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INFLUÊNCIA DA TEMPERATURA DA ESTUFA DE SECAGEM NA COMPOSIÇÃO QUÍMICA DE UM MALTE DE CEVADA GREGO E PROPRIEDADES DO SEU MOSTO

INFLUENCE OF KILNING TEMPERATURE ON CHEMICAL COMPOSITION OF A GREEK BARLEY MALT AND ITS WORT PROPERTIES

LA INFLUENCIA DE LA TEMPERATURA DE SECADO EN LA COMPOSICIÓN QUÍMICA DE UNA MALTA DE CEBADA GRIEGA Y LAS PROPIEDADES DE SU MOSTO

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RESUMO

Introdução: É reconhecido que o caráter e a qualidade do malte são obtidos durante a secagem. Além disso, as mudanças ocorridas durante a secagem afetam a qualidade do esmagamento e do mosto.
Objetivos: Até agora, na Grécia, para a produção de malte, são comumente usadas outras variedades de cevada, em vez de nativas. Assim, esta pesquisa tem como objetivo avaliar o efeito da temperatura de estufa na melhoria da capacidade de maltagem para cultivo da cevada grega.
Métodos: Os grãos de cevada foram maltados no Food Process Engineering Laboratory - Pilot Plant of ATEITh (Thessaloniki). A cevada maltada foi primeiro seca a 40-45 °C (malte seco) e depois seca em estufa. A secagem da cevada germinada foi realizada a três temperaturas diferentes (80, 90 e 100 °C, por 6h), a fim de produzir três maltes diferentes. A cevada, malte seco e malte foram analisados quanto ao teor de humidade, cinzas, proteína e seus respetivos teores em β-glucana, teor de extrato do malte, cor, densidade e viscosidade específica. Também foram determinados o total de açúcares fermentáveis, bem como o seu perfil. Finalmente, os maltes foram comparados com um malte comercial.
Resultados: Fatores como a humidade do malte, o teor de β-glucana, a cor do malte, o extrato de malte e a viscosidade específica foram significativamente afetados pelo processo de secagem em estufa. Pelo contrário, os teores de cinzas e proteínas no malte não foram significativamente afetados. O malte produzido a partir da cultivar de cevada grega mostrou um teor de β-glucana, gravidade específica e valores de viscosidade específicos semelhantes aos da amostra comercial.
Conclusões: O presente estudo revelou que a qualidade do malte e do mosto da cultivar de cevada grega (Seirios) pode ser significativamente melhorada pela otimização da temperatura de estufa.

Palavras-chave: malte de cevada; teor de β-glucana, estufa, qualidade de mosto, HPLC

ABSTRACT

Introduction: It is recognized that the character and the quality of the malt is obtained during kilning. Moreover, the changes occurring during kilning affect mashing and wort quality.
Objectives: Till now, in Greece, for the malt production other barley varieties rather than native ones are commonly used. Thus, this research aims to evaluate the effect of kilning temperature in improving malting ability of a Greek barley cultivar.
Methods: Barley kernels were malted in the Food Process Engineering Laboratory - Pilot Plant of ATEITh (Thessaloniki). The malted barley was first dried at 40-45°C (dry malt) and then kilned. The kilning of germinated barley was performed at three different temperatures (80, 90 and 100°C for 6h), in order to produce three different malts. Barley, dry malt and malted kernels were analysed for their moisture, ash, protein and β-glucan content whereas their respective worts, for extract content of the malt, colour, specific gravity and specific viscosity. The total fermentable sugars as well as their profile were also determined. Finally, the malts were compared with a commercial malt.
Results: Factors such as malt moisture, β-glucan content, malt colour, malt extract and specific viscosity were significantly affected by the kilning process. Contrary, the ash and protein contents in the malt were not significantly affected. The malt produced from the Greek barley cultivar showed β-glucan, specific gravity and specific viscosity values similar to the commercial sample.
Conclusions: The present study revealed that the malt and wort quality of the Greek barley cultivar (Seirios) can be significantly improved by optimizing the kilning temperature.

Keywords: barley malt; β-glucan content, kilning, wort quality, HPLC

RESUMEN

Introducción: Se reconoce que el carácter y la calidad de la malta se obtienen durante el secado. Además, los cambios que ocurren durante el secado en estufa afectan la maceración y la calidad del mosto.
Objetivos: Hasta este momento, en Grecia, se usan habitualmente otras variedades de cebada en lugar de las nativas para la producción de malta. Por lo tanto, esta investigación tiene como objetivo evaluar el efecto de la temperatura del secado en estufa en la mejora de la capacidad de malteado de un cultivar de cebada griego.
Métodos: Los granos de cebada fueron malteados en el laboratorio de Food Process Engineering - Pilot Plant de ATEITh (Thessaloniki). La cebada malteada se secó primero a 40-45 ° C (malta seca) y luego se tostó. El secado en estufa de la cebada germinada se realizó a tres temperaturas diferentes (80, 90 y 100 ° C durante 6 h), con el fin de producir tres maltas diferentes. La cebada, la malta seca y los granos malteados se analizaron por su contenido de humedad, cenizas, proteínas y β-glucano, mientras que sus respectivos mostos, por el contenido de extracto de la malta, el color, la gravedad y la viscosidad específica.

También se determinaron los azúcares fermentables totales, así como su perfil. Finalmente, las maltas se compararon con una malta comercial.
Resultados: Factores como la humedad de la malta, el contenido de β-glucano, el color de la malta, el extracto de malta y la viscosidad específica fueron afectados significativamente por el proceso de secado en estufa. Al contrario, los contenidos de cenizas y proteínas en la malta no se vieron afectados significativamente. La malta producida a partir del cultivar de cebada griego mostró β-glucano, gravedad específica y valores de viscosidad específicos similares a la muestra comercial.
Conclusiones: : El presente estudio reveló que la calidad de malta y mosto del cultivar de cebada griego (Seirios) puede mejorarse significativamente al optimizar la temperatura del secado en estufa.

Palabras Clave: malta de cebada, el contenido de β-glucano, secado en estufa, calidad de mosto, HPLC

INTRODUCTION

Barley (*Hordeum vulgare* L.) is classified among the most important cereals in terms of production quantity and cultivation areas. The annual world production reached over 141 million tons in 2016 (FAOSTAT, 2018). Barley shows a great diversity in the morphological form and is cultivated across a wide range of production areas in the world (Horsley & Hochhalter, 2004). Malting has been used to impart distinctive flavours and colours in barley. Brewing is the most common area of use, in order to get fermentable sugars for alcoholic fermentation. Another potential use of malting is to increase the bioavailability of nutrients and change flour texture (Bamforth & Barclay, 1993; Stanca, Gianinetti, Rizza, & Terzi, 2016).
Malting procedure includes three steps: steeping, germination and kilning. During steeping, the barley is soaked in water until reach the defined moisture content. The aim of steeping is the moisture content of the grain will reach the required level for germination to begin. During germination, hydrolytic enzymes are synthesized by the aleurone cell. The content of hydrolytic enzymes (α-amylase, endo-β-glucanase and proteinase) increase in amount during the second or third day after steeping (MacLeod, Duffus, & Johnston, 1964). Kilning is heating of grain with increasing temperature regime above 50°C in order to obtain desired properties for the malters. The desired properties include enzyme survival, removal of moisture for stabilization, removal of raw flavours, development of malty flavours and colour (Bamforth, 2003). The kilning of malt is not simply a drying process but also a chemical process in which the character and quality of the malt are generated (Johnston, 1954). The reaction of sugars and amino acids induced by heating during the kilning process and wort boiling leads to the formation of melanoidins via the Maillard reaction responsible for imparting colour to beer (Bamforth, 2003).
Generally, all barley cultivars could be used to produce malt. However, different properties of the barley should be taken into account in order to obtain high quality malt. The barley should be of high vitality to be used for malt since it is obligatory for the germination process. Moreover, it should have a chemical composition that makes it suitable for having high yield during brewing. Too much protein content lowers the extract yield and can give a beer that is not clear or may slow down the start of germination. On the other hand, too little protein results in lower enzyme activity and slows growth of the yeast in the brewery. In fact, there is evidence that uneven protein content represents quality problems in malt (Palmer, 2000).
β-Glucan and arabinoxylans, the major constituents of barley endosperm cell walls, are hydrolysed during malting, allowing release of the entrapped starch granules (Briggs, 1998). As a consequence, soluble β-glucan and arabinoxylans that are released in the wort have the ability to increase its viscosity, due to their physicochemical properties, such as molecular weight and concentration (Lazaridou, Biliaderis, Micha-Screttas, & Steele, 2004; Skendi, Biliaderis, Izydorczyk, Zervou, & Zoumpoulakis, 2011; Skendi, Biliaderis, Lazaridou, & Izydorczyk, 2003).
Differences in β-glucan content and composition depend on the variety and growing conditions (Skendi et al., 2003). The content of β-glucan is lower, is more soluble and possibly of lower molecular in malt than in barley (Teixeira, Nyman, Andersson, & Alminger, 2016). It was found that modification of the steeping conditions during malting can possibly preserve β-glucan content of barley malt (Rimsten et al., 2002). The degradation of the starch during mashing of the malt as well as other carbohydrate polymers by endogenous enzymes produced during malting of barley results in a vast diversity of fermentable sugars. The final composition of wort is very complex and mainly attributed to the enzyme activity and the process control (MacGregor, Bazin, Macri, & Babb, 1999). The major fermentable sugars is maltose, with lesser quantities of glucose, maltotriose, sucrose and fructose (Bamforth, 2003).
The aim of the present work was to study the extent to which chemical characteristics of a Greek barley variety could be modified by using different kilning temperatures, on brewing. For this purpose, β-glucan, protein, moisture and minerals were investigated in laboratory-scale malting experiments for Seirios barley cultivar followed with comparison with a commercial barley cultivar used for brewing.

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1. METHODS

1.1 Materials and Chemicals

The barley kernels, cultivar Seirios (SB) (two-rowed), were obtained from the ELGO-DEMETER, Institute of Plant Breeding and Genetic Resources (Thessaloniki, Greece). Commercial malt (CM) (in the form of kernels), was generously donated by the "Macedonian Thrace Brewery S.A.".

All reagents used for the determination of the fermentable sugars were of analytical grade purity. The standard of D-(-)-fructose was purchased from Riedel-deHaen (Seelze, Germany), the D-(+)-glucose, sucrose and maltose were from Merk KGaA (Darmstadt, Germany), whereas the maltotriose was obtained from Megazyme (Bray, Ireland). Acetonitrile was obtained from Chem-Lab NV (Zedelgem, Belgium). For the HPLC measurements ultrapure water was used. For the determination of the β-glucan content in the unmalted barley and malts a Megazyme β-glucan assay kit (Bray, Ireland) was used.

1.2 Malting and mashing

Barley kernels (SB) were malted in the Food Process Engineering Laboratory - Pilot Plant of ATEITh (Thessaloniki). For the malting of the samples the method used involves steeping the barley at 15°C following several circles of soaking and air rests for 2 days, then the barley was evenly spread out on trays and allowed to germinate at 15°C. After germination, barley was dried (pre-kilning) at 40-45°C in order to obtain the dry malt (SDM) in an air circulatory electrically heated oven. Then, the pre-kilned sample was divided in three sub -samples, each one kilned for 6 hours at a different kilning temperature (80°C, 90°C and 100°C) in order to obtain three different malt samples (SM80, SM90 and SM100, respectively). Before analysis, barley and malt kernels were ground to pass through a 0.8mm sieve on a mill (Falling Number AB Type 120 grinder, S-12611, Stockholm, Sweden). The analyses were performed in both untreated barley and malted samples.

The milled samples of barley and malt were used to obtain mash. The wort was performed as reported to the congress mash procedure according to the EBC method 4.5.1 (2004) in the Cereal Laboratory of ATEITh (Thessaloniki). There were obtained the following worts: from unmalted barley (SB-W), from dry malt (SDM-W), from commercial malt (CM-W) and from malts prepared at 80, 90 and 100°C (SM80-W, SM90-W and SM100-W).

1.3 Malt and wort analyses

The protein content of unmalted, commercial malt and malted barley samples were determined using the EBC-methods (3.3.1 for barley and 4.3.1 for malt). The factor used to convert nitrogen to protein was 6.25. β-Glucan content was measured following the assay procedure for barley (EBC Method 3.10.1) and malt (EBC Method 4.16.1) described in the Megazyme β-glucan assay kit (K-BGLU, Megazyme International, Ireland), respectively. Moisture content was resulted by drying milled samples in an oven at 105°C till constant weight. Ash content was determined by using AACC method 08-01 (1983) approved method. All analyses were made at least in duplicate.

The mash was filtered according to EBC-method 4.5.1, through filter paper (Whatman® prepleated qualitative filter paper, Grade 597 1/2) into graduated cylinders. The filtered wort was used to measure colour, viscosity, specific gravity and malt extract. Congress wort viscosity was measured according to EBC-method 8.41 whereas the malt extract was calculated based on the EBC-method 4.5.1.

1.4 HPLC analysis of fermentable sugars in wort

The obtained wort samples (filtered ones) were immediately frozen and then freeze-dried. For the analysis of the fermentable sugars, a portion of dried sample was diluted in ultrapure water and then filtered through a 0.22μm nylon syringe filter (Simplepure). All the samples were daily prepared.

Fermentable sugars were determined by high-performance liquid chromatography (HPLC). The HPLC consisted in a SpectraSYSTEM liquid chromatography system (Thermo Finnigan, San Jose, CA) equipped with SpectraSYSTEM SCm1000 degasser, a Marathon series IV SSV pump, a refractive index detector (ERC-7515A, ERC Inc), a chromatographic column (Separon SGX NH2 5μm, size: 4.6 × 250mm RigasLab, Thessaloniki, Greece) and a rheodine injector equipped with 20μl loop.

A quantity of 100mg of each standard fermentable sugar (fructose, glucose, sucrose, maltose and maltotriose) was added to a 10mL volumetric flask and dissolved in ultrapure water to obtain the stock solution. Then, six different concentrations were obtained by diluting stock solution with ultrapure water in order to prepare the standard curve. An isocratic elution mode was employed with the mobile phase consisting of acetonitrile/water (85:15, v/v) at a flow rate of 2.3mL/min. The detector temperature was 35°C and the chromatographic column temperature was 30°C.

1.5 Statistical analysis

Analytical procedures were carried out at least in duplicate and the means and standard deviation of all results were calculated. The data are shown as the mean and standard deviation of the mean (SD). ANOVA and Duncan's post hoc tests were used to evaluate the difference between each kilning condition and untreated barley. A two-tailed Pearson's test was used to evaluate

the existing correlations. Statistical significance was established when $P < 0.05$. Statistical analysis was performed using SPSS Statistics software.

2. RESULTS AND DISCUSSION

2.2 Variation in the malt composition

Drying of green malt represent an important step during which there are stopped different modifications in the structure of the barley and make malt stable for storage ensuring in the same time the survival of enzymes for the mashing step. Variation in the moisture content of the malt is shown in Figure 1. The final moisture 14.38% in the dried malt was the result of the moisture reduction during first drying at 40-45°C of the green malt. The reduction was lower than the one generally recommended for the green malt (about 12%) when the water in the kernels is firmly "bound" (Briggs, Hough, Stevens, & Young, 1981). Briggs reports that in European lager malts the moisture values vary 5-6% with some high-diastatic power malts having moisture contents of around 8% (Briggs, 1998). In general, the moisture content of the malts obtained at 80, 90 and 100°C is significantly lower (5.72, 7.45 and 5.63%, respectively) than that of the unmalted barley (10.70%). The obtained values are within the limits observed for the commercial malts (Briggs, 1998).

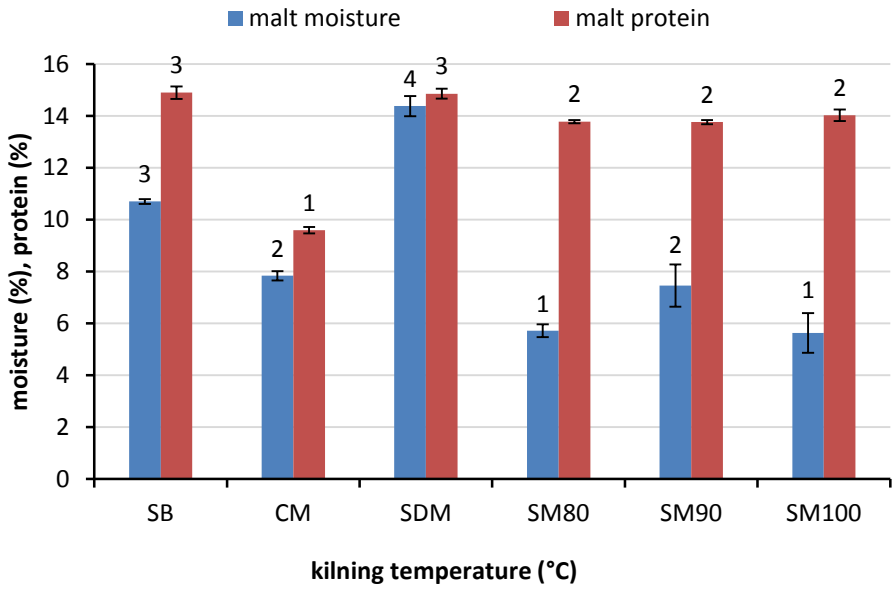

Figure 1. Variation of the moisture and protein content (dry basis) in barley, dried malt and malts.

According to the literature barley for malting should have a protein content of from 9.5 to 11.5% (dry basis)(Ingvordsen et al., 2016; Pettersson & Eckersten, 2007). The cultivar Seirios has a much higher protein content (14.90%). This value is much higher than the values reported from Plant Breeding and Genetic Resources Institute for the same cultivar (10.5-11.5%). This deviation may be due to the very adverse condition of growing observed in the growing season (2016). Malting barley with high protein content results in lower extracts. Grain protein content was negatively related with the grain size and grain yield (Magliano, Prystupa, & Gutiérrez-Boem, 2014). Generally it is believed that a barley with a high protein content will give a malt with a lower yield of extract than a malt from a barley having a lower protein content (Briggs, 1998).

During malting, proteins are mainly solubilized and hydrolysed into smaller peptides and amino acids through a range of proteolytic enzymes (Baxter, 1981; Jones, Marinac, & Fontanini, 2000), but the absolute crude protein content was not expected to change significantly. There was a small relative decrease in protein content for all malted samples (Figure 1) as a result of respiration of carbohydrates. Since the protein-rich roots and shoots are removed from the malt there is a decrease in protein content. No difference due to the different kilning temperature (80, 90, 100°C) was observed in the protein content. The similar protein content in the dried malt compared to the unmalted barley may be related to partial removal of the roots in the dried malt due to its high moisture content.

There is observed a slight decrease in the ash content of the malt during malting (Figure 2). This is possibly because of the solubilization and removal of some metals during malting. Moreover, the values observed in the commercial barley are similar to those observed in the unmalted barley and malted samples.

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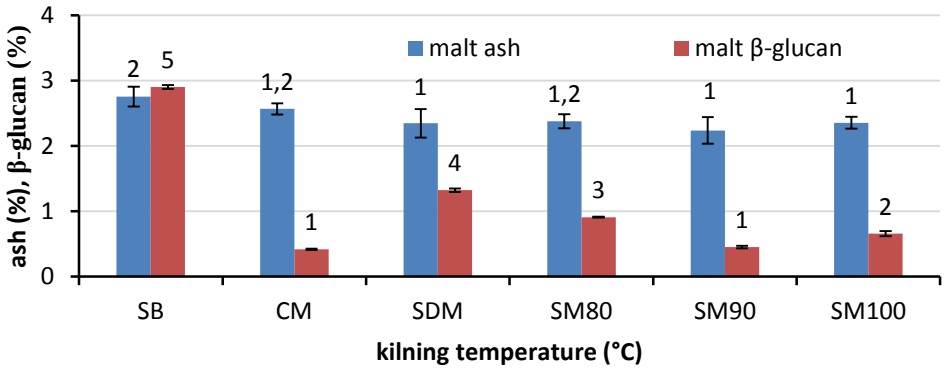

Figure 2. Variation of the ash and β-glucan content (dry basis) in barley, dried malt and malts.

Presence of high amount of β-glucan is found responsible for giving rise to a filtration problem in the brewery causing chill haze, on the maturing of beer (Gupta, Abu-Ghannam, & Gallaghar, 2010). Changes in β-glucan levels during malting are related to a great extent to β-glucanase activity, which is found responsible for the depolymerization of β-glucan (Etokakpan, 1993).
The content of β-glucan decreased significantly during malting (Figure 2). The decrease was higher in the malt kilned at 90°C; this value being similar to that of commercial malt. It was reported that β-glucanases, that develop during the germination of barley, are rapidly and extensively destroyed in kilning (Bamforth & Martin, 1983). It is observed that the decrease in the β-glucan content is not stopped on the first drying step (40-45°C). It continues during kilning step suggesting that β-glucanases are not deactivated if low drying temperatures were applied and continue to be active also in the dried kernels.

2.2 Variation in the wort parameters

Congress mashing is generally used for routine malt analysis. Figure 3 and 4 highlight the parameters which were determined during congress mashing.
Barley β-glucans are recognized as important factors that determine wort viscosity and beer filtration rates (Stewart, Freeman, & Evans, 2000). The wort of unmalted barley exhibited a specific viscosity value of 9.58 (Figure 3). This is possibly due to the high concentration of β-glucans present in the wort. All the worts from the malted samples showed lower specific viscosity than the wort from unmalted barley. The values of specific viscosity varied in the range 0.45-0.58 (Figure 3). It was observed that the dried malt (SDM) and malt kilned at 90°C (SM90) produces wort with the lowest specific viscosity among the malts, possibly due to the low content of β-glucan and presence of the high content of the degrading enzymes that break down β-glucans. Kilning temperature 100°C inactivated part of the enzymes responsible for the decrease of the specific viscosity since the respective wort showed the highest specific viscosity among the malts. The wort from malt produced at 90°C showed specific viscosity values similar to that made from commercial malt.
Correlation analysis performed between the β-glucan content and the specific viscosity revealed a very strong (0.934) positive relation at the 0.01 level (2-tailed). This relation explains the very high specific viscosity value observed in the barley wort as well the variation in the specific viscosity of the worts.

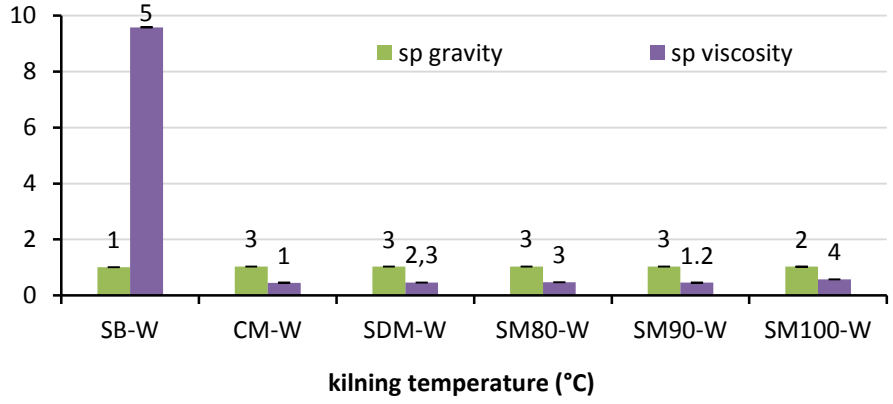

Figure 3. Variation of the specific gravity and specific viscosity in the worts obtained from barley, dried malt and malts.

According to Briggs et al., the specific gravity of the extract is a measure of the "preformed soluble substances" present in the malt (Briggs, Boulton, Brookes, & Stevens, 2004). The specific gravity is related with the presence of fermentable sugars present in the wort. Generally, higher the specific gravity higher the amount of fermentable sugars in the wort. Similarly, the higher the specific gravity the higher concentration of wort solids in the solution (Briggs et al., 2004). The specific gravity of the wort from dried malt and barley kilned at 80 and 90°C was similar to the commercial barley sample whereas the malt kilned at 100°C showed lower value (Figure 3). In the case of malt kilned at 100°C the low value of specific gravity may be related to the lower content of the degrading enzymes that reduce the amount of starch transformed in fermentable sugar, as well as to the negative effect of high specific viscosity in the extraction of the fermentable sugars.
The weight of extract in the wort is calculated assuming that the dissolved extract solids change the specific gravity to the same extent as sucrose (Briggs et al., 2004). The malt extract is calculated from the specific gravity using tables that relate the strengths of sucrose solutions with their specific gravities. As expected, malt extract values among the samples showed the same trend as the values of the specific gravity with values ranging from 64.91 to 77.99% (Figure 4). Worts from malts kilned at 80 and 90°C showed statistically similar values to the wort from commercial malt. Extracts of pale malts determined by the EBC method are usually in the range 77-83% whereas the typical ranges for darker malts are 75-78% (Briggs et al., 2004).
In general, colour specifications are set based upon the final product being produced. It was observed that malt processing can have a large impact on colour development; increasing modification or kilning temperatures can lead to more intense wort colour (Bamforth, 2006). Indeed, increasing the kilning temperature increased the colour of the wort (Figure 4). The wort colour of dried malt and malt kilned at 80°C was found to be lighter than that of the commercial malt wort.

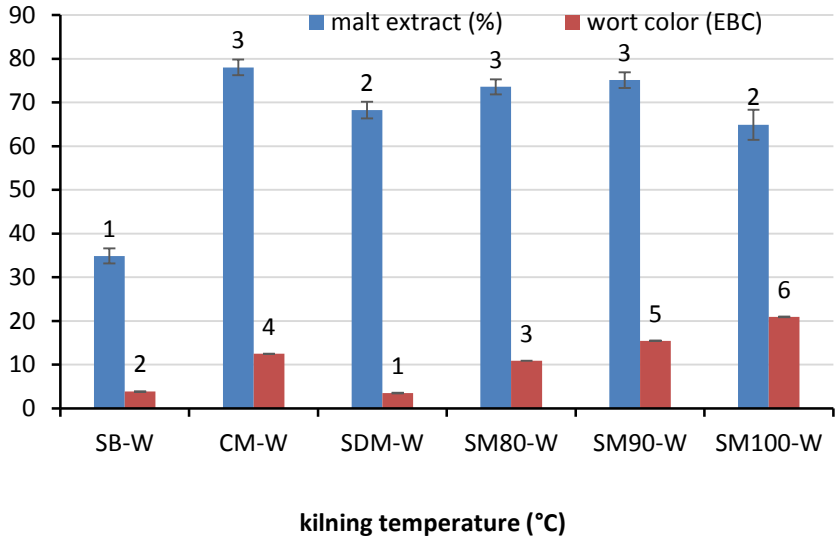

Figure 4. Variation of the malt extract and colour in the worts obtained from barley, dried malt and malts.

2.3 Variation in the fermentable sugars in wort

In general, the amount of fermentable sugars present in the obtained congress wort decreases with increasing of the kilning temperature (Figure 5A). The values for the total fermentable sugars varied in the range 4.3 to 20.76%. The wort prepared with the dry malt and the malt kilned at 80°C showed similar content of fermentable sugar per liter as the wort with the commercial barley malt. As expected the unmalted barley released less than 5g/L fermentable sugars in the respective wort.
Standard brewery wort consists of the following : sucrose, fructose, glucose, maltose and maltotriose, together with dextrin material (He et al., 2014). The main component of the total fermentable sugars was maltose with values ranging from 58.6% to 72% followed by glucose at the range 14.2 to 21.7% (Figure 5B). The maltose/maltotriose ratio varied between 6 and 11. The literature reports that maltose and maltotriose are the most abundant sugars (He et al., 2014). Fructose represents the compound with the lowest concentration (1.8-2.9%). It is evident that the amount of sucrose in the wort of malted samples of Seirios variety was more than the double of the sucrose content in the wort from the commercial malt.

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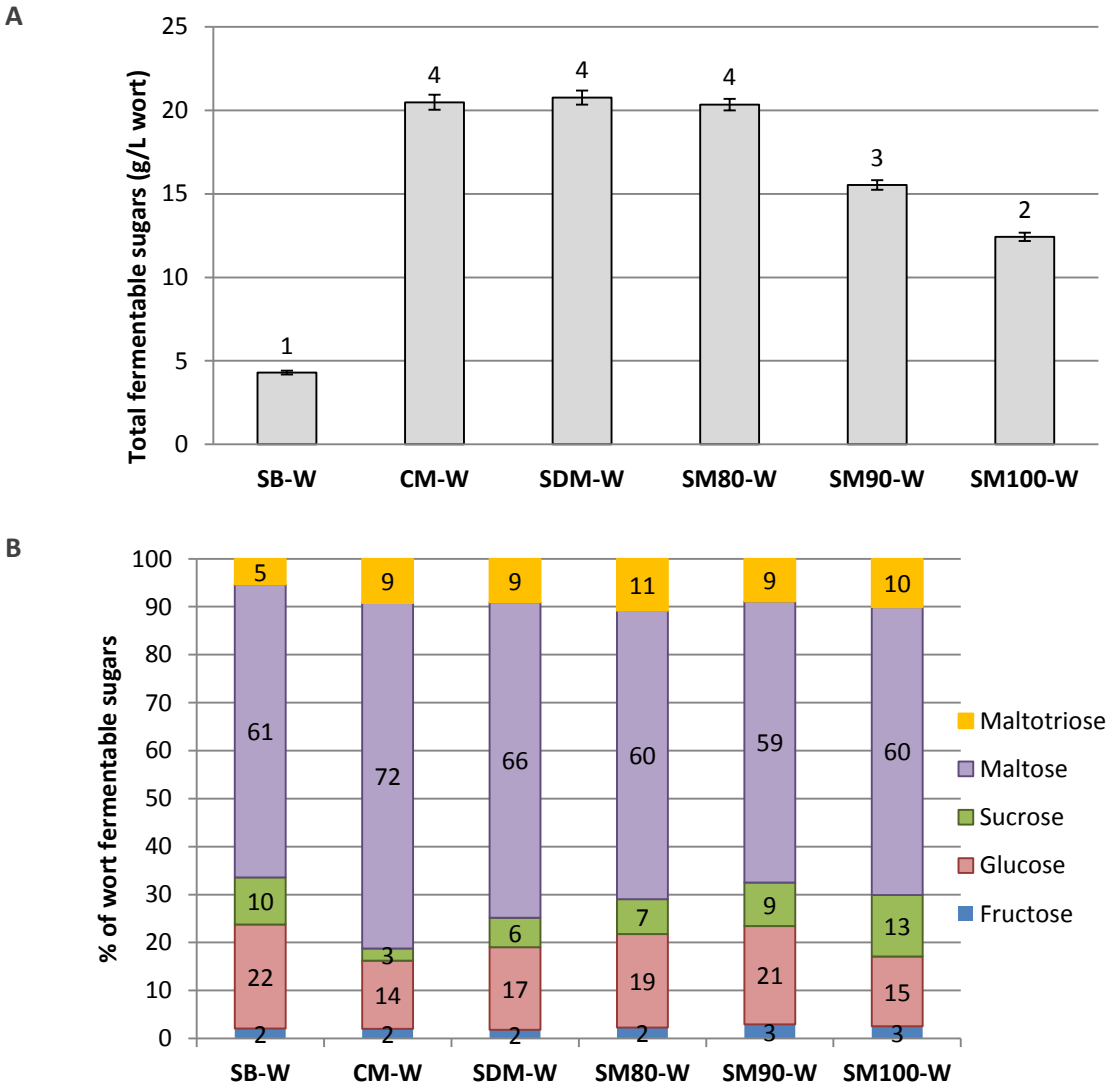

Figure 5. (A) Content of the total fermentable sugars present in the wort and (B) percentage of the five fermentable sugars in the commercial malt (CM), unmalted barley (SB), and three different malts SM80, SM90 and SM 100 obtained by kilning at 80, 90 and 100°C, respectively.

CONCLUSIONS

This study elucidates the impact of kilning temperature on chemical composition of the malt in terms of moisture, ash, protein and β-glucan content, as well as the respective wort characteristics specifically specific viscosity, specific gravity, wort colour and malt extract. Moreover, it was studied the profile of the fermentable sugars present in the wort.

Based on the results of this study, it can be concluded that prolonged kilning at 40°C does not necessary causes inactivation of β-glucanase activity. The content of β-glucan was decreased substantially at a kilning temperature of 90°C. Possibly the presence of high moisture could favour the β-glucanase activity reducing further the β-glucan content and producing wort with specific viscosity, specific gravity and malt extract similar to that of the commercial sample. However, the total fermentable sugars were decreased with the kilning temperature. Although wort samples SM80 and SM90 showed similar specific gravity and malt extract, kilning at 80°C produced wort with a content of total fermentable sugars similar to that of commercial malt. This suggests that at 90°C the presence of high solid matter can be explained by the presence as well as the amount of the enzymes that survived the kilning process. The combination of both factors may be responsible for the production, through fractional hydrolysis during wort production, of soluble components able to give higher specific gravity. As expected the wort colour was darker with increasing kilning temperature.

In general, by altering the kilning temperature it was possible to obtain malt from a Greek barley that reassembles the chemical composition and characteristics of wort from the commercial malt. Further optimization of kilning conditions, could introduce potential to ensure survival of the enzymes.

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