difference ($P > 0.05$) during the malting process. However, peak 28,400 and Gli-A tended to increase during malting. Our results are in agreement with those of Faltermaier et al. (2013), who described the effect of malting on wheat gliadin proteins as not significant. It can be assumed that gliadins function as storage proteins, which supply the embryo with peptides and amino acids during germination (Lasztity 1995; Faltermaier et al. 2013); however, its degradation may occur after five days of germination.

Glutenin Fraction. The protein profile of the glutenin fraction during malting is depicted in Figure 6A. The glutenin fraction presented a band pattern with a total of 13 protein peaks characterized by typical bands at 19,800, 22,100, 44,200, 50,700, 60,200, 71,400, 93,900, 113,900, 129,900, 136,500, 144,800, 173,900, and 215,300. Owing to overlapping, the glutenin fraction was divided into three areas and two additional peaks: Glu-A (19,800 and 22,100), Glu-B (44,200, 50,700, 60,200, and 71,400), and Glu-C (93,900, 113,900, 129,900, 136,500, and 144,800), and peaks at 173,900 and 215,300. Figure 6B shows the relative concentration of the glutenin bands during malting. This fraction underwent a sharp degradation after kilning. Glu-A and Glu-B reached their highest concentration by 5D and 4D, respectively, and decreased thereafter during kilning. Glu-C did not show a significant variation during the malting process. Peaks 173,900 and 215,300 were completely degraded by 3D of germination. The degradation process of the glutenin fraction during malting was also described in other cereals such as wheat (Faltermaier et al. 2013), oats (Klose et al. 2008), and barley (Weiss et al. 1992). These authors described an almost complete degradation of the glutenin fraction, which was also found in the results obtained here.

Depending on the authors, the glutenin fraction of spelt ranges between 51.8 and 54.6% (Belitz et al. 1989) or between 12.5 and 17.4% (Koenig et al. 2015). Radić-Miehle et al. (1998) compared the electrophoretic glutenin patterns of spelt and wheat. The patterns were characterized by MWs from 30,000 to 113,000 with some of the peaks being exclusive to wheat or spelt. These authors divide the glutenin pattern into three areas: 80,000–115,000, 24,000–54,000, and 3,000–16,000. Analogous to Radić-Miehle et al. (1998), the major present protein area in our results ranged between 44,000 and 60,000.

A high similarity between gliadin and glutenin fractions could be observed. This phenomenon has already been described by other authors (Pomeranz 1988; Abdel-Aal et al. 1996; Ovesná et al. 2001) who defined 1) the overlapping of LMW glutenins and gliadins, and 2) the possibility of transition of the HMW (and partially also the LMW) fraction of glutenins into gliadins in the medium SDS-PAGE. However, although both fractions share peaks, the HMW glutenin peaks underwent degradation during malting, whereas the same peaks in the gliadin fraction did not suffer any degradation. Moreover, several reports (Abdel-Aal et al. 1996; Harsch et al. 1997; Radić et al. 1997; von Büren et al. 2000; Abdel-Aal and Hucl 2005) have shown that spelt contains gliadins and glutenins with structures distinct from those of common wheat.

Composition of Free Amino Acids. The free amino acid composition of spelt was analyzed during the different stages of malting (Table III). The results reveal an increase in the content of free amino acids during malting, except for asparagine, which had the tendency to decrease. This is owing to the hydrolysis of the native proteins, which causes both HMW protein breakdown products and LMW proteolysis products (peptides and amino acids)

TABLE II
α-Amylase Activity ± SD (U/kg, dry matter) of Spelt Affected by Germination

Sample	α-Amylase (Ceralpha units/kg d.m.)
Raw material	4 ± 0.2
Day 1	5 ± 0.6
Day 2	12 ± 3.2
Day 3	23 ± 0.0
Day 4	34 ± 0.2
Day 5	101 ± 0.9
Malt	71 ± 0.4

TABLE III
Free Amino Acid Composition (mg/L) of Spelt Affected by Germination[z]

Amino Acid	Raw Material	Day 1	Day 2	Day 3	Day 4	Day 5	Malt
Aspartic acid	247.2 ± 16.7a	332.3 ± 3.0b	300.0 ± 29.1b	354.3 ± 63.7b	412.7 ± 14.0c	603.5 ± 31.0d	615.7 ± 77.6d
Glutamic acid	219.7 ± 2.6a	325.6 ± 27.6b	533.5 ± 26.9c	764.1 ± 116.7c	889.0 ± 77.7d	1,173.7 ± 156.4d	1,054.4 ± 202.6d
Cysteine	33.6 ± 1.2a	33.2 ± 0.7a	34.0 ± 1.2a	35.5 ± 1.2a	36.2 ± 1.4a	43.3 ± 2.1b	38.5 ± 1.4b
Asparagine	314.6 ± 12.6a	384.4 ± 12.4b	266.6 ± 25.7c	146.2 ± 34.7d	107.6 ± 44.7d	274.3 ± 57.8e	168.1 ± 97.3e
Serine	44.6 ± 1.1a	80.1 ± 1.6b	139.3 ± 2.6c	187.8 ± 8.9d	212.2 ± 2.9e	394.1 ± 1.5f	310.1 ± 6.6g
Glutamine	44.4 ± 2.5a	126.5 ± 8.5b	205.4 ± 3.7c	285.7 ± 27.4d	295.1 ± 65.8d	966.3 ± 102.1e	573.9 ± 112.2f
Histidine	320.7 ± 11.9a	344.9 ± 29.6a	388.9 ± 34.6a	469.0 ± 73.3a	440.6 ± 25.2b	525.0 ± 32.7c	510.2 ± 41.1c
Glycine	49.6 ± 1.6a	48.3 ± 4.5a	60.3 ± 2.3b	77.1 ± 7.5b	88.3 ± 4.3c	139.4 ± 9.9d	128.0 ± 9.4d
Threonine	38.8 ± 1.5a	58.5 ± 7.6b	94.5 ± 1.8c	124.3 ± 4.9d	134.5 ± 4.8d	232.4 ± 7.5e	199.2 ± 13.1f
Arginine	302.3 ± 6.1a	354.1 ± 17.5b	411.6 ± 17.2c	529.0 ± 20.8d	555.5 ± 3.5d	752.9 ± 0.7e	673.3 ± 1.1f
Alanine	109.6 ± 1.5a	182.1 ± 6.4b	357.7 ± 5.4c	366.8 ± 13.6c	367.2 ± 6.0c	450.3 ± 2.6d	506.0 ± 11.6e
Tyrosine	129.3 ± 85.3a	135.0 ± 94.7a	190.5 ± 63.0a	245.6 ± 63.2a	245.6 ± 43.8a	390.2 ± 4.2b	345.0 ± 2.1c
Valine	156.6 ± 4.3a	172.4 ± 2.4b	221.0 ± 9.5c	281.2 ± 12.0d	278.0 ± 13.2d	401.0 ± 53.7d	372.4 ± 42.4d
Methionine	11.4 ± 0.7a	14.5 ± 4.5a	32.0 ± 2.0b	57.2 ± 12.1b	64.7 ± 11.0c	136.2 ± 12.7d	116.3 ± 15.0d
Tryptophan	474.4 ± 1.5a	479.8 ± 6.4a	523.9 ± 5.4b	575.5 ± 13.6c	601.1 ± 6.0c	646.6 ± 2.6d	644.1 ± 11.6d
Phenylalanine	52.2 ± 0.9a	70.9 ± 6.0b	135.1 ± 0.2c	216.4 ± 12.0d	254.1 ± 4.1e	558.2 ± 9.3f	463.7 ± 12.0g
Isoleucine	237.8 ± 18.5a	250.3 ± 25.8a	292.8 ± 19.5b	362.9 ± 31.1c	360.8 ± 1.0c	519.8 ± 32.8d	481.2 ± 25.7d
Leucine	602.7 ± 1.5a	627.7 ± 6.4b	728.7 ± 5.4c	862.5 ± 13.6d	922.9 ± 6.0e	1,281.8 ± 2.6f	1,204.4 ± 11.6g
Lysine	287.6 ± 45.3a	314.0 ± 46.9a	364.1 ± 56.7a	444.4 ± 58.4b	465.3 ± 41.6b	618.7 ± 49.8c	548.6 ± 48.9c

[z] Days of germination. Mean ± SD. Different letters following numbers indicate significant differences between malting stages.

(Narziß and Back 2012). Although asparagine tended to decrease, it described an irregular behavior. This amino acid is the amide of aspartic acid that is easily hydrolyzed to the latter. This process may explain the irregular behavior of asparagine found in the results.

The majority of amino acids except aspartic acid, alanine, and tryptophan increased until 5D and decreased in concentration afterward during kilning, which is a site of heat sensitivity. Between 5D and the kilned malt the total free amino acid concentration decreased by about 11%. Nie et al. (2010) analyzed the effects of malting conditions on the amino acid composition of barley and found also an increase of almost all amino acids during malting except for arginine. Narziß and Back (2012) suggested that the increase in the amino acid content is owing to Strecker aldehyde reactions and the decrease to aminocarbonyl reactions.

Abdel-Aal and Hucl (2005) and Cubadda and Marconi (2002) summarized the results of different authors investigating the amino acid content of spelt and wheat. Both comparisons showed a slight difference in the amino acid composition of proteins from spelt and modern wheat. However, in both cereals a higher presence of glutamic acid, proline, and leucine as well as a lower content of cysteine, methionine, and tryptophan was found. When comparing spelt and wheat lysine content, some authors define it as lower (Bonafaccia et al. 2000; Abdel-Aal and Hucl 2002) and others (Grela 1996) as higher. Abdel-Aal and Hucl (2002) also found an adverse influence on the lysine content of the end product owing to milling and processing of spelt and common wheat.

RM presented high concentrations of leucine, tryptophan, histidine, asparagine, and arginine (Table III). Although the concentrations of these amino acids (except for asparagine) increased during malting, their distribution in different process stages varied. In the final malt, leucine, glutamic acid, arginine, tryptophan, and aspartic acid accounted for the majority of free amino acids found. Amino acids threonine, methionine, and cysteine were found in low quantities in RM and (together with asparagine) in malt.

CONCLUSIONS

This paper covers the changes in the protein levels concerning the patterns of spelt as well as its Osborne fractions and the free amino acid content during malting. Spelt proteins were found in a MW range of 13,000–236,000. About 40–50% of the proteins were characterized by a pI range between 5.84 and 6.89 and MW between 35,000 and 55,000. In general, degradation of proteins to small peptides and amino acids could be observed. The Osborne fractionation revealed an albumin band pattern with MWs between 14,000 and 131,700, a globulin band pattern ranging from 13,400 to 130,500, a gliadin band pattern from 14,500 to 220,800, and a glutenin band pattern from 19,000 to 215,000. Although the malting process depicts a strong influence on the albumin, globulin, and glutenin fraction, no significant changes could be found in the gliadin fraction of spelt. The determination of free amino acids indicated an increase in concentration except for the amino acid asparagine, which decreased during malting. This paper contributes to the understanding of the protein modifications and metabolic changes occurring during the malting process of spelt and contributes to determining the potential of this cereal for the production of cereal-based products.

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3.4. Influence of Malting on the Protein Composition of Triticale (× *Triticosecale Wittmack*) Trigold

Influence of Malting on the Protein Composition of Triticale (× *Triticosecale* Wittmack) 'Trigold'

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ABSTRACT

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Triticale (× *Triticosecale* Wittmack) is a promising cereal for malting and brewing owing to its high levels of α-amylase and proteolytic activity and low gelatinization temperatures. However, almost no investigation about its protein modifications during malting has been conducted. In this work, triticale proteins during the malting process were separated and analyzed, first, according to their isoelectric point and molecular weight (MW) and, second, according to their solubility and MW. Moreover, the composition of free amino acids was determined. The results describe the modification changes of the different protein fractions during malting. Triticale proteins were found between 4,700 and 64,000. The majority of the proteins were characterized as having an isoelectric point between 5.08 and 6.63. The Osborne fractionation revealed an albumin band pattern of between 13,400 and 153,800, globulin from 12,700 to 152,300, gliadin from 14,500 to 230,100, and glutenin from 11,200 to 102,200. Free amino acids increased in concentration during malting except for asparagine, which decreased. Asparagine exhibited varying trends during malting. This paper contributes to the understanding of the protein modifications and metabolic changes during the malting process of triticale and indicates the potential of this cereal for the production of cereal-based products.

Triticale (× *Triticosecale* Wittmack), the first man-made cereal, is an intergenic hybrid between female wheat (*Triticum aestivum* L.) and male rye (*Secale cereale* L.). Triticale was designed to combine the productivity, uniformity, and superior grain quality of wheat with the hardiness, disease resistance, and better nutritional quality of rye (Sehgal et al. 1983; Jonnala et al. 2010). This cereal is capable of producing much higher agricultural yields and biomass than other cereals and has better tolerance to many diseases and pests than its parental species (Grujić et al. 2009b), which thereby reduces the necessity of chemical protection against such harmful agents (Oettler 2005).

Triticale offers several advantages over other cereal grains, such as better quality protein in terms of amino acid composition and digestibility, higher content of soluble dietary fiber and minerals, and a pleasing, nutty, mild rye flavor (Au et al. 2010). The protein content of whole grain triticale is often higher than that of wheat (Ruckman et al. 1973). Some authors (Pomeranz et al. 1970; Ramírez et al. 2003) report the protein content of triticale as between 8.0 and 22.5%. This fact is apparently owing to the combination of the protein fractions from wheat and rye (Unrau and Jenkins 1964; Bushuk 1974; Burgos-Hernández et al. 1999). The gluten content of triticale is lower and of inferior quality (weak and less elastic) to that of wheat (Oettler 2005). In addition, some triticale lines display high levels of amylolytic and proteolytic activities in their unmalted form (Rao et al. 1976; Lorenz et al. 1981; Glatthar et al. 2003).

The current uses of triticale are largely determined by its chemical composition (McGoverin et al. 2011). McGoverin et al. (2011) reviewed the uses of triticale. The unsuitable nature of triticale for baking, breadmaking specifically, was acknowledged early, and subsequently efforts were made to improve triticale for baking (McGoverin et al. 2011). Triticale flours produce weak doughs owing to a low gluten content, inferior gluten strength, and high levels of α-amylase activity (Macri et al. 1986; Mergoum and Macpherson 2004). However, Lorenz et al. (1972) demonstrated the possibility of producing triticale bread of acceptable quality by making only minor modifications in the mixing procedure. In contrast, the ease with which triticale produces high levels of α-amylase (Mergoum and Macpherson 2004) and high proteolytic activity, in combination with the low gelatinization temperatures (59–65°C), has its positive side: it allows triticale to perform well in malting and brewing. Intense research has been done on the use of triticale as an adjunct for brewing (Glatthar et al. 2003, 2005; Grujić et al. 2009a, 2010) and for its suitability in the malting and brewing process (Pomeranz et al. 1970; Tomdros and Briggs 1984; Gupta et al. 1985; Blanchflower and Briggs 1991a, 1991b, 1991c; Creydt et al. 1999; Grujić and Pejin 2007; Grujić et al. 2009b; Zarnkow et al. 2009).

Triticale malt is described, in general, as having higher malt losses but also higher malt extracts, higher diastatic power, and higher α- and β-amylase activity than barley malt (Pomeranz et al. 1970; Varughese et al. 1996b; Grujić et al. 2009b). Gupta et al. (1985) and Pomeranz et al. (1970) found that worts obtained from mashing triticale malts were high in nitrogenous compounds and had a dark color, originating from the high proteolytic activity. Moreover, wort from triticale malt has a higher viscosity than barley malt worts (Blanchflower and Briggs 1991b, 1991c; Grujić et al. 2009b; Zarnkow et al. 2009; Rakha et al. 2012). Blanchflower and Briggs (1991c) investigated the contribution of various wort constituents to wort viscosity and turbidity, and they found that the high viscosity was caused by pentosans and that the turbidity was proteinaceous in origin.

Chen and Bushuk (1970a, 1970b, 1970c) studied the proteins and amino acid composition of triticale and its parents, and they showed that both solubility characteristics and amino acid composition of triticale proteins are, in general, intermediate between those of its parent species. However, Lei and Reeck (1986) indicated that the electrophoretic patterns of triticale proteins are generally similar to, but not identical to, those of the corresponding combined protein fractions of its parental species. One of the most important physicochemical changes during malting is the degradation of the proteinaceous matrix (storage proteins) that surrounds the starch granules within the cells of the endosperm and their conversion into soluble peptides and amino acids to provide substrates for the synthesis of proteins in the growing embryo (Briggs 1998; Klose et al. 2009). Moreover, amino acids influence the aroma and flavor profile of the final product (e.g., beer) by taking part in different reactions.

Understanding the protein modifications and metabolic changes that occur during the malting process of triticale may help in determining the potential of this cereal for the development of, among other things, cereal-based beverages. The aim of this study was to determine the triticale protein modifications that occur during the malting process. The proteins were analyzed at the different malting stages according to their isoelectric point (pI), molecular weight (MW), solubility, and free amino acid composition.

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MATERIALS AND METHODS

Materials. Triticale (× *Triticosecale* Wittmack) 'Trigold' with a moisture content of 13.5%, a protein content of 14%, and germination capacity of >95% was procured from KWS Lochow GmbH (Bergen, Germany). The raw material was grown in 2011 and stored under cool and dry conditions until further use.

Triticale was malted according to the optimum malting parameters given by Zarnkow et al. (2009) and according to the Mitteleuropäische Brau- und Analysenkommision (MEBAK) malting method 2.5.3.1 (Anger 2006): five days of germination, 45% moisture content, and a set germination temperature of 15°C in temperature-controlled chambers with 75% relative humidity. The steeping water was equilibrated by placing it for 24 h prior to steeping in the climate chambers. The degree of steeping was determined by weighing the samples and calculating the water content of each one, taking into account the moisture content at the beginning of the malting process.

Samples were taken every 24 h: raw material (RM), first (1D), second (2D), third (3D), fourth (4D), and fifth (5D) day of malting, and final malt. Samples 1D, 2D, 3D, 4D, and 5D were dried by means of a BETA 2-16 freeze dryer (Martin Christ GmbH, Osterode am Harz, Germany). Sample malt was kilned according to MEBAK method 2.5.3.1 (Anger 2006). All the samples had a water content between 4.5 and 5.0%. After freeze drying or kilning, rootlets and sprouts were removed. Before analysis, the samples were milled with a disc mill (Bühler, Braunschweig, Germany) set at a fine setting of 0.05 mm.

Characterization of Proteins According to pI and MW. The proteins of every sample were characterized according to the pI and MW by using, in combination, both the 3100 OFFGEL electrophoresis system (Agilent Technologies, Santa Clara, CA, U.S.A.) and the Agilent 2100 bioanalyzer. First, the proteins present in the sample were extracted by mixing 40 mg of milled sample with 400 µL of buffer. The buffer used was composed of 2M urea, 15% glycerol, 0.1M Tris HCl, and 0.1M dithiothreitol with a pH of 8.8, and it is normally used for the protein extraction for samples analyzed with the bioanalyzer. The mixture was vortexed briefly and immediately placed in an ultrasonic water bath for 5 min. Afterward, the samples were centrifuged at 14,000 × *g* for 15 min.

The pI-based separations were performed on the 3100 OFFGEL fractionator in combination with the OFFGEL kit at pH 3–10 with a 24-well setup according to the supplier's protocol. The MW-based separations were then performed on the Agilent 2100 bioanalyzer combining both the Protein 230 kit and the Agilent 2100 Bioanalyzer Expert software. All samples were prepared according to the supplier's protocol. Each run included a ladder comprising reference proteins with MWs of 7,000, 15,000, 28,000, 46,000, 63,000, 95,000, and 150,000 plus an upper marker of 240,000 and a lower marker of 4,500. Each sample contained an internal standard comprising the upper and lower markers to allow for quantification. The results obtained were standardized to the same upper marker and plotted with Matlab version 7.10.0.499, R2010a (MathWorks, Natick, MA, U.S.A.). All samples were analyzed in triplicate ($n = 3$).

Characterization of Proteins According to Solubility—Modified Osborne Fractionation. Osborne fractions were separated as previously described (Faltermaier et al. 2013). The samples were then MW separated by employing the lab-on-a-chip technique without any further preparation by using the Agilent 2100 bioanalyzer. For this, the Protein 230 kit and the Agilent 2100 Bioanalyzer Expert software were used. All samples were prepared according to the supplier's protocol. Each run included a ladder comprising reference proteins with MWs of 7,000, 15,000, 28,000, 46,000, 63,000, 95,000, and 150,000 plus an upper marker of 240,000 and a lower marker of 4,500. Each sample contained also an internal standard comprising the upper and lower markers to allow for quantification. All samples were analyzed in triplicate ($n = 3$). The fractions were assigned according to the MW.

Composition of Free Amino Acids. Sample preparation for amino acid analysis by HPLC was carried out by mixing 0.15 g of milled sample with 1.5 mL of distilled water. The mixture was stirred for 60 min and 329 × *g* at 65°C and finally centrifuged at 2,900 × *g* for 10 min. The supernatant was diluted 1:5 with distilled water. The amino acids present in the diluted supernatant were quantified by HPLC (U3000, Dionex, Sunnyvale, CA, U.S.A.) as described by Krömer (2006). Quantification was done by a six-point external calibrating curve for each amino acid. All samples were analyzed in triplicate ($n = 3$).

α-Amylase Activity. The α-amylase activity was measured by using a Megazyme enzyme kit (Megazyme, Bray, Ireland) according to the standard methods of the European Brewery Convention (Eerde 1998). Analytical procedures were carried out in triplicate.

Statistical Analysis. Statistical analysis was performed by one-way ANOVA to test for differences within the malting process. Additionally, unpaired two-way *t* tests were applied to determine differences between the individual malting stages. The software used for both tests was Microsoft Excel 2010 (Microsoft, Seattle, WA, U.S.A.). In all cases, statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Characterization of Proteins According to pI and MW. Triticale proteins during the malting process were fractionated and analyzed according to their pI and MW. The combination of OFFGEL fractionation and the bioanalyzer offers an alternative technique to the conventional two-dimensional gel electrophoresis (2D-PAGE). Other authors (Faltermaier et al. 2013) compared the results of analyzing wheat proteins by means of 2D-PAGE and by the combination of OFFGEL and bioanalyzer, and they obtained comparable results.

Figure 1 shows the changing triticale protein levels during the malting process. Triticale proteins could be divided into 16 zones named from A to P. The majority of the protein zones were found in a pI between 5.08 and 6.63. Table I displays the protein zones, the pI, the MW, and the concentration. Zones A, C, and H–P accounted for the majority of triticale proteins. Most of the polypeptides increased gradually, only to decline thereafter. Zones B, H, I, and L increased by 5D and then decreased in concentration in the final malt. Zone D increased until 4D, zones C and O until 3D, and zones F and G until 2D. Proteins in zone L were increasingly synthesized during the malting process. This zone is not present in the RM, and by 3D it increased its concentration seven times; by 5D it decreased and also afterward during kilning.

The mobilization pattern of zones A, J, and K depicted a degradation process throughout; however, these areas are still present in the final malt. The results showed a 60, 49, and 40% decrease in their proportion during malting, respectively, suggesting a storage role. The low (or absent) concentration of zone A by 1D and 5D was probably owing to the high pI (9.26–9.52) of zone A, which was found in the limits of the analysis (pH 3–10), denoting a poor fixation. Zone E was the only polypeptide that increased steadily during malting, its concentration in the final malt being two times higher than in the RM. Zones M, N, and P showed varying trends all through the malting process.

The mobilization pattern of the polypeptides H and I, J and K, and M and N indicated a similarity between them. These protein couples were found at the same pI but with slightly different MWs (Table I), suggesting a relation between them, probably formed by methylation or glycosylation.

Modified Osborne Fractionation. To provide a fundamental understanding of the degradation of triticale proteins during malting, the proteins were separated according to their solubility in water (albumin), salt solutions (globulin), alcohol (gliadin), or acidic solutions (glutenin). Thereafter, each fraction was separated according to its MW by means of the bioanalyzer. Differences in both the solubility distribution and the nature of proteins are responsible for

the low (relative to wheat) gluten-like protein content of triticale (Varughese et al. 1996a).

Albumin Fraction. The albumin fraction consists of a mixture of structural, metabolic, and storage proteins (Gupta et al. 1991; Shewry and Halford 2002). The triticale albumin fraction is defined to be larger than that of wheat: an important fact, because many proteins in the water-soluble fraction exhibit biological activity such as enzymes and exogenous enzyme inhibitors (Burgos-Hernández et al. 1999). Some authors (Chen and Bushuk 1970a; Wu 1982) have reported an albumin fraction between 17 and 26.4% in triticale. Wu (1982) analyzed the proteins of triticale during sprouting (0, 3, 6, and 8 days of germination) and found no changes in the albumin content.

Figure 2 shows the changes during malting of the protein profile and the peak relative concentrations of the albumin fraction. The MWs of the albumin fraction ranged between 13,400 and 153,800. The electropherograms showed a band pattern with a total of 14 protein peaks characterized by typical bands with MWs of 13,400, 13,700, 19,600, 23,900, 27,400, 32,900, 39,400, 46,400, 52,400, 62,200, 75,900, 98,900, 122,500, and 153,800. Overlapping peaks were quantified together as Alb-A (13,400 and 13,700), Alb-B (19,600, 23,900, and 27,400), Alb-C (32,900, 39,400, and 46,400), and Alb-D (62,200 and 75,900). According to the statistical analysis, Alb-A, Alb-C, and Alb-D did not show significant changes during malting. Alb-B and peaks 52,400 and 153,800 increased

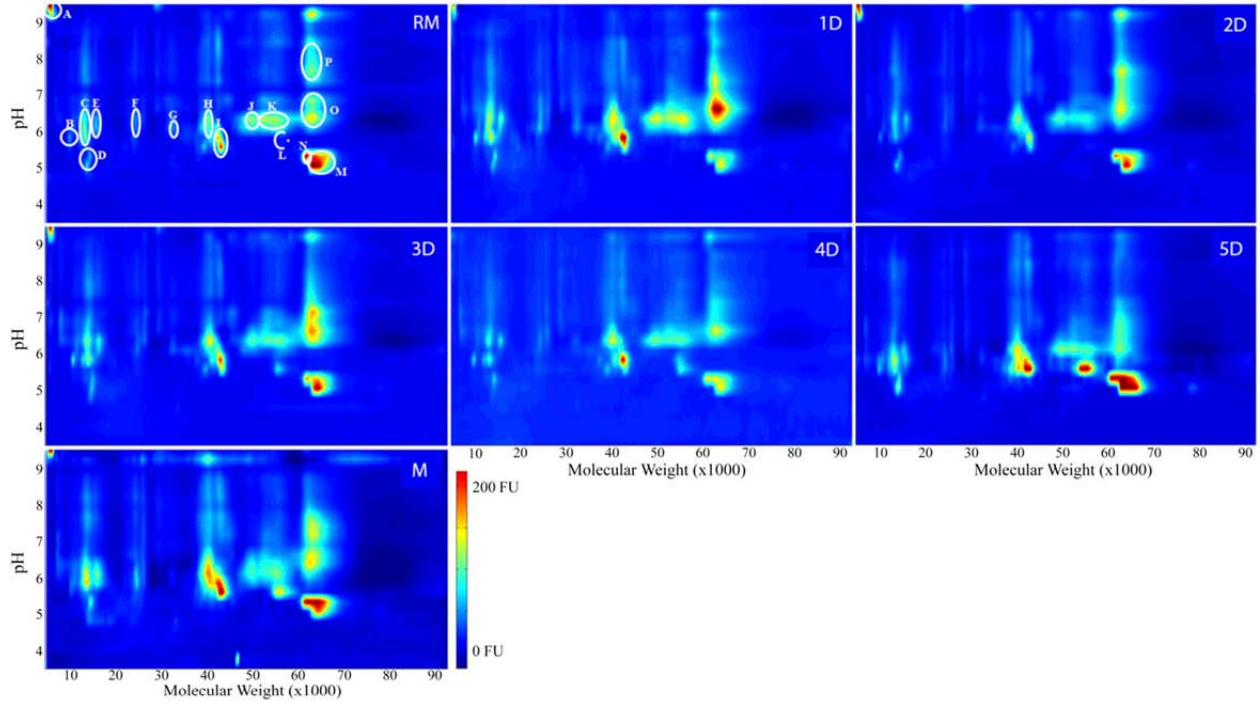

Fig. 1. Two-dimensional separation of triticale proteins as affected by germination. The *y* axis shows separation according to isoelectric point, and the *x* axis represents molecular weight. RM = raw material; D = days of germination; and M = malt.

TABLE I
Triticale Protein Zones and the Corresponding Isoelectric Point (pI), Molecular Weight, and Concentration[y]

Zone	pI	MW (×10³)[z]	Raw Material (FU)	Day 1 (FU)	Day 2 (FU)	Day 3 (FU)	Day 4 (FU)	Day 5 (FU)	Malt (FU)
A	9.26–9.52	4.7–6.7	14,860 ± 160.1a	6,263 ± 154.6b	16,920 ± 36.0c	14,308 ± 95.5d	10,302 ± 158.0e	0 ± 0.0	6,705 ± 169.6f
B	5.58–5.84	9–10	378 ± 95.4a	763 ± 56.4b	333 ± 94.6c	860 ± 31.6d	603 ± 36.8e	780 ± 53.8f	317 ± 195.4g
C	5.84–6.89	12–14	4,660 ± 537.6a	8,706 ± 175.3b	5,991 ± 400.3c	10,941 ± 240.9d	7,712 ± 179.7e	5,168 ± 249.3f	7,505 ± 325.3g
D	5.06–5.32	12.7–14.8	889 ± 135.4a	1,206 ± 152.8a	1,274 ± 99.3b	2,351 ± 49.3c	1,407 ± 164.4d	1,308 ± 255.2d	589 ± 33.9e
E	6.11–6.37	15–16.2	910 ± 166.7a	2,111 ± 76.3b	1,286 ± 135.1c	2,229 ± 142.8d	1,271 ± 172.3e	1,367 ± 47.9e	2,668 ± 23.2f
F	6.11–6.37	23.7–24.7	578 ± 88.3a	1,661 ± 54.0b	927 ± 92.1c	816 ± 80.9c	446 ± 230.2c	275 ± 115.2d	570 ± 199.5d
G	6.11	31.3–33.3	409 ± 53.6a	557 ± 83.1a	397 ± 24.5a	429 ± 101.0a	221 ± 50.4b	116 ± 17.1b	0 ± 0.0
H	5.58–6.63	38–41	4,514 ± 96.3a	8,693 ± 359.8b	5,216 ± 463.6c	9,609 ± 262.5d	7,703 ± 430.7e	8,244 ± 166.2e	9,776 ± 111.8f
I	5.58–6.11	41–44	4,245 ± 172.6a	7,782 ± 182.5b	4,763 ± 232.8c	7,797 ± 157.9d	7,024 ± 192.7e	6,371 ± 172.5f	6,691 ± 52.6f
J	6.11–6.38	47–51	4,193 ± 149.8a	6,636 ± 80.6b	3,878 ± 50.4c	4,505 ± 39.2d	4,526 ± 110.9d	3,083 ± 194.7e	3,217 ± 168.8e
K	6.11–6.38	52–58	6,897 ± 115.2a	11,209 ± 185.5b	6,190 ± 253.5c	9,181 ± 350.9d	7,332 ± 236.4e	4,832 ± 92.3f	6,135 ± 114.0g
L	5.58–5.84	54–57	0 ± 0.0	0 ± 0.0	0 ± 0.0	1,483 ± 58.9a	2,514 ± 80.5b	6,913 ± 199.4c	3,724 ± 18.4d
M	5.05–5.32	62–68	6,960 ± 32.9a	3,508 ± 94.3b	6,517 ± 40.4c	8,849 ± 125.3d	6,022 ± 126.7e	7,953 ± 69.0f	5,868 ± 33.6g
N	5.32	60–61	2,264 ± 20.4a	1,340 ± 31.5b	2,389 ± 45.5c	2,947 ± 23.7d	2,301 ± 23.7e	3,214 ± 43.1f	1,319 ± 113.5g
O	6.37–6.89	59–65	6,897 ± 206.1a	14,439 ± 244.5b	10,146 ± 220.8c	16,033 ± 164.3d	11,008 ± 150.4e	8,165 ± 126.5f	9,184 ± 97.9g
P	7.16–8.47	61–64	5,965 ± 230.6a	7,720 ± 492.2b	7,255 ± 293.3b	10,905 ± 265.6c	4,776 ± 289.6d	2,563 ± 790.2e	8,180 ± 222.7f

[y] Days of germination. Mean ± SD. Different letters following numbers indicate significant differences between malting stages. FU = fluorescence units.
[z] Molecular weight in thousands.

throughout the entire malting process up to 5D (Fig. 2B). Peak 122,500 underwent a total degradation by 2D, and peak 98,900 was visible at 3D, increasing in concentration throughout.

Chen and Bushuk (1970b) determined the protein fractions of triticale to be intermediate between those of durum wheat and rye. Faltermaier et al. (2013) analyzed the Osborne fractions of wheat during the malting process and found several protein peaks and peak areas in the following ranges: 14,000–23,000, 25,000–30,000, 38,000–50,000, and 55,000–63,000. Our results are in agreement with the latter. Triticale albumins can be divided into five main peak areas: Alb-A, Alb-B, Alb-C, peak 52,400, and Alb-D.

The increasing accumulation of various polypeptides (Fig. 2) revealed an increase in the albumin fraction during germination. Peak 52,400 represented at the beginning of the malting process about 2% of its concentration after kilning. The same behavior was found by the separation of proteins according to the pI and MW for zone L, which shares the MW with this peak. Muralikrishna and Nirmala (2005) characterized the enzyme α-amylase by a MW between 41,500 and 54,000 in cereals such as wheat, barley, sorghum, and rice. The α-amylase activity was measured to prove its correlation with the increase in peak 52,400 concentration. The enzyme activity during the malting process is shown in Table II. The steady increase of α-amylase activity during germination as well as the decrease from 5D to malt corresponded with the behavior of peak 52,400. According to the MW of this peak and the known increase of α-amylase during germination, it may be possible to determine this peak as α-amylase.

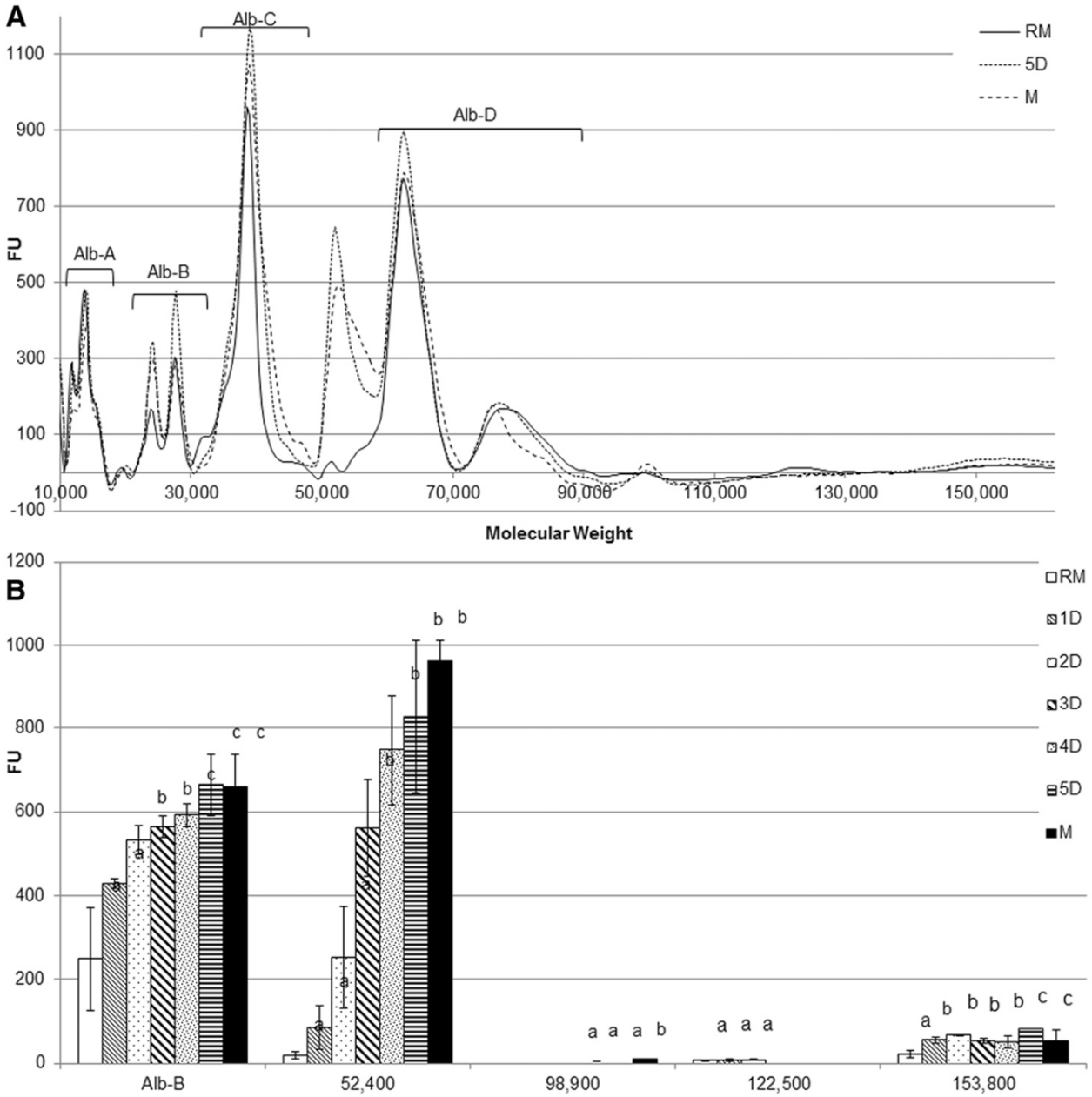

Fig. 2. A, Molecular weight protein profile; and **B,** peak relative concentration of the albumin fraction of triticale affected by germination. FU = fluorescence units; RM = raw material; D = days of germination; and M = malt. Lowercase letters indicate significant differences between malting stages. Peaks not plotted in B did not show significant differences between the malting stages.

TABLE II
α-Amylase Activity ± SD (U/kg, dry matter) of Triticale Affected by Days of Germination

Sample	α-Amylase (Ceralpha units/kg d.m.)
Raw material	0 ± 0.0
Day 1	3 ± 0.8
Day 2	8 ± 0.16
Day 3	35 ± 0.58
Day 4	89 ± 2.35
Day 5	100 ± 1.19
Malt	93 ± 2.1

Globulin Fraction. Chen and Bushuk (1970a) quantified the globulin fraction in triticale as 6.5%, lower than that of rye (10.7%) but higher than that of wheat (4.7%). However, the globulin fraction is normally quantified together with the albumin fraction. Jonnala et al. (2010) and Naeem et al. (2002) analyzed triticale cultivars and determined an albumin plus globulin fraction as between 11.7 and 19.0%. These results do not agree with those of Varughese et al. (1996a), who summarized the results of various studies and found the albumin plus globulin fraction to range between 27.9 and 32.2%. Although there is a discrepancy, all authors agree that this cereal's albumin plus globulin fraction is larger than wheat. Taking into

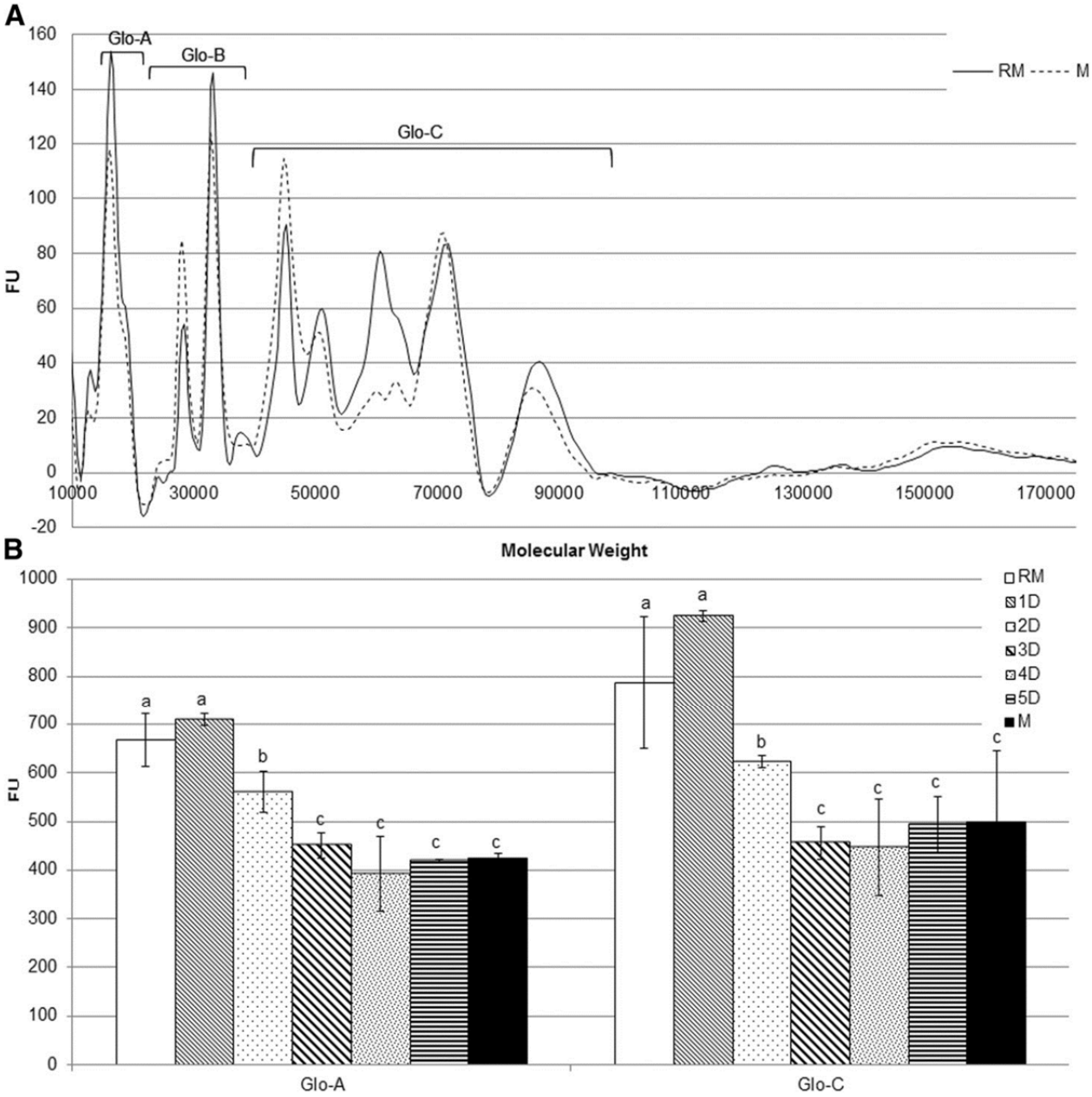

Fig. 3. A, Molecular weight protein profile; and **B,** peak relative concentration of the globulin fraction of triticale affected by germination. FU = fluorescence units; RM = raw material; D = days of germination; and M = malt. Lowercase letters indicate significant differences between malting stages. Peaks not plotted in B did not show significant differences between the malting stages.

consideration the high presence of the albumin fraction with respect to the total protein content of triticale, it can be said that the globulin fraction is the smallest Osborne fraction.

The globulin fraction was characterized by a protein band pattern between 12,700 and 152,300 and characterized by 12 peaks with MWs of 12,700, 16,400, 24,800, 28,000, 33,300, 37,600, 44,900, 50,700, 60,500, 71,800, 86,200, and 152,300. Overlapping peaks were quantified together in the following areas: Glo-A (12,700 and 16,400), Glo-B (24,800, 28,000, and 33,300), and Glo-C (37,600, 44,900, 50,700, 60,500, and 71,800). The areas of Glo-A and Glo-C decreased steadily during malting (Fig. 3B). The degradation process to which Glo-C is subjected was not equal in every peak within this fraction. Peak 60,500 suffers greater degradation than the other peaks within Glo-C. Glo-B and peaks 86,200 and 152,300 showed varying trends. The behavior of Glo-A and Glo-C indicated these are storage proteins that provide substrates for the synthesis of proteins during germination.

Gliadin Fraction. The gliadin fraction of triticale has been quantified as about 24.4–33.3% (Chen and Bushuk 1970a; Varughese et al. 1996a) lower than that of wheat. Figure 4A depicts the changes to the protein profile of the gliadin fraction between RM and malt. This fraction showed 13 polypeptides in a range between 14,500 and 230,100: 14,500, 15,800, 17,600, 27,700, 40,700, 47,900, 53,100, 60,800, 133,400, 154,600, 187,700, 214,900, and 230,100. Balázs et al. (2012) described a molecular size overestimation of HMW glutenins owing to their highly asymmetric linear structure

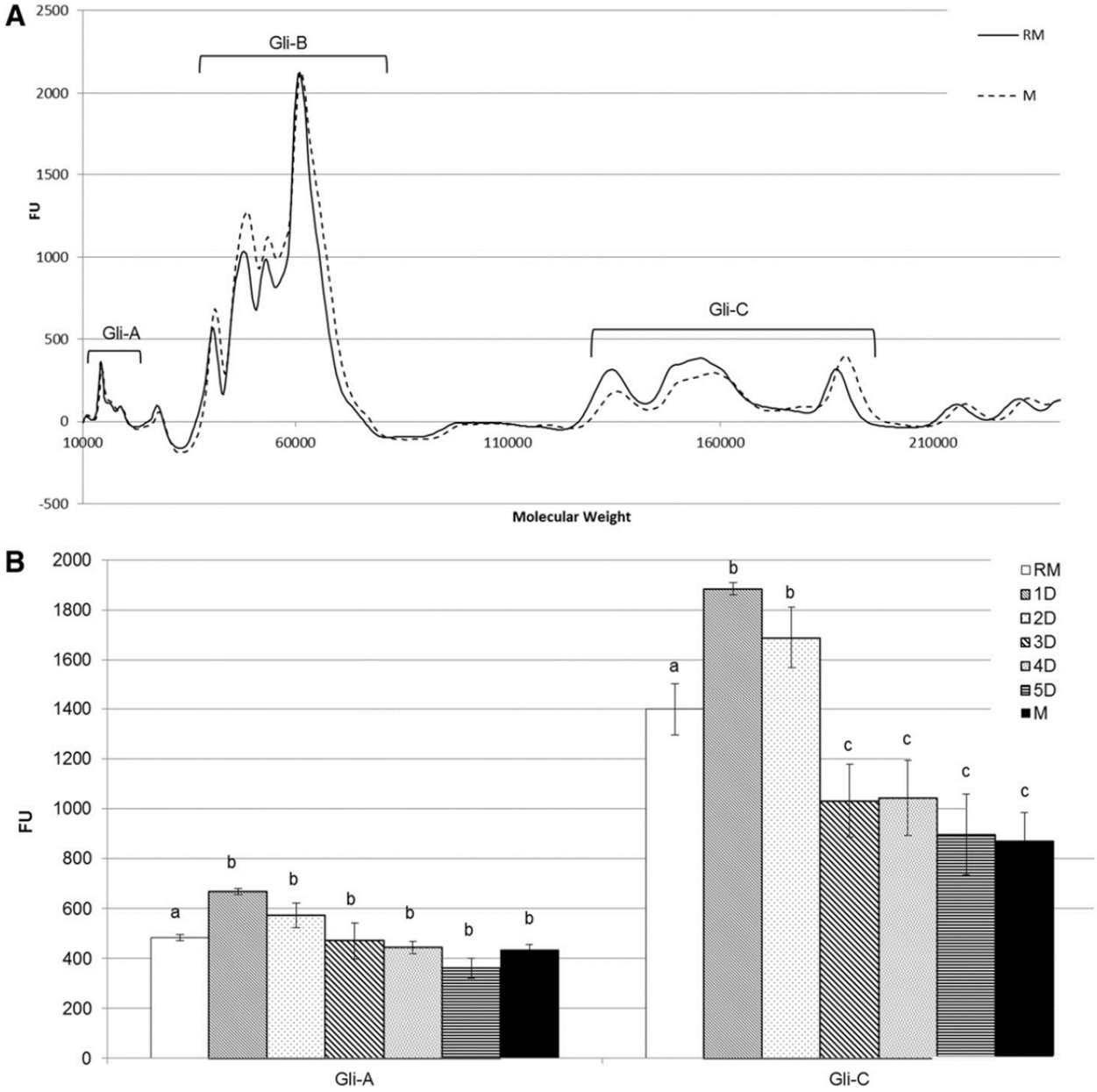

Fig. 4. A, Molecular weight protein profile; and **B,** peak relative concentration of the gliadin fraction of triticale affected by germination. FU = fluorescence units; RM = raw material; D = days of germination; and M = malt. Lowercase letters indicate significant differences between malting stages. Peaks not plotted in B did not show significant differences between the malting stages.

when using lab-lab-on-a-chip methodology, which may explain the presence of protein peaks in the HMW gliadin fraction.

The gliadin fraction could be divided into three areas and three peaks: Gli-A (14,500, 15,800, and 17,600), peak 27,700, Gli-B (40,700, 47,900, 53,100, and 60,800), Gli-C (133,400, 154,600, and 187,700), peak 214,900, and peak 230,100.

Figure 4B shows the degradation pattern of Gli-A and Gli-C. The other areas and peaks did not show any significant variation during the malting process. Both areas increased in concentration in the first 24 h of malting and then underwent degradation. This degradation pattern agrees with the results of Wu (1982), who found a decrease in the gliadin fraction from the beginning of the germination process, the strongest decrease being between days 3 and 6. Drzymała et al. (2008) analyzed the quantitative and qualitative changes occurring in the gliadin fraction of triticale grains and found that the most intensive hydrolysis of gliadin was observed after the second and third day of the process. The gradual degradation of this fraction indicates the presence of storage proteins used for the development of the grain.

Glutenin Fraction. The glutenin fraction has been shown to represent between 9.4 and 17.3% of the proteins in triticale, lower than that in wheat (Chen and Bushuk 1970a; Varughese et al. 1996a). During the extraction process of the gliadin fraction, samples RM, 1D, and 2D formed a gel that made it impossible to separate the glutenin fraction from the rest of the proteins. It was not, therefore, possible to analyze them. This gel was not formed for

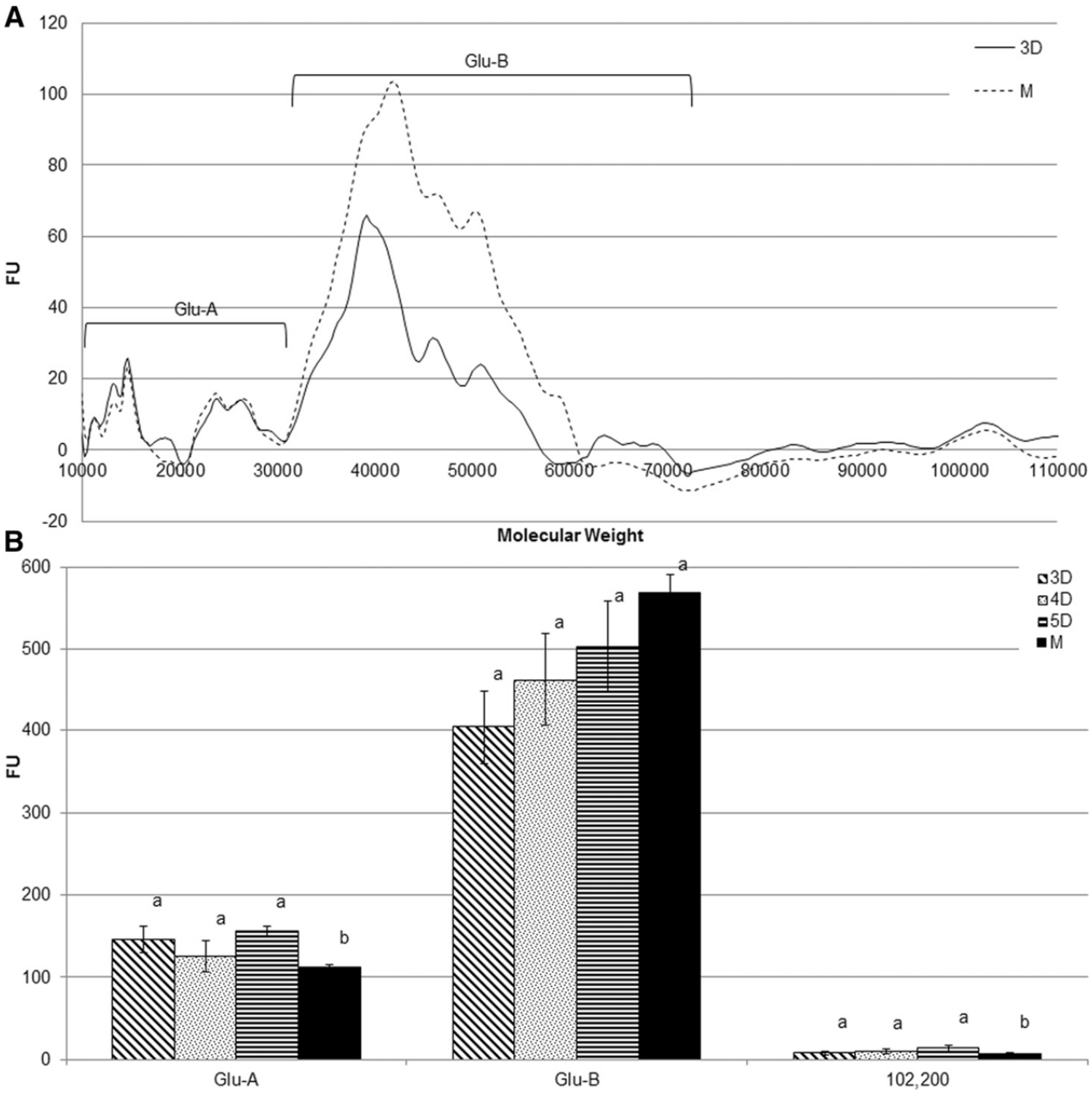

Fig. 5. A, Molecular weight protein profile; and **B,** peak relative concentration of the glutenin fraction of triticale affected by germination. FU = fluorescence units; D = days of germination; and M = malt. Lowercase letters indicate significant differences between malting stages. Peaks not plotted in B did not show significant differences between the malting stages.

samples 3D, 4D, 5D, and malt. This phenomenon may be owing to the presence of insoluble arabinoxylans and the formation during malting of an enzyme that is able to degrade them by 3D to malt but does not exhibit any such effect at the beginning of the malting process.

The glutenin fraction showed a protein pattern between 11,200 and 102,200 composed of 11 peaks with MWs of 11,200, 13,400, 14,600, 18,700, 23,900, 25,800, 39,600, 46,000, 50,800, 63,300, and 102,200. Owing to overlapping, peaks 11,200, 13,400, 14,600, 18,700, 23,900, and 25,800 were quantified together as Glu-A, and peaks 39,600, 46,000, 50,800, and 63,300 as Glu-B. Figure 5 shows the changes to the protein profile and to the peak relative concentration of the glutenin fraction from 3D up to malt. Glu-A and peak 102,200 slightly decreased in intensity during kilning, the relative concentration in malt being lower than in the other three malting stages. Although no significant difference ($P > 0.05$) was found for Glu-B, this area showed a tendency to increase during malting.

Composition of Free Amino Acids. The composition of free amino acids of triticale during the different stages of malting was analyzed (Table III). The results revealed an increase in the content of amino acids during malting. This is owing to the hydrolysis of the native proteins, which results in both HMW protein breakdown products and LMW proteolysis products (peptides and amino acids) (Narziß and Back 2012). Aspartic acid and asparagine are the only two amino acids that did not increase in concentration during malting and exhibit varying trends. Asparagine is the amide of aspartic acid that is easily hydrolyzed to the latter. This hydrolysis and deamination may explain the irregular behavior of both amino acids found in the results.

The amino acids glutamic acid, serine, glutamine, threonine, arginine, methionine, and tryptophan increased in concentration until 5D of germination and then decreased in the final malt, probably owing to the formation of Maillard products. The amino acids cysteine, leucine, and lysine increased until 4D, and histidine, tyrosine, and valine increased until 3D. These amino acids decreased thereafter, except for histidine, which remained almost constant until the final malt. Narziß and Back (2012) suggested that the increase in the amino acid content is owing to Strecker aldehyde reactions and the decrease to aminocarbonyl reactions.

Our results are in agreement with those presented in the literature. Wu (1982) and Robbins and Pomeranz (1971) analyzed the amino acid composition of triticale at different stages of germination and found a large increase in the lysine content. The same behavior was found by Dalby and Tsai (1976), who identified an increase in the amounts of lysine and tryptophan in triticale and other cereals.

According to Lorenz and Maga (1972), triticale has a better balanced amino acid composition than wheat, particularly owing to a generally higher amount of lysine. Many authors (Chen and Bushuk 1970a; Ruckman et al. 1973; Hulse and Laing 1974; Wu et al. 1976; Sikka et al. 1978; Grela 1996; Varughese et al. 1996a) agree about the high content of lysine in triticale; however, the information regarding the content of the other amino acids is confusing. According to Chung et al. (1983), tryptophan, threonine, cysteine, and glycine contents in triticale are higher than those of either parent. Chen and Bushuk (1970a) found the amount of glycine, cysteine, tyrosine, and arginine slightly higher in triticale than in the parent species. Mossé et al. (1988) established a high glycine and valine content but a lower proline content. Additionally, Wu et al. (1976) identified high levels of glutamic acid and proline. In our samples, RM had a high content of free leucine, aspartic acid, asparagine, and tyrosine. However, the free amino acid distribution in the final malt was not the same. Although the concentration of almost all amino acids increased during malting, the distribution in the different process stages varied. For example, the sample malt exhibited a high concentration of leucine, glutamic acid, isoleucine, and valine.

CONCLUSIONS

This paper covers the changes in the protein levels, the Osborne fractions, and the free amino acid contents of triticale during malting. Triticale proteins were found between MWs 4,700 and 64,000. The majority of the proteins were characterized as having a pI between 5.08 and 6.63. Triticale proteins underwent qualitative and quantitative changes that led to an increase of amino acid content. The Osborne fractionation revealed an albumin band pattern with MWs between 13,400 and 153,800, a globulin band pattern ranging from 12,700 to 152,300, a gliadin band pattern from 14,500 to 230,100, and a glutenin band pattern from 11,200 to 102,200. The amino acid concentration increased, except for the aspartic acid, which decreased during malting. Asparagine exhibited varying trends during malting. The amino acid composition in wort in particular is thought to be linked to the aroma compound profile of fermented beverages. This paper contributes to the understanding of the protein modifications and metabolic changes occurring during the malting process of triticale and contributes to determining the potential of this cereal for the production of cereal-based products.

TABLE III
Free Amino Acid Composition (mg/L) of Triticale During Malting[z]

Amino Acid	Raw Material	Day 1	Day 2	Day 3	Day 4	Day 5	Malt
Aspartic acid	639.2 ± 5.5a	527.9 ± 4.6b	502.1 ± 2.4c	463.5 ± 4.8d	478.8 ± 9.6d	490.7 ± 14.8d	449.6 ± 12.4e
Glutamic acid	465.5 ± 32.1a	496.4 ± 4.7a	709.4 ± 9.5b	704.8 ± 2.6b	753.9 ± 18.7c	840.7 ± 16.5d	740.9 ± 54.5d
Cysteine	36.3 ± 1.8a	43.9 ± 9.1a	46.3 ± 9.5a	53.5 ± 10.9a	61.4 ± 5.5b	39.1 ± 3.5c	51.1 ± 0.9d
Asparagine	595.4 ± 22.8a	319.6 ± 6.9b	269.8 ± 13.9c	269.8 ± 12.8c	343.7 ± 4.8d	297.2 ± 6.8e	480.6 ± 3.9f
Serine	108.7 ± 31.8a	124.2 ± 7.2a	191.0 ± 0.3b	268.7 ± 8.8c	343.4 ± 3.7d	375.2 ± 7.6e	346.3 ± 2.4f
Glutamine	112.8 ± 5.4a	245.1 ± 9.2b	533.0 ± 4.7c	905.0 ± 9.1d	1,273.5 ± 46.0e	1,386.7 ± 23.4f	1,205.9 ± 44.3g
Histidine	421.2 ± 15.2a	449.7 ± 16.9a	449.3 ± 1.3a	523.1 ± 0.3b	515.0 ± 14.7b	489.0 ± 6.6c	545.7 ± 6.5d
Glycine	88.6 ± 3.5a	58.5 ± 3.9b	72.6 ± 3.9c	104.9 ± 4.9d	123.3 ± 3.7e	121.4 ± 4.4e	142.4 ± 0.7f
Threonine	67.5 ± 3.0a	87.2 ± 1.2b	129.6 ± 3.4c	183.2 ± 0.2d	225.6 ± 2.1e	247.7 ± 10.4e	235.2 ± 11.8e
Arginine	311.1 ± 6.1a	280.0 ± 11.4b	318.4 ± 14.4c	400.3 ± 17.3d	492.2 ± 23.4e	611.6 ± 17.1f	560.0 ± 11.6g
Alanine	209.8 ± 1.8a	213.3 ± 2.6a	260.6 ± 11.6b	313.3 ± 15.0c	322.5 ± 19.2c	309.3 ± 15.1c	433.7 ± 5.6d
Tyrosine	516.0 ± 5.1a	503.5 ± 20.2a	590.4 ± 22.4b	684.5 ± 20.9c	384.4 ± 8.0d	403.7 ± 10.2d	407.0 ± 51.2d
Valine	448.5 ± 15.4a	439.9 ± 18.4a	466.7 ± 14.4a	614.8 ± 12.0b	612.1 ± 11.4b	598.3 ± 10.5b	618.7 ± 14.5b
Methionine	9.4 ± 2.4a	17.4 ± 0.1b	37.0 ± 1.7c	76.4 ± 0.5d	96.7 ± 2.1e	98.5 ± 2.4e	97.8 ± 7.4e
Tryptophan	330.9 ± 8.0a	336.5 ± 8.3a	379.2 ± 2.7b	431.6 ± 10.9c	507.3 ± 13.1d	507.7 ± 18.7d	489.1 ± 16.3d
Phenylalanine	65.1 ± 7.4a	81.9 ± 0.3a	175.3 ± 3.0b	291.3 ± 8.7c	434.9 ± 1.5d	437.3 ± 34.9d	439.2 ± 11.5d
Isoleucine	496.9 ± 4.5a	471.3 ± 17.5a	543.4 ± 28.3b	611.2 ± 4.6b	615.3 ± 8.1c	603.7 ± 18.2c	622.8 ± 16.0c
Leucine	1,460.1 ± 10.9a	1,488.4 ± 17.5a	1,635.6 ± 81.7a	1,821.3 ± 111.7b	1,861.7 ± 84.9b	1,778.8 ± 100.1b	1,810.6 ± 84.7b
Lysine	275.7 ± 5.6a	309.0 ± 11.6b	391.2 ± 16.5c	536.8 ± 4.1d	642.7 ± 15.2e	557.2 ± 5.9f	566.6 ± 16.9f

[z] Days of germination. Mean ± SD. Different letters following numbers indicate significant differences between malting stages.

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3.5. Variation of Sunstruck Flavor-Related Substances in Malted Barley, Triticale and Spelt

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ORIGINAL PAPER

Variation of sunstruck flavor-related substances in malted barley, triticale and spelt

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Abstract Sunstruck flavor is an off-flavor formed when beer is exposed to light. Although isohumulone is known as the main substrate, the degradation of substances present in malt also plays a role in its formation. Riboflavin, phenylalanine, sulfur-containing amino acids and the sulfur-containing peptide glutathione have been described as sunstruck flavor initiators, promoting the formation of sunstruck flavor (Charpentier and Maujean in Flavour ´81, 3rd Weurman symposium, Munich, 1981; Huvaere et al. in J Agric Food Chem 53(5):1489–1494, 2005; Kuroiwa and Hashimoto in Am Soc Brew Chem 19:28–36, 1961). Moreover, tryptophan and polyphenols are known to reduce the formation of sunstruck character in beer (inhibitors) (Pozdrik et al. in J Agric Food Chem 54(17):6123–6129, 2006). The initiators and inhibitors of sunstruck flavor originate in the raw material used, and their content can be influenced during manufacturing. The aim of this paper was to define the influence of the malting process parameters on the content of initiators and inhibitors involved in the formation of sunstruck flavor in different cereals. Barley, spelt and triticale were malted under different parameters, and the content of the sunstruck flavor initiators and inhibitors was analyzed. The initiator and inhibitor contents were described by linear, two-factor interaction or quadratic models. The malting parameters exert in most of the substances a positive influence. The germination temperature had a negative influence on the content of cysteine, methionine and tryptophan of triticale. The germination time and temperature showed a negative influence on the tryptophan content of spelt. With the knowledge gained, the potential of different cereals for its use in the development of malted cereal-based beverages can be determined. Moreover, the influence of malting process on the content of these substances as well as differences between the raw materials was defined.

Keywords Sunstruck flavor · Barley · Triticale · Spelt · Malting process

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Introduction

Sunstruck flavor is an off-flavor formed when beer, bottled in translucent or green bottles without UV-protection, is exposed to light. This off-flavor, detected as early as 1875 by Lintner [5], is described to have meaty, sulfury and skunky or foxy notes. Kuroiwa and Hashimoto [3] and Kuroiwa et al. [6] described in the early 1960s the aroma active compound 3-methylbut-2-ene-1-thiol (MBT), out of odorless precursors, as being the key compound responsible for sunstruck flavor. This substance, with an aroma threshold in water of 0.2–0.4 ng/L [7] and of 4–35 ng/L [8] in beer, is considered one of the most important aroma active compounds. Komarek et al. [8] identified in beer after exposure to sunlight, besides MBT, other substances such as 3-(methylthio)-propanal, phenylacetaldehyde, 2-methylpropanal, 3-methylbutanal and an unknown compound with a sulfury odor note. Moreover, a recent study [9] described in illuminated beer an increased content of these substances as well as of methanthiol. However, the results of these investigations showed that the content of

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phenylacetaldehyde and 3-methylbutanal in a beer with sunstruck flavor is still under their corresponding thresholds. Therefore, their contribution to the off-flavor aroma is not relevant. However, if the concentration increases, phenylacetaldehyde and 3-methylbutanal may play a role.

Kuroiwa et al. [3, 6] suggested isohumulone as the main initiate substrate in the formation of MBT as they had exposed some model solutions containing isohumulones, riboflavin and cysteine to light. Under these conditions, riboflavin is the only light-absorbing species [10, 11]. Therefore, photo-reactivity can only occur via intervention of riboflavin [12]. A couple of years later, Huvaere et al. [10] were able to determine the reaction products formed on photo-oxidation of isohumulones and described the complete reaction. Hence, the photochemical sensitization of riboflavin can be considered indispensable for the formation of MBT from isohumulones [13]. Together with riboflavin, some substances have also been described as sunstruck flavor initiators, promoting the formation of sunstruck flavor, and others as sunstruck flavor inhibitors, preventing its formation. Sulfur-containing amino acids, cysteine [1, 13–16] and methionine [1, 8, 13, 15, 17], and the sulfur-containing peptide glutathione [9, 14] have also been identified as playing a role in the formation of sunstruck flavor (initiators). These amino acids serve as a sulfur source for the development of the sunstruck flavor, accelerating the formation of MBT, 3-(methylthio)-propanal and methanthiol. Also the amino acid phenyalanine takes part in the formation of phenylacetaldehyde [9].

Substances like tryptophan, ascorbic acid or tryptophol reduced the formation of the sunstruck character in beer (inhibitors) by protecting riboflavin from photo-degradation, protecting beer against sunstruck flavor formation [4, 18]. Some authors [1, 4, 8, 18–23] described also the polyphenols such as catechin, epicatechin and quercitin, as protectors of the formation of this sunstruck flavor. The protective effect of polyphenols was described as (1) quenching both the singlet- and triplet-excited states of riboflavin with rates close to the diffusion control [22], (2) to preserve riboflavin absorbance in light-exposed model beers by quenching its excited energy state, and (3) a possible extraction of electrons by isohumulones from the polyphenols and the latter from photo-reduced riboflavin returning this to its original state [4]. However, these aromatic compounds have also been described as catalyzing the transformation of amino acids to their corresponding Strecker aldehydes, methional and phenylacetaldehyde [8, 20], which may provide an aged flavor or even increase their presence in illuminated beer.

The initiators riboflavin, cysteine, methionine, phenylalanine and glutathione, and the inhibitors tryptophan and polyphenols have their origins in the raw material used, and their content is influenced during malting. Germination or malting of cereals has been used for centuries to soften kernel structure, to increase nutrient content and availability, to decrease the content of anti-nutritive compounds, and to add new flavors [24, 25]. Changes occurring during germination influence the content, absorption of nutrients and bioavailability of functional components [24]. One of the most important physical–chemical changes during malting is the degradation of the proteinaceous matrix and the conversion into soluble peptides and amino acids to provide substrates for the synthesis of proteins in the growing embryo [26, 27]. Variation of the malting parameters, for example, longer germination times, exerts an influence on the degradation of proteins, normally increasing the content of free amino acids [28]. About 80 % of phenolics present in beer are derived from barley malt [29]. The polyphenol content of barley depends on the variety and increases during malting [30]. Also riboflavin present in beer is derived mainly from malt. This vitamin, known to increase during germination, is located in the epithelium of seedlings and the aleurone layer of the barley grain and together with other vitamins is involved in enzyme synthesis [31].

Amino acids play an important role in the production of malt and beverages. They influence the aroma and flavor profile of the final product by taking part in different reactions. Amino acids (1) are involved in the formation of Maillard products during kilning with the already formed sugars, (2) may react with dicarbonyl compounds producing the aroma-intensive Strecker aldehydes, (3) containing sulfur may suffer deamination, decarboxylation and reduction, respectively, and form hydrogen sulfide and higher alcohols, excreted as fermentation by-products, and moreover, (4) SO_2, known to improve the flavor and flavor stability of the beer, is produced as an intermediate product of the synthesis of sulfur-containing amino acids toward the end of fermentation.

Also polyphenols have an influence on the final beer quality. Whereas some authors point to an increase in mouthfeel due to proanthocyanidins [32, 33], others associate a harsh astringent flavor, and thus, a detriment in the mouthfeel [34]. Furthermore, polyphenols contribute partly to the color of beer, act as natural antioxidants and play a significant role in the colloidal stability of beer [35].

The aim of this paper is to define the influence of the malting process on the content of initiators and inhibitors involved in the formation of sunstruck flavor in barley, spelt and triticale. With the knowledge gained, the potential of different cereals for its use in the development of malted cereal-based beverages can be determined. Moreover, the influence of the malting process on the content of these substances as well as the differences between the raw materials may be defined.

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Table 1 CCD matrix of malting parameters of barley, spelt and triticale

Sample/parameter	Barley			Spelt/triticale		
	Time (day)	Temperature (°C)	Degree of steeping (%)	Time (day)	Temperature (°C)	Degree of steeping (%)
1	5	13	38	5	13	43
2	5	13	38	5	13	43
3	5	13	44	5	13	47
4	5	13	44	5	13	47
5	5	15	41	5	15	45
6	5	17	38	5	17	43
7	5	17	38	5	17	43
8	5	17	44	5	17	47
9	5	17	44	5	17	47
10	6	13	41	6	13	45
11	6	15	38	6	15	43
12	6	15	41	6	15	45
13	6	15	41	6	15	45
14	6	15	41	6	15	45
15	6	15	44	6	15	47
16	6	17	41	6	17	45
17	7	13	38	7	13	43
18	7	13	38	7	13	43
19	7	13	44	7	13	47
20	7	13	44	7	13	47
21	7	15	41	7	15	45
22	7	17	38	7	17	43
23	7	17	38	7	17	43
24	7	17	44	7	17	47
25	7	17	44	7	17	47

Materials and methods

Raw material

Barley, variety Grace, with a moisture content of 12.8 % was procured from Weyermann (Bamberg, Germany) in 2013. Triticale, variety Trigold, with a moisture content of 13.5 % was procured from KWS Lochow GmbH (Bergen, Germany) in 2011. Dehulled spelt, variety Frankenkorn, with a moisture content of 14 % was procured from and dehulled by Schapfen Mühle (Ulm, Germany) in 2011.

Experimental design and statistical analysis

To analyze the impact of the malting parameters (degree of steeping, germination temperature and time) on the content of riboflavin, polyphenols, cysteine, methionine, phenylalanine and tryptophan on three different cereal malts, two factorial ANOVAs was applied. Maximum and minimum predictor levels were defined by preliminary malting experiments. Face-centered cube design was used with double replicated factorial and three replicated center points. Values of malting parameters are shown in Table 1.

Malting was conducted in 25 of 1-kg batches in micro-malting systems for each cereal by varying the degree of steeping, germination time and germination temperature (independent variables). The steeping water was equilibrated by placing it 24 h prior to steeping in the climate chambers. Each parameter was varied to three different levels. The degree of steeping of the grains was adjusted to 38, 41 and 44 % for barley and 43, 45 and 47 % for spelt and triticale by conducting a standard steeping procedure as described in the "Mitteleuropaeische Brau- und Analysenkommission" (MEBAK) method 1.5.3 [36]. Thereafter, germination of steeped grains was carried out in micro-climate chambers controlled at different temperatures 13, 15 and 17 °C for different periods of germination 5, 6 and 7 days. Steeping and germination temperatures were kept constant in temperature-controlled chambers with 75 % relative humidity (RH). Standard kilning was done at 50 °C for 16 h followed by an hour at 60 °C, 1 h at 70 °C and 5 h

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rest at 80 °C according to the MEBAK method 2.5.3.1 [36]. After kilning, rootlets and sprouts were removed and the malt was allowed to rest for 7 days before analysis.

The software package Design Expert version 8.0.6 by StatEase (Stat-Ease Corporation, Minneapolis, MN, USA) was used to analyze the data obtained from the 25 micro-malting trials that were conducted for the three different cereals. The data thus obtained were analyzed using the analysis of variance (ANOVA). Different indexes were used to analyze and evaluate the adequacy of the calculated statistical models. The coefficient of determination R^2 is the proportion of variation in the response attributed to the model rather than to random error [37]. For a good fit of a model, a R^2 value higher than 80 % is recommended [38]. The studied parameter is considered to have a significant effect on the response, if p value <0.05. F value is used to describe the influence of the model, and the lack of fit to describe the scatter of the data around the fitted model.

Analytical procedures

Analytical procedures were carried out in duplicate ($n = 2$), and the means of all results were calculated.

Riboflavin

The determination of riboflavin was done using the whole-blood test kits from Chromsystems (Gräfelfing, Germany). These kits proved to be suitable for beer, wort and aqueous extracts from malt because no interference with other substances occurred during the analysis. The chromatographic separation of the samples was done on a HPLC (U3000 Dionex, Dionex, Sunnyvale, USA). Riboflavin for the calibration of the system was obtained from Sigma-Aldrich (St. Louis, USA). For the vitamin extraction from the samples, 100 mg of finely ground cereal grist was weighed accurately into Eppendorf vials, extracted with 1.5 mL water at 45 °C for 1 h, centrifuged and analyzed following the instructions for the whole-blood analysis.

Free amino acids

Sample preparation for amino acid analysis with HPLC was carried out by mixing 0.15 g of milled sample with 1.5 mL distilled water. The mixture was stirred for 60 min and 10,000 g at 65 °C and finally centrifuged at 2900 g for 10 min. The supernatant was diluted 1:5 with distilled water. The amino acids present in the diluted supernatant were quantified by a HPLC (U3000 Dionex, Dionex, Sunnyvale, USA) following a modified method of Krömer [39]. This method uses a Gemini C18 column (5 μm, 110A, 150 × 4.6 mm; Phenomenex, Aschaffenburg, Germany) with automated online derivatization (o-phtaldialdehyde and 3-mercaptopropionic acid) at a flow rate of 1 mL/min and fluorescence detection. Instead of using an internal standard for quantification, as Krömer [39] suggests, in this case quantification was done by a six-point external calibrating curve for each amino acid. The following amino acids: cysteine, methionine, phenylalanine and tryptophan were quantified. All samples were analyzed in triplicate ($n = 3$).

Total polyphenol content

Sample extracts for the measurement of total phenol content were prepared from 1.25 g freshly ground sample in 25 mL methanol as described by Alvarez-Jubete et al. [40]. Samples were homogenized for 2 min at 12,000 rpm using an Ultra-Turrax T-25 tissue homogeniser (IKA-group, Saufen, Germany), shacked for 20 min and centrifuged for 10 min at 2000×g (Megafuge 16R, Thermoscientific, Waltham, Massachusetts, USA). The final extracts were obtained by filtering 10 mL of the supernatant through 0.22 μm PTFE syringe filters (Phenomenex, Macclesfield Cheshire, UK). The total phenolic content of methanolic grain extracts was evaluated using a modified version of the Folin–Ciocalteu assay as described by Singleton, Rossi [41]. Briefly, 100 μL of methanolic grain extract or standard, 100 μL of MeOH, 100 μL of Folin–Ciocalteu reagent and 700 μL of Na_2CO_3 were added into a 1.5-mL micro-centrifuge tube. The samples were vortexed immediately, and the tubes were incubated in the dark for 20 min at room temperature. After incubation, all samples were centrifuged at 13,000×g (Megafuge 16R, Thermoscientific, Waltham, Massachusetts, USA) for 3 min. The absorbance of the supernatant was then measured at 735 nm. Gallic acid was used as a standard, and a calibration curve was prepared with a range of concentrations from 10 to 200 mg/L. The results are expressed in mg gallic acid equivalent per 100 g dry-weight basis (mgGAE/100 g dm).

Glutathione

The glutathione determination was done as previously described by Zielinski et al. [42]. Malted cereal grains (3 g) were ground in a TissueLyser II (Qiagen, Hilden, Germany). The flour was transferred to a centrifuge tube and mixed with phosphate buffer (15 mL; 0.2 M, with EDTA, 1 mM, pH 7.5) and potassium chloride (KCl, 0.33 g). The mixture was homogenized for 30 s using an Ultra-Turrax T-25 tissue homogeniser (IKA-group, Saufen, Germany). Polyvinylpolypyrrolidone (PVPP; 0.25 g) was added, and after thorough mixing, the mixture was centrifuged at 2000×g, 10 min; 4 °C (Megafuge 16R, Thermoscientific, Waltham, Massachusetts, USA). After recentrifugation, the supernatant was kept on ice and assayed for the reduced

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glutathione content. In order to determine the GSH content, 60 µL of the extract was mixed with 1.84 mL 0.1 M sodium phosphate, 0.005 M EDTA buffer (pH 8.0) and 100 µL of the OPT solution, containing 100 µg of OPT (1 % w/v) in reagent-grade absolute methanol. After thorough mixing and incubation at room temperature for 15 min, the solution was transferred to a quartz cuvette. Fluorescence at 420 nm was determined with the excitation at 350 nm. A series of GSH standards were prepared in a phosphate–EDTA buffer, pH 8.0, which ranged from 0.033 to 6.51 nmol/60 µL. The data were calculated in grams of wet basis of cereal grains.

Results

The aim of this work is to study the single or interaction effects of the malting parameters on the content of initiators and inhibitors involved in the formation of sunstruck flavor in barley, spelt and triticale, and also to define the differences between the three cereals. Therefore, 25 samples of each cereal were malted under different malting parameters. Tables 2 and 3 show the main results of the ANOVA of the sunstruck flavor initiators and inhibitors, respectively. Based on the statistical analysis, the models were highly significant with very low p values (from <0.0001 to 0.0023). The R^2 values for all attributes fitted models were higher than 0.79, indicating that the regression models explained the reaction well. The lack of fit of all the attributes was not significant, relative to pure error; it reflected how the fitted models can be used to explain the responses.

Riboflavin

The attribute riboflavin was described for barley and triticale by a quadratic model and for spelt by a 2FI model (see Table 2). Barley and triticale showed a similarity in significant terms. The terms degree of steeping, germination temperature as well as germination time and the quadratic effect of germination temperature were significant ($p < 0.05$) for the models of both these cereals. The quadratic effect of the degree of steeping was also significant for the triticale model. On the other hand, spelt model had as significant terms the germination temperature and time, the interaction effect of degree of steeping and germination time, and temperature and time.

Figure 1 shows the influence of temperature and degree of steeping after 7 days of germination on the riboflavin content of barley. The barley, spelt as well as triticale models showed the same behavior; the malting parameters exerted a positive influence on the riboflavin content present in malt. Higher degrees of steeping, temperatures and times lead to higher riboflavin contents. The increase

Table 2 Analysis of variance (ANOVA) of the sunstruck flavor initiators fitted models

Initiators	Riboflavin			Cysteine			Methionine			Phenylalanine			GSH		
	Ba	S	T	Ba	S	T	Ba	S	T	Ba	S	T	Ba	S	T
Model	Q	2FI	Q	Q	2FI	2FI	L	2FI	Q	Q	2FI	2FI	L	Q	Q
Model p value	<0.0001	0.0002	0.0010	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	0.0001	<0.0001	<0.0001	<0.0001	0.0023	0.0008
A	<0.0001	0.3514	0.0140	<0.0001	0.6107	0.2275	<0.0001	0.3946	0.0010	0.5713	0.5021	<0.0001	<0.0001	0.7751	0.7518
B	<0.0001	0.0021	0.0422	0.0046	0.0022	0.0055	0.0763	0.1797	0.0004	0.6515	0.0037	0.0095	0.0009	0.2251	0.3807
C	<0.0001	<0.0001	0.0008	0.0011	<0.0001	0.0079	<0.0001	<0.0001	0.0003	0.0008	<0.0001	<0.0001	0.5892	0.1247	0.0663
A x B	0.8365	0.2017	0.8345	0.0329	0.0033	0.1292	–	0.0004	0.0763	0.7	0.0112	0.0131	–	0.5516	0.0065
A x C	0.0621	0.003	0.173	0.0668	0.6184	<0.0001	–	0.5466	0.0346	0.0462	0.5479	0.0201	–	0.0198	0.7178
B x C	0.1493	0.0015	0.31	0.7702	0.0002	0.0009	–	<0.0001	0.038	0.0149	0.0007	0.2058	–	0.0036	0.5475
A^2	0.5259	–	0.007	<0.0001	–	–	–	–	0.0042	0.3889	–	–	–	0.0046	0.0004
B^2	0.0244	–	0.0057	0.7279	–	–	–	–	0.4317	0.116	–	–	–	0.6041	0.062
C^2	0.608	–	0.0533	0.9187	–	–	–	–	0.0315	0.0002	–	–	–	0.0002	<0.0001
R^2	0.91	0.80	0.83	0.98	0.85	0.86	0.92	0.86	0.87	0.86	0.88	0.88	0.80	0.82	0.85
Lack of fit	0.13	0.85	0.28	0.95	0.07	0.13	0.42	0.05	0.83	0.36	0.21	0.67	0.72	0.64	0.40

A degree of steeping, *B* temperature, *C* time, *Ba* barley, *S* spelt, *T* triticale

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Table 3 Analysis of variance (ANOVA) of the sunstruck flavor inhibitors fitted models

Inhibitors	Tryptophan			TPP		
	Barley	Spelt	Triticale	Barley	Spelt	Triticale
Model	Q	2FI	Q	Q	L	L
Model *p* value	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	<0.0001
A	<0.0001	0.0959	0.1406	<0.0001	0.4385	<0.0001
B	<0.0001	0.0404	0.0018	0.0005	0.0252	0.131
C	<0.0001	0.001	<0.0001	0.6793	<0.0001	0.001
A × B	0.7141	0.0035	0.9791	<0.0001	–	–
A × C	0.0222	0.0331	0.2594	0.0052	–	–
B × C	0.0008	0.0015	0.0113	0.0004	–	–
A^2	0.0305	–	0.824	0.0093	–	–
B^2	0.0017	–	0.0013	<0.0001	–	–
C^2	0.2934	–	0.7654	0.0802	–	–
R^2	0.92	0.79	0.89	0.91	0.79	0.81
Lack of fit	0.90	0.70	0.72	0.79	0.58	0.83

A degree of steeping, *B* temperature, *C* time

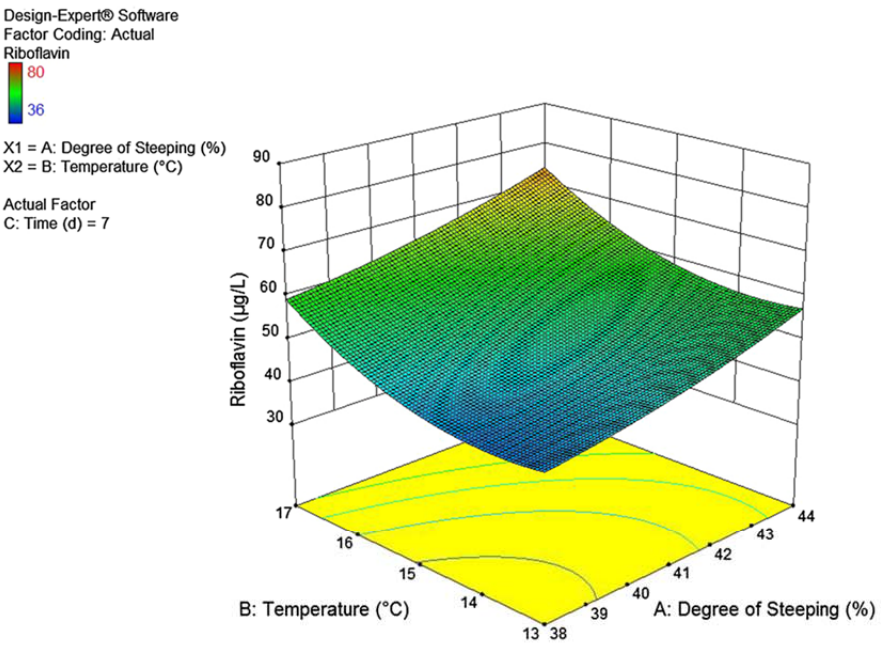

Fig. 1 Influence of the germination temperature and degree of steeping on the riboflavin content of barley malt after 7 days of germination

in riboflavin content in different cereals during germination has been determined by many authors [26, 28, 43–45]. The maximum riboflavin content is found in barley (B) by 44 %/17 °C/7 days, in spelt (S) 43 %/17 °C/7 days and in triticale (T) 47 %/15 °C/7 days. On the opposite, the lowest riboflavin content was found in the low modified malts, i.e., malted with a low degree of steeping and under low temperature and short times. Spelt presented the highest riboflavin content, on average, followed by triticale and last barley. Due to the increase in riboflavin content, the higher the degree of steeping, the lower the riboflavin content presented in barley malt may be, which is possibly explained by the lower degrees of steeping at which barley was malted (38–44 %).

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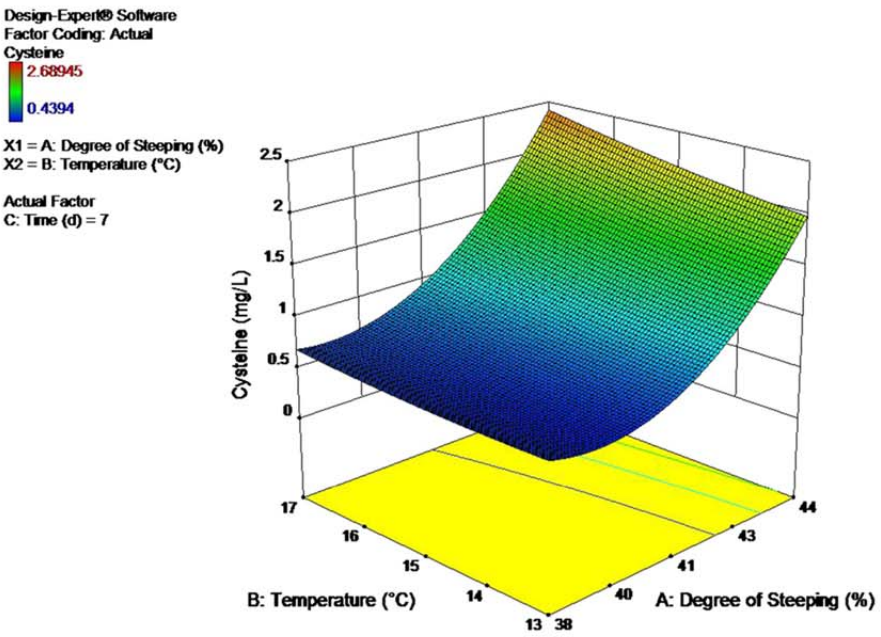

Fig. 2 Influence of the germination temperature and degree of steeping on the free cysteine content of barley malt after 7 days of germination

Cysteine

Cysteine was described in spelt and triticale malt by a 2FI model. The significant terms influencing the free cysteine content are germination temperature, germination time, and the interaction effect B × C for both cereals and the interaction effect A × B for spelt and A × C for triticale. The cysteine content in barley malt was described by a quadratic model with germination temperature, germination time, the degree of steeping, the interaction effect A × B and the quadratic effect of degree of steeping as significant terms.

Figure 2 shows the influence of the temperature and the degree of steeping after 7 days of germination on the cysteine content of barley. Although the models describing the cysteine content in barley, spelt and triticale malts differentiate, the influence of the malting parameters was similar among all three cereals. Higher degrees of steeping, temperatures and times lead to higher cysteine contents except for the germination temperature by triticale which exerted a negative influence. The maximum cysteine content was found in B 44 %/17 °C/7 days, in S 47 %/17 °C/7 days and in T 47 %/13 °C/7 days. According to the values measured, triticale malt has the highest cysteine content on average, and spelt and barley malt have similar average contents. The higher cysteine concentration of triticale compared to spelt was also proved by Grela [46].

Methionine

The free methionine content in barley, spelt and triticale malt was described by a linear, 2FI and quadratic models, respectively. The parameter germination time was significant in all models. Moreover, the degree of steeping was a significant term for the barley model, and the interaction effects A × B and B × C were significant for the spelt model. Degree of steeping, germination temperature, the interaction effects A × C and B × C and the quadratic effects of degree of steeping and germination time were significant for the triticale model.

Figure 3 shows the influence of the germination temperature and the degree of steeping after 7 days of germination on the free methionine content of barley malts. Similar to the content of free cysteine, the higher the degrees of steeping, temperatures and times, the higher the methionine contents except for the germination temperature for triticale which exerted a negative influence. The maximum methionine content was found in B 44 %/17 °C/7 days, in S 47 %/17 °C/7 days and in T 47 %/13 °C/7 days. According to the values measured, barley malt has the highest methionine content on average, and spelt and triticale malt have similar average contents.

Phenylalanine

Similar to the cysteine model, the free phenylalanine content was described by a quadratic model for barley malt

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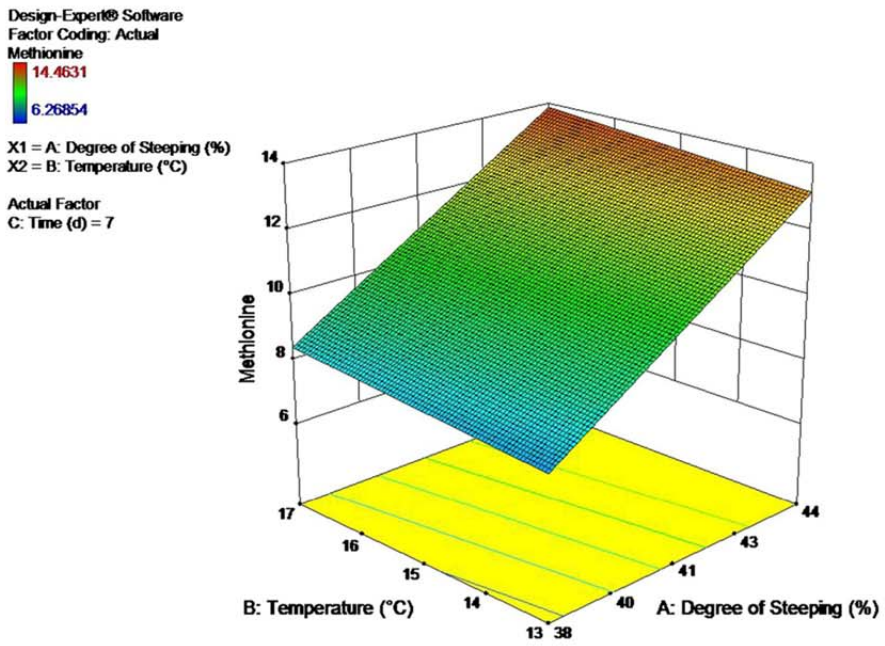

Fig. 3 Influence of the germination temperature and degree of steeping on the free methionine content of barley malt after 7 days of germination

and by a 2FI model for spelt and triticale malts. The significant terms were for barley germination time, the interaction effects A × C and B × C and the quadratic effect of germination time. Germination temperature, germination time and the interaction effect A × C were significant terms for both spelt and triticale malts. Moreover, the degree of steeping and the interaction effect were also significant terms of the phenylalanine content model of triticale malt, and the interaction effect B × C was also a significant term of the phenylalanine content model of spelt malt.

All significant terms exerted a positive influence on the free phenylalanine content present in the malts.

Figure 4 shows the influence of the germination time and temperature at 47 % degree of steeping on the free phenylalanine content of spelt malt. The maximum free phenylalanine content was found in B under 42 %/17 °C/6 days, in S 47 %/17 °C/7 days and in T 47 %/17 °C/7 days. According to the values measured, barley malt had the highest phenylalanine content, on average, followed by triticale and spelt malts, respectively.

Glutathione

Glutathione is a tripeptide involved in the process of overcoming dormancy of the grain. Belderok [47] described a correlation between the total amount of glutathione in embryos after 24 h wetting and the percentage of grain germinated in 3 days. The model in barley malt describing the glutathione content differs widely from those of spelt and triticale malts. Glutathione content in barley malt is described by a linear model with the degree of steeping and germination temperature as significant terms. The malting parameters exert a positive influence on the glutathione content of barley malt. Due to this influence, the maximum glutathione content in barley malt was found in the over-modified malts (44 %/17 °C/7 days) and the minimum in the under-modified malts.

On the contrary, the glutathione content of spelt and triticale malts was described by quadratic models, the significant terms being the interaction effects A × B and B × C, A^2 and C^2 for spelt malt and the interaction effects A × B and A^2 and C^2. All significant terms exert a positive influence on the glutathione content of spelt and triticale malt except for the quadratic term of the germination time which exerts a negative influence. Therefore, the behavior of glutathione content depending on the germination time is described by a parabola. A maximum as well as a minimum glutathione content was not found within the analyzed area. However, the maximum analyzed glutathione content was found under 47 %/17 °C/6 days for spelt, and 43 %/13 °C/6 days for triticale, and the minimum under 45 %/15 °C/5 days for both cereals. The results correspond with those found in the literature where some authors [47, 48] reported a decrease followed by an increase in the glutathione content of germination barley.

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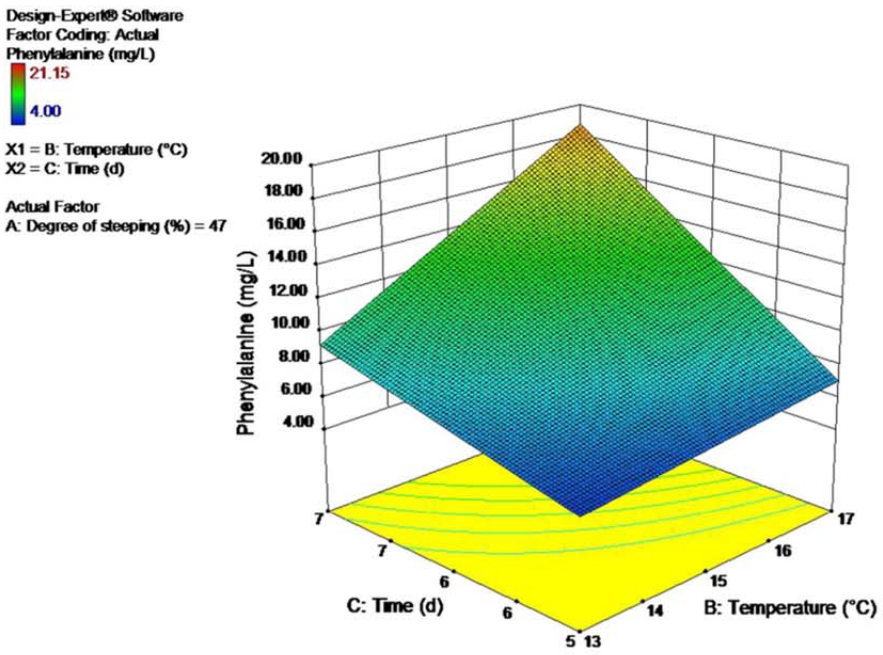

Fig. 4 Influence of the germination time and germination temperature on the free phenylalanine content of spelt malt by a degree of steeping of 47 %

Fig. 5 Influence of the germination temperature and degree of steeping on the glutathione content of barley malt after 5 days of germination

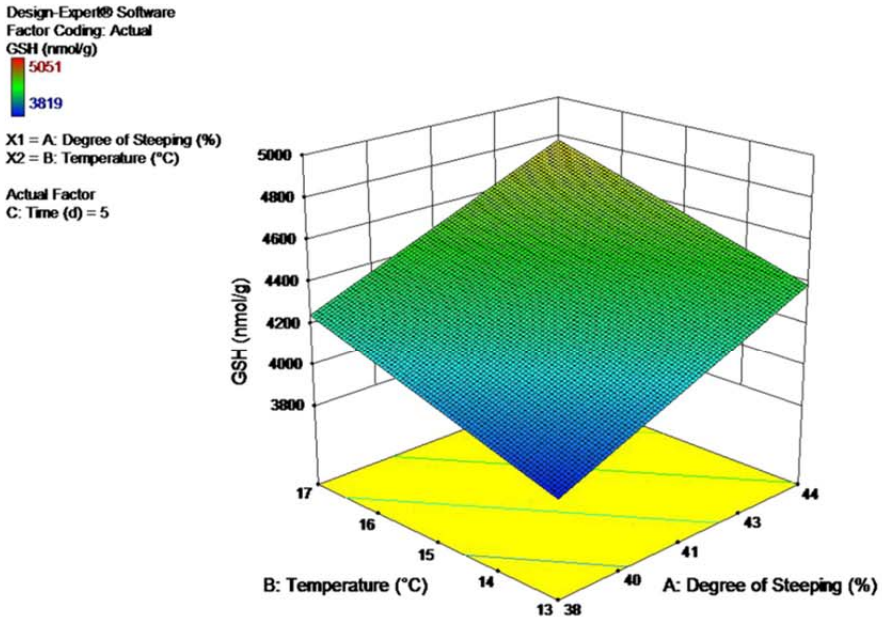

Figure 5 shows the influence on glutathione content of barley malt of the degree of steeping and germination temperature. On average, triticale malt showed higher glutathione content followed by barley malt and spelt malt.

Tryptophan

The attribute tryptophan was described by a quadratic model for barley and triticale malts and by a 2FI model for

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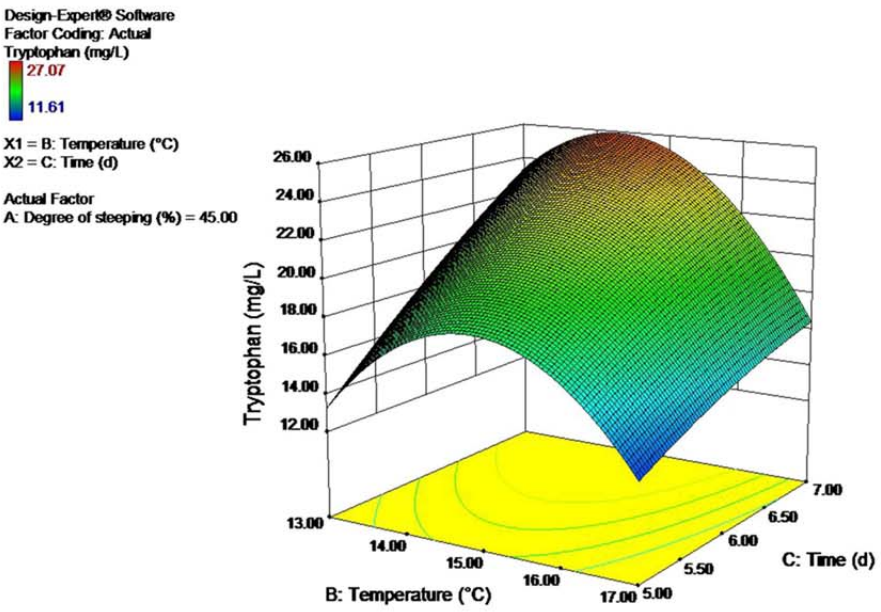

Fig. 6 Influence of the germination temperature and germination time on the tryptophan content of triticale malt at 45 % degree of steeping

spelt malt. Germination time, germination temperature and the interaction effect B × C were significant in all models. Also the terms, the degree of steeping, the interaction effect A × C and the quadratic effects of degree of steeping and germination temperature were significant in the model of barley. The interaction effects A × C and A × C were significant in the spelt model and the quadratic effect of the germination temperature in the triticale model.

The significant terms had a positive influence on the content of free tryptophan of the malts except for the germination time and temperature for spelt and germination temperature for triticale.

Figure 6 shows the tryptophan content of the triticale malts influenced by the germination time and temperature by a degree of steeping of 47 %. The influence of the significant terms can be further substantiated by the tryptophan content; the maximum was found in B under 43 %/17 °C/7 days, in S 47 %/13 °C/5 days and in T 47 %/14 °C/7 days. Spelt showed the higher average content of free tryptophan followed by triticale and barley.

Total polyphenol content

The total polyphenol model of barley malt differs widely from those of spelt and triticale malt. A quadratic model described the total polyphenol content in barley malt. The significant terms of this model were the single and quadratic terms of degree of steeping and temperature as well as all three interaction effects A × B, A x C and B x C. The degree of steeping exerted a negative influence on the total polyphenol content, whereas the germination temperature exerted a positive influence. The maximum polyphenol content was found under 39 %/17 °C/7 days and the minimum under 44 %/13 °C/5 days.

Total polyphenol content of spelt and triticale malts was described by linear models. By spelt malt, the single terms germination temperature and time were significant. By triticale malt, the single terms degree of steeping and germination time were significant. For both cereals, all malting parameters exerted a positive influence.

Figure 7 depicts the influence of the germination temperature and time on the total polyphenol content of spelt malt by a degree of steeping of 47 %. The maximum polyphenol content for both cereals was found under 47 %/17 °C/7 days.

Barley malt has, on average, higher polyphenol content than that of spelt and triticale malt. The presence of husk in barley and the absence in spelt (dehulled) and triticale (hulless) may underline the higher total polyphenol content of barley. Polyphenols are deposited in the husk, the pericarp and the aleurone layer of barley [49–52]. Moreover, Isoe et al. [53] indicate a particularly high polyphenol content in hulled spelt and the possibility to reduce it by removing the husk. Glatthar et al. [54] described a decrease in the polyphenol content in beers brewed with some triticale instead of barley malt. However, the triticale employed was unmalted.

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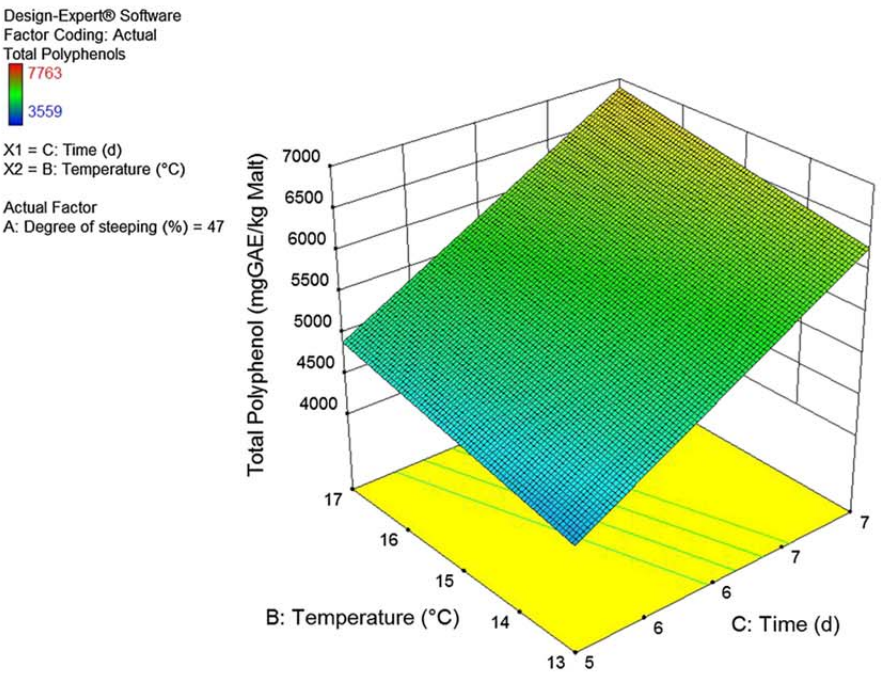

Fig. 7 Influence of the germination temperature and germination time on the total polyphenol content of spelt malt by 47 % degree of steeping

Discussion

With the knowledge gained, the potential of different cereals for their use in the development of malted cereal-based beverages can be determined. Moreover, the influence of the malting process on the content of these substances as well as the differences between the raw materials was defined.

Although sunstruck flavor is a well-known off-flavor in beverages containing hops, malted cereal-based beverages exhibit this problem when exposed to light. The main substrate in the formation of sunstruck flavor is formed by isohumulones, which are present in hops as humulones and are isomerized during boiling. However, the degradation of other substances present in malt also plays a vital role. Riboflavin, phenylalanine, sulfur-containing amino acids and the sulfur-containing peptide glutathione have been described as sunstruck flavor initiators, and tryptophan and polyphenols as sunstruck flavor inhibitors. The content of these substances can be influenced during the malting and brewing process, thus influencing the formation of sunstruck flavor in beer or in malted cereal-based beverages. The aim of this paper is to define the influence of the malting process parameters on the content of initiators and inhibitors involved in the formation of sunstruck flavor in different cereals. In general, it can be said that the content of the inhibitors and initiators was increased during malting. Solely, the germination temperature had a negative influence on the content of the amino acids cysteine, methionine and tryptophan of triticale. The germination time and temperature showed a negative influence on the tryptophan content of spelt.

Comparing the content of initiators of the three cereals, barley had, on average, the highest methionine and phenylalanine content, spelt the highest riboflavin content, and triticale the highest cysteine and glutathione content. On the contrary, barley had the highest total polyphenol content and spelt the highest tryptophan content. According to these results, it is not possible to determine one of the cereals as the best cereal for a free sunstruck flavor beer. When using a hop product with humulone, the wort produced from each of these cereals has the potential to develop sunstruck flavor.

Although it was possible to decrease the content of initiators to undermine the formation of sunstruck flavor under variation of the malting parameters, the content of sunstruck flavor inhibitors decreases concurrently. The lower content of initiators and inhibitors was found, in general, in under-modified malts rather than in over-modified. A compromise should be found in order to aim for a beverage (containing hops) free of sunstruck flavor. The balance could be found by using an under-modified malt (with low content of initiators and inhibitors) together with a rich polyphenol hop product (with low, if any,

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humulone content). However, the use of under-modified malts may cause technological problems during the brewing process.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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4. Discussion

Flavor stability has always been of particular interest in the brewing industry. Besides the well-known beer oxidation process caused by oxygen, the off-flavor known as “sunstruck flavor” may occur if beer is exposed to light without protection. All fermented non-distilled beverages suffer from light-induced development of off-flavors and deterioration of the product. When beer is exposed to sunlight, a group of compounds develops from the degradation of substances present in it. The main compound responsible for the sunstruck flavor is 3-methyl-2-butene-1-thiol but methanethiol, methional, 3-(methylthio)-propanal, phenylacetaldehyde, 2-methylpropanal, 3-methylbutanal also play a role in the cereal-based beverages exposed to light.

The formation of the aroma active compounds responsible for the sensory perception of sunstruck flavor is due to the presence of the initiators isohumulones, riboflavin, cysteine, methionine, phenylalanine and glutathione. Also, the Osborne fractions albumin and glutenin are known to increase the formation of sunstruck flavor as they probably serve as a source of the amino acids involved in the process. However, cereal-based beverages have also substances able to prevent the formation of sunstruck flavor. These substances are known as sunstruck flavor inhibitors and are the polyphenols catechin, epicatechin and quercitin, and the amino acid tryptophan. Whereas the cereal or malt used in the brewing process serves as a source of all sunstruck flavor initiators and inhibitors, the hop product use serves as a source of polyphenols. Table 4.1 shows the sunstruck flavor initiators and inhibitors and the raw material where they are to be found.

Table 4.1: Origin of sunstruck flavor initiators and inhibitors

	Substance	Cereal/Malt	Hop
Initiators	Riboflavin	x	
	Cysteine	x	
	Methionine	x	
	Phenylalanine	x	
	Glutathione	x	
Inhibitors	Polyphenols	x	X
	Tryptophan	x	

The brewing industry offers physical and compositional solutions to prevent the formation of sunstruck flavor. The physical options to prevent the formation of sunstruck flavor are the use of brown bottles or cans and the use of sunscreen on the bottles to protect the beer. However, a prestigious rational psychology poll states that people prefer to drink beer filled in white glass bottles [58].

As compositional solutions to prevent sunstruck flavor formation offered by the industry apply the use of chemically modified isohumulones. Also, many patents have been registered suggesting the use of flavoproteins or riboflavin-binding proteins, N-heterocyclic substances, absorbent clay, or 1,8 epoxy compounds in order to prevent the formation of sunstruck flavor in beers bottled in transparent bottles. However, the addition of these substances stops only the formation of 3-methyl-2-butene-1-thiol but not the formation of the other aroma active compounds also responsible for the sensory perception of sunstruck flavor and which are formed due to the presence of amino acids. Moreover, none of these products is permitted by the German Purity Law, and many brewers consider them as non-natural.

The fact that people prefer to drink beer filled in white glass bottles, the growing trend towards natural products (clean label) and the higher costs of protected bottles and reduced isomerized products as well as brewing in accordance to the German Purity Law motivate the search for other natural products to help towards the isohumulone photo-degradation.

The raw materials used in the cereal-based beverage production contain substances capable of preventing the formation of sunstruck flavor, the so-called sunstruck flavor inhibitors. Higher contents of sunstruck flavor inhibitors together with a lower content of sunstruck flavor initiators could lead to a lower formation of sunstruck flavor in a natural way. The aim of this research was to analyze the potential of different raw materials (hops as well as cereals) for the production of cereal-based beverage by increasing or decreasing the content of sunstruck flavor inhibitors and initiators in the medium, respectively. Moreover, the raw materials chosen should also ensure a good brewing performance.

Hops are a big source of polyphenols in beer. In wort and beer, up to 20–30 % of the polyphenols present are derived from hop, despite the fact that hops are added in much shorter amounts than malt. During the extraction procedure of some products, the total polyphenol content is discarded or increased. However, the polyphenol composition of the different hop products is normally unknown. Based on this idea, it was necessary to find a hop product with a high polyphenol content, specially catechin, epicatechin and quercitin, and no humulones or isohumulones. Therefore, commercially available hop products were analyzed for the content of total and single polyphenols, bittering acid content, and sunstruck flavor perception in beer after exposure to light.

The different commercially available hop products analyzed were Isohop, spent hops from the CO_2 extraction, spent hops from the pellets type 45 production, pellets type 90, tannin extract, and polyphenol enriched extract. Figure 4.1 shows the Hopsteiner hop product family-tree.

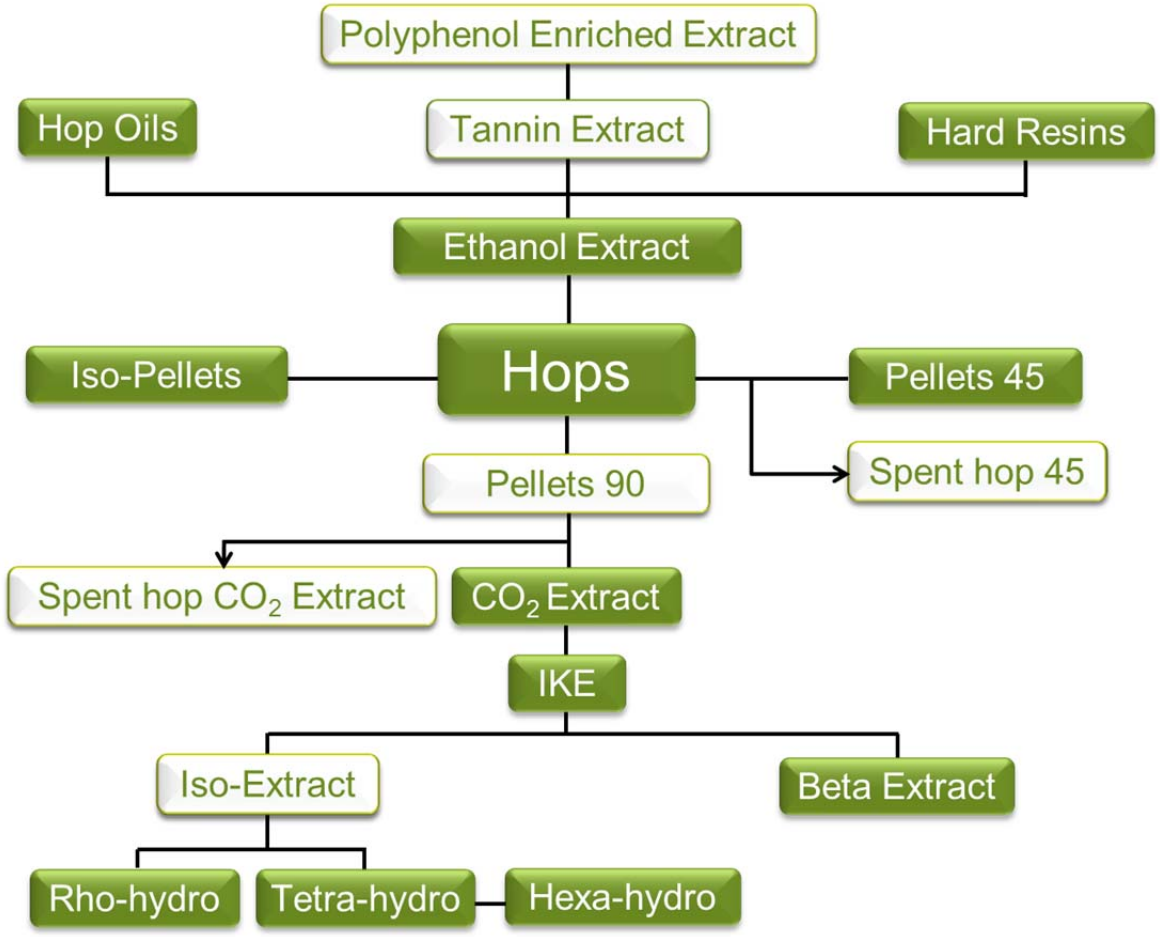

Figure 4.1: Hopsteiner hop product family-tree. The white elements represent the products used.

The results reveal a removal of the bittering acids in spent hops by the CO_2 extraction, spent hops type 45, tannin extract, and polyphenol enriched extract. Also, the total polyphenol content accounted for 10 mg/g for Isohop, 41 mg/g for spent hop CO_2, 64 mg/g for spent hop type 45, 67 mg/g for pellets type 90, 144 mg/g for tannin extract, and more than 845 mg/g for polyphenol enriched extract. These results show the separation of polyphenols from the product during the CO_2 extraction and during the ethanol extraction. The enrichment on the polyphenol content in tannin extract compared to pellets type 90 in the ethanol extract production is clearly high. The total content of polyphenols in spent hops type 45 and pellets 90 shows that in the production of pellets type 45 a higher amount of polyphenols is discarded and ends up in the spent hops.

On the other hand, the total polyphenol content of spent hops from CO_2 production is lower than that in pellets type 90. These results showed that there exists a separation

of the polyphenols in the pelletizing process of pellets type 45, in the ethanol extract production and in the CO_2 production which entails that further products in the production line will not be suitable for approaching sunstruck flavor reduction.

Due to the fact that the products either originate from different hop plant fractions or are manufactured in a different manner the single polyphenol profile is also different between products. The polyphenols catechin, epicatechin, and quercetin were of special interest as they are known to protect beer against the formation of sunstruck flavor. Therefore, the hop products were examined on their content of single polyphenols. The highest catechin content was found in pellets type 90 followed by spent hops type 45 and tannin extract. The latter presented the highest epicatechin content. Although polyphenol enriched extract presented the highest quercetin glycoside content, neither catechin nor epicatechin nor quercetin were detected. The results showed a loss of the catechin and epicatechin content and an increase in the quercetin glucoside content in the production of polyphenol enriched extract.

The evaluation of the illuminated beers brewed with single hop products showed that the beer with Isohop had the highest scores for sunstruck flavor descriptors owing to the high presence of iso-α-acid and tannin extract, and spent hops type 45 presented the lowest off-flavor perception. The results show no correlation between polyphenol content and sensory perception of the sunstruck flavor. Although pellets type 90 and spent hops from the CO_2 extraction have similar polyphenol content as spent hops type 45, both products showed a higher sunstruck flavor perception. The results indicate the potential of the tannin extract for the reduction of sunstruck flavor in beer. Therefore, the influence of the concentration of tannin extract and different boiling times were tested. Tannin extract showed a reduction of the sunstruck flavor perception in final beer, probably due to the higher presence of the polyphenols catechin, epicatechin and quercitin. Moreover, the tannin extract also increased partially the color of the beers (1.5 EBC), which may also contribute to the absorption of light. Fatty acid and fatty acid ester content increased with the content of tannin extract. However, the concentration of these compounds was within the average concentrations of lager beer.

Further steps in this research were the comparison of the content of amino acids and Osborne protein fractions during the malting process in different cereals. The protein fraction of the cereals used in the brewing process plays also an important role in the

formation of sunstruck flavor. The amino acids methionine, cysteine, phenylalanine as well as the peptide glutathione and the Osborne fractions albumin and glutenin are known to promote the formation of sunstruck flavor, whereas the amino acid tryptophan is known to quench the triplet state and inhibit the formation of light-struck character in beer. The cereals spelt and triticale were chosen as they offer different concentrations of sunstruck flavor initiators and inhibitors than that of barley and, at the same time, they have shown good brewing performance in previous work [104, 118]. Spelt has lower content of riboflavin, cysteine, methionine and phenylalanine than barley, and triticale has lower concentrations of phenylalanine and higher tryptophan and polyphenol concentrations than barley. These differences in the composition offer the possibility to employ new raw materials with less sunstruck flavor appearance and good brewing characteristics for cereal-based beverage production.

Also, the malting process exerts an influence on the content of the sunstruck flavor initiators and inhibitors. One of the most important physical–chemical changes during malting is the degradation of the proteinaceous matrix and their conversion into soluble peptides and amino acids. Spelt and triticale were analyzed during malting to broaden the knowledge of protein modifications and metabolic changes during this process. For this purpose, samples during the malting process of spelt and triticale (from raw material to malt) were taken every 24 h and the proteins as well as the amino acid composition were analyzed. The proteins were characterized according to their pI and MW during the different stages of the malting process.

The results showed that the majority of the spelt proteins ranged between 13 and 236 kDa and had a pI between 6 and 7, and of triticale between 4.7 and 64 kDa and which had a pI between 5.08 and 6.63. Although these results revealed a protein degradation process during malting of spelt, in contrast in triticale most of the polypeptides increased gradually, only to decline thereafter.

The Osborne fractionation shows some fundamental understanding of the modification of the proteins during malting. Although the protein composition of both cereals, spelt and triticale differs, the behavior of the albumin fraction during the malting process was very similar. For both cereals, the majority of the proteins of the albumin fraction increased during malting until the last days of germination. However, some of the proteins of higher molecular weight underwent a total degradation after

some days of germination. Also in the glutenin fraction of spelt and triticale the same behavior can be found. This fraction underwent a sharp degradation after kilning. The same behavior has been found in the albumin and glutenin fractions of barley [131, 189].

The albumin fraction consists of a complex mixture of structural, metabolic and storage proteins with a high metabolic activity. Celus *et al.* [190] analyzed the effects of malting and mashing on barley proteins and stated that the apparent increase in albumins and globulins during malting is the result of the proteolytic breakdown of the disulfide-linked hordeins and the secretion of enzymes in the endosperm. This breakdown of disulfide-linked proteins may serve as a source of sulfur, and thus having an impact on the formation of sunstruck flavor.

The degradation of the protein fraction has also an impact on the amount of amino acids involved in the formation of sunstruck flavor. The results reveal an increase in the content of the amino acids cysteine, methionine and phenylalanine during malting, increasing until the last day of germination and decreasing in concentration afterwards during kilning, which is a sight of heat sensitivity. Just the content of phenylalanine in triticale increased steadily all through the malting process. The same behavior has been found in barley by other authors [191]. According to these results the content of the sunstruck flavor precursors cysteine, methionine, phenylalanine and those peptides resulting from the degradation of the proteins increases during malting, having, therefore, a higher potential of develop sunstruck flavor. Moreover, the content of cysteine in triticale (and triticale malt) is higher than in spelt (and spelt malt), whereas the content of methionine and phenylalanine is higher in spelt (and spelt malt) than in triticale and triticale malt.

The malting parameters such as germination temperature, germination time and degree of steeping have an influence, not only on the content of the amino acids and peptides but also on the content of riboflavin and polyphenols. Therefore, further steps in this research were the investigation of the influence of the malting parameters on the content of sunstruck flavor initiators and inhibitors. The definition of the behavior of the content of these substances under the variation of the malting process parameters allows a better understanding of the origin and content of sunstruck flavor initiators and inhibitors in different cereals and the potential of achieving a high light stability together with high quality products. Therefore, the

changes in the content of sunstruck flavor initiators (cysteine, methionine, phenylalanine, glutathione, and riboflavin) and inhibitors (tryptophan and polyphenols) of barley, spelt and triticale were analyzed under the influence of different germination temperatures, germination times and degrees of steeping.

The results showed an increase of the inhibitors and initiators of sunstruck flavor the more intense the malting parameters are (higher temperature, longer germination times and higher degree of steeping). Solely, the germination temperature had a negative influence on the content of the amino acids cysteine, methionine and tryptophan of triticale. The germination time and temperature showed a negative influence on the tryptophan content of spelt. In general, although the lowest content of sunstruck flavor initiators was found under lower germination temperatures, shorter germination times and lower degrees of steeping, concurrently, the content of sunstruck flavor inhibitors is also low under these conditions.

Comparing the content of initiators of the three cereals, barley had, on average, the highest methionine and phenylalanine content, spelt the highest riboflavin content, and triticale the highest cysteine and glutathione content. On the contrary, barley had the highest total polyphenol content and spelt the highest tryptophan content.

The results of this work show an influence of the raw material used (cereal and hop product) on the content of sunstruck flavor initiators and inhibitors. Although the content of amino acids, polyphenols, and riboflavin was different between cereals, neither barley, neither spelt nor triticale could be defined as more prone for the formation of the sunstruck flavor.

However, some of the hop products investigated show more potential for the reduction of sunstruck flavor as they do not contain any humulones or isohumulones and have higher content of polyphenols in special catechin, epicatechin, and quercetin. Any product after the CO_2 extraction or ethanol extraction is not suitable for the inhibition of sunstruck flavor as the polyphenols are removed during the production process.

The content of tryptophan increased during the malting process, which has a positive effect on the inhibition of sunstruck flavor. However, the content of cysteine, methionine, phenylalanine and the albumin fraction also increased compared to that prior to malting. For this reason, it can be concluded that the malting process does

not exert a positive effect on the inhibition of sunstruck flavor appearance in cereal-based beverages.

By changing the malting parameters, the content of sunstruck flavor initiators and inhibitors can be decreased and increased. The last step in this work was to identify the changes in the content of sunstruck flavor initiators and inhibitors due to different malting parameters in spelt, triticale and barley. Under variation of the malting parameters, it was possible to decrease the content of initiators to undermine the formation of sunstruck flavor. However, the content of sunstruck flavor inhibitors decreases concurrently. Malts produced under more intense malting parameters (higher germination temperatures and degrees of steeping and longer germination times) tend to have higher content of sunstruck flavor inhibitors but also higher content of sunstruck flavor initiators, and malts produced under less intense malting parameters (lower germination temperatures and degrees of steeping and shorter germination times) tend to have lower content of sunstruck flavor inhibitors but also lower content of sunstruck flavor initiators. Therefore, a compromise should be found in order to aim for a beverage (containing hops) with no potential of developing sunstruck flavor, for example, using under-modified malts (with low content of initiators and inhibitors) together with a rich polyphenol hop product (with no humulone content).

However, under these parameters, the formation of the sunstruck flavor will not be completely inhibited. To avoid the formation of sunstruck flavor further research should be carried out in order to understand the behavior of riboflavin during fermentation. Some research [79] has shown that the ability of yeast to synthesize riboflavin appears to be genetically determined and that the medium composition influences the synthesis or depletion of riboflavin by the yeast. Also, the absorption of the amino acids by the yeast has an influence on the final content in beer but was not taken into consideration in this work. Moreover, other cereals should be tested in order to find a raw material with higher potential in the production of light stable malt-based fermented beverages.

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- Influence of malting on the protein composition of triticale (× Triticosecale Wittmack) Muñoz Insa, A., Gastl, M., Becker, T. Jan/Feb 2016 Journal of Cereal Chemistry
- Influence of malting on the protein composition of spelt (Triticum spelta L.) Muñoz Insa, A., Gastl, M., Becker, T. Jan/Feb 2016 Journal of Cereal Chemistry
- Variation of sunstruck flavor-related substances in malted barley, triticale and spelt Muñoz Insa, A., Gastl, M., Becker, T. August 2015 Food Research and Technology
- Use of polyphenol rich hop products to reduce sunstruck flavor in beer. Muñoz Insa, A., Gastl, M., Becker, T. 2015 Journal of the American Society of Brewing Chemists
- Malting Process Optimization of Spelt (Triticum spelta L.) for the Brewing Process. Muñoz Insa, A., Selciano, H., Zarnkow, M., Becker, T., Gastl, M. 2012 LWT- Food Science and Technology
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- Brewing Summit Chicago, USA: Lightstruck flavor reduction by increasing polyphenol content in beer. American Society of Brewing Chemists. Muñoz Insa, A., Gastl, M., Becker, T., 2014
- 11. Rohstoffseminar Freising, Germany: Einfluss des Rohstoffproteingehaltes auf die Bierschaumstabilität. Muñoz Insa, A., Gastl, M., Becker, T., 2014
- 46. Technologisches Seminar, Freising, Germany: Einsatz von Hopfenprodukten zur Vermeidung von Lichtgeschmack. Muñoz Insa, A., Gastl, M., Becker, T., 2013
- World Brewing Congress, Portland, USA: Oat: Substrate for Malted Cereal Fermented Beverages. Muñoz Insa, A., Gastl, M., Becker, T., 2012
- 45. Technologisches Seminar, Freising, Germany: Technologische Vermeidung qualitätsmindernder Aromastoffe in hopfenhaltigen Getränken. Muñoz Insa, A., Gastl, M., Becker, T., 2012
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- MBAA Annual Conference, Minneapolis, USA: Identification of barley varieties by Lab-on-a-Chip capillary electrophoresis. Muñoz Insa, A., Gastl, M., Becker, T., 2011
- 33rd EBC Congress Glasgow, UK: Evaluation of different hop products and boiling parameters to reduce sunstruck flavor in non-alcoholic fermented beverages. Muñoz Insa, A., Gastl, M., Becker, T., 2011
- 44. Technologisches Seminar, Freising, Germany: Optimierung des Mälzungsregimes von alternativen Getreiden hinsichtlich des Gehalts an Photoinhibitoren und -initiatoren zur Vermeidung von Lichtgeschmack. Muñoz Insa, A., Gastl, M., Becker, T., 2011
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