POLYPHENOLS AND VITAMIN E AS POTENTIAL ANTIOXIDANTS IN BARLEY AND MALT

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Abstract

Barley (*Hordeum vulgare L.*) is an ancient and important cereal grain crop. Whole grain products are recommended for healthy diets as being recognized sources of dietary fiber and antioxidant substances – such as polyphenols and vitamin E. The aim of current research is to compare the content of total phenolic compounds (TPC) and vitamin E of different barley varieties and malt types. One flaky, malting barley variety 'Klass', four hull-less barley lines from Latvia and theirs corresponding malt and for comparison four types of industrial malt – Pilsner, Munich, light, caramel and black malt were used for analyses. The total phenolic content was determined by spectrophotometer according to the Folin-Ciocalteu colorometric method with some modifications. Total phenolics were expressed as gallic acid equivalents (mg GAE g^{-1} dry weight). During research vitamin E content was detected according to standard method AOAC 971.30. All barley varieties and malt samples exhibited significant content of vitamin E, and contained significant levels of phenolic compounds. The content of vitamin E in all barley samples was similar and average increase during malting was 34% for all varieties. Increase of content of vitamin E during malting can be explained by synthesis of vitamin E in germination process of grains. Content of vitamin E in malt depends on kilning temperature: higher kilning temperature, higher losses of vitamin E. A significant increase in TPC (from 2.017 to 3.406 mg GAE g^{-1} DW) in all malt samples were observed after malting, i.e. steeping, germinating and kilning samples showing greater effect. It increases during malting probably not only by the modification or release of phenolic compounds, but also by the formation of new antioxidants, such as Maillard reaction products.

Key words: Vitamin E; hull-less barley; malt; total phenolic content

Introduction

Cereals and their derivatives are the most important foods in the human diet mainly because of the energy that they provide, due to their high carbohydrate content. However, in recent years, researchers have also begun to study their antioxidant profiles. Barley is widely consumed cereal, because of its dietary and technological properties. In fact, barley meals and fractions are now gaining renewed interest as ingredients for the production of functional foods, due to its bioactive compounds, such as β-glucans and tocols (Bonoli et al., 2004). Moreover, there are several classes of compounds in barley that have a phenolic structure, such as benzoic and cinnamic acid derivatives, proanthocyanidins, flavonols, flavones and many other phenolic compounds (Bonoli et al., 2004). Phenolic compounds in cereals are either in free or bound form.

Plant phenolics, including, flavonoids and phenolic acid and tocopherols, are known to protect plants against tissue injuries, high levels of oxygen, free radicals and reactive oxygen species formed by the byproducts of photosynthesis. These molecules also play an important role in the protection of food against lipid oxidation and in human health by counteracting the risk of cardiovascular diseases, cancer and cataract, among other degenerative diseases of aging (Peyrat-Maillard et al., 2001).

In the food industry, hull-less barley (*Hordeum vulgaris* L.) is acknowledged as more valuable and more economical, compared with flaky barley. The hull-less barley has elevated content of ß-glucans. Soluble dietary fibre, mainly ß-glucans, provides the formation of viscosity; as a result, cholesterol and fat absorption are decreased (Bhatty, 1999; Belicka and Bleidere, 2005). Selected hull-less barley varieties are able to pass flaky barley criteria: moreover, the amount of extract substances in hull-less barley is higher by 4–5% compared to malting barley (Dabina-Bicka et al., 2010).

Attention needs to be focused on the protection of endogenous antioxidant in beer and its raw materials, that is, barley and hop. About 80% of phenolic compounds present in beer are derived from barley malt, and the remaining comes from hop (Goupy et al., 1999). They contribute to astringency and colour (Shahidi and Naczk, 1995), serve as browning substrates, participate in chill haze formation and are responsible for overall beer stability. Malt contains various compounds of barley (endogenous phenolic compounds) and from the malting process (Maillard reaction products) which can play significant role in malting and brewing through their antioxidant properties (Goupy et al., 1999) In this area, Maillard reaction products (MRP) which are naturally produced in food during thermal processing and home cooking operations by reducing sugars interacting with available amino groups, modify important food properties such as colour, flavour and stability during processing and storage (Maillard et al., 2007).

Vitamin E (α-tocopherol) is also a monophenolic compound present in barley and malt (Bamforth et al., 1993) which can quench free radicals. Their antioxidant activity is based mainly on the tocopherol-tocopheril quinone redox system (Randhir et al., 2008). The vitamin E content of cereal grains is influenced by plant genetics and is adversely affected by too much rain and humidity during harvest (Ball, 2006).

Antioxidants are generally thought to play a significant role in malting and brewing due to their ability to delay or prevent oxidation reactions and oxygen free radical reactions (Zhao et.al., 2008). Antioxidant compounds present in barley extracts are complex, and their activities and mechanisms would largely depend on the composition and conditions of the test systems. Extraction solvent had significant effect on barley total phenolic content (TPC) evaluation, and 80% acetone (v/v) was recommended as antioxidants extraction solvent from malting barley for TPC evaluation (Zhao et al., 2006).

The aim of current research is to compare the content of total phenolic compounds (TPC) and vitamin E of different barley varieties and malt types.

Materials and Methods

Barley and malt samples

The research was carried out on four hull-less barley lines '3528'; 'L-400'; '3475'; '3537' 9further in text abbreviated: A; C; D; B, respectively) and flaky barley (one line 'Klass') grains, which were harvested in Latvia in 2010, with germination capacity above 95%. The following technology was used for malt production from the tested grains: washing and steeping of grains (H_2O t=17±2 °C) until moisture content in grains reached 38–40%. Then the grains were placed for germination. The grains were germinated for 4 days at 19±1 °C temperature. An 8 hour kilning of the germinated grains was completed in a laboratory kiln. Grains in a thin layer were spread on sieves in a chamber-type drier with hot air circulation at the temperature from +50 °C to +80 °C till a constant moisture content was achieved in the grains (5±1%).

In this study experimentally produced malt from hull-less barley was compared to commercial sorts of malt. Four kinds of malt, which are produced in "Viking Malt" (Lithuania) – Pilsener, and "Slodownia Strzegom" (Poland) – Munich, Light caramel and Black. Kilning and roasting temperatures were following: Pilsener – 75 °C; Munich – 100 °C; Caramel – 150 °C; Black – 230 °C.

Chemicals

Gallic acid and Folin-Ciocalteus phenol reagent were purchased from Sigma-Aldrich (Switzerland). All other chemicals and solvents were of the highest commercial grade and obtained from BARTA a CIHLAR spol.s.r.o. (Czech Republic) - Na_2CO_3, ethanol, acetone.

Preparation of extracts from barley and malt

Barley and malt was finely ground in a laboratory mill CIATRONIC KSW 2669. Four grams of ground samples were extracted 10 minutes in ultrasound bath (ULTRASONS, SELECTA P) with 40 ml of solvent. To reach a compromise between alcoholic and acetone extractions, a 7/7/6 ethanol/acetone/water (v/v/v) mixture was tested (Bonoli et al. 2004). After centrifugation at 3000 min^{-1} for 10 min using a centrifuge MEDITRONIC BL-C, the supernatant was removed and the extraction was repeated once more. The supernatant was collected in a 50 ml volumetric flask and refilled by solvent till mark (Jakobsone, 2008).

Total phenolic content (TPC)

The TPC of the barley and malt extract was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999) with some modifications. First, 0.25 ml of sample was transferred to a 25.0-ml volumetric flask containing 6 ml of H_2O, to which was subsequently added 1.25 ml of undiluted Folin-Ciocalteu reagent. After 1 min, 3.75 ml of 20% aqueous Na_2CO_3 was added, and the volume was made up to 25.0 ml with H_2O. The control sample contained all the reaction reagents except the extract. After 2 h of incubation at 25 °C, the absorbance was measured at 760 nm using a spectrophotometer JENWAY 6300. Total phenols were expressed as gallic acid equivalents (Damien Dorman et al., 2004).

Vitamin E

The analyses of vitamin E content were carried out by the AOAC Official Method 971.30 "α-Tocopherol and α-Tocopheryl Acetate in Foods and Feeds" standard colorimetric method (1971–1972). The term "vitamin E" is the generic descriptor for all tocol and tocotrienol derivatives that exhibit qualitatively the biological activity of α-tocopherol (Ball, 2006).

Statistical analysis

The differences in the total phenol and vitamin E content were analyzed using the analysis of variance (ANOVA). Tukey's test was applied to compare the mean values and p-value at 0.05 was used to determine the significant differences.

Results and Discussions

Phenolic compounds were considered as a major group of compounds that contributed to the antioxidant activity of cereal (Zhao et al., 2008). Significant amounts of total phenolics were detected in all barley varieties. The content of TPC of the different barley varieties ranged from 1.96 to 2.43 mg GAE g^{-1} DW (Fig. 1). The results were similar to those reported by Zhao et al. (2008) but higher than those reported by Maillard et al. (1996). This might be due to the differences of the barley varieties and the extraction methods used in studies. The differences of TPC content between hulled barley "Klass" and hull-less barley lines A, B, C were not significant (P>0.05), but higher amount of TPC in line D was detected. Comparing hull-less barley lines, significantly lower content of TPC in line C was detected.

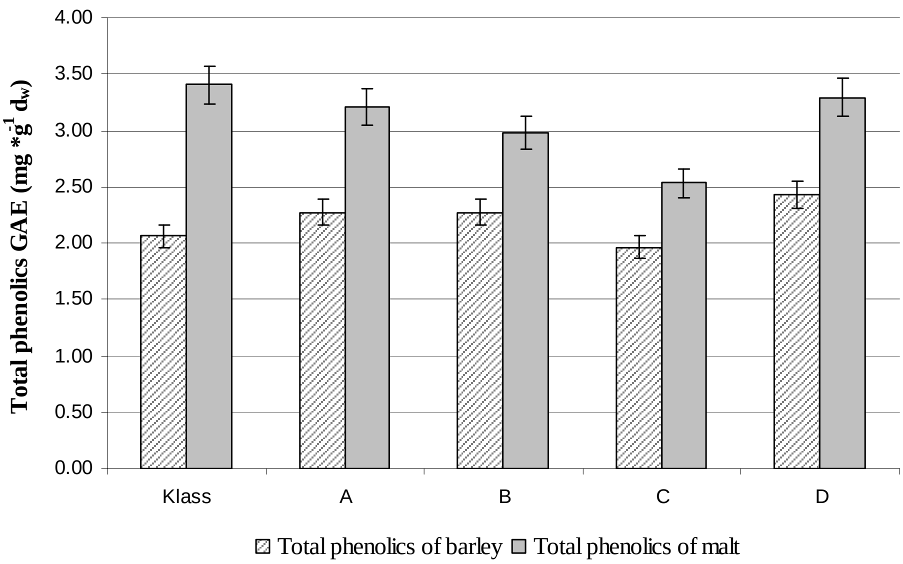

Figure 1. Total phenolic contents in barley grains and malt

Content of TPC in malt was higher than in unprocessed barley (Fig. 1), and the increase ranged from 28% till 65%. Lower content in hull-less barley line C was detected and also lower increase after barley malting – 28%. Increase can be explained by biochemical reactions that occur during germination process. Overall increase of phenolic compounds in legumes, peas after germination was reported (Lopez-Amoros et al., 2006). Also results could be explained by formation of phenolic compounds during Maillard reaction (Maillard. et al., 2007).

Content of vitamin E in barley ranged from 34.96 to 39.2 mg kg^{-1} DW (Fig. 2) and these results are in compliance to Hall (2001). The current research showed that vitamin E content increased during barley malting by 38% comparing to unprocessed barley. Literature studies showed that germination influence the content of tocopherols. In lupins during germination increase of α-tocopherol, whereas decrease of γ-tocopherol were observed, but did not affect content of δ-tocopherol (Frias et al., 2005).

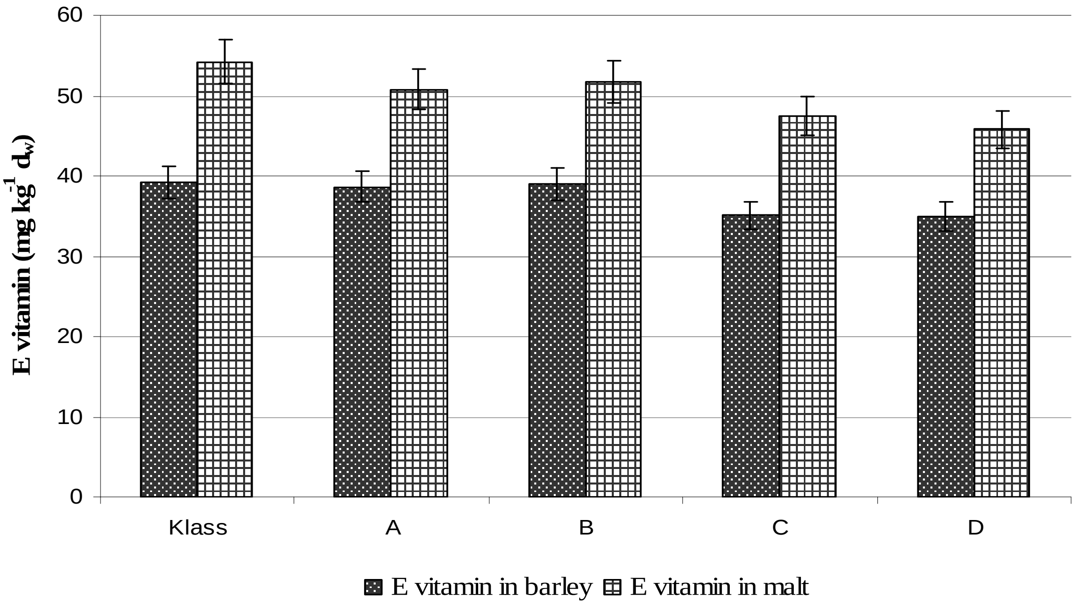

Figure 2. Vitamin E in in barley grains and malt

The thermal stability of the vitamin E in food vary from the heating time, heating methods, and food composition. Content of vitamin E in the commercial malt types (Pilsener, Munich, Caramel, Black) was lower than in experimental malt samples (Table 1).

Table 1

Content of vitamin E and TPC in commercial malt types

Malt	Total phenolics GAE (mg g^{-1} DW)	Vitamin E (mg kg^{-1} DW)
Pilsener	3.529±0.072	37.7±0.1
Munich	4.354±0.184	41.7±0.3
Caramel	6.773±0.293	40.8±0.5
Black	6.782±0.369	24.2±0.7

Pilsener malt kilning temperature was the same that for experimental samples, but content of vitamin E was lower. In malt produced from varieties Klass, A, B, C, D content of vitamin E ranged from 45.8 to 54.2 mg kg^{-1}, but in Pilsener malt only 37.7 mg kg^{-1}. Other two types of

commercial malts Munich and Caramel showed higher amount of vitamin E than Pilsener malt, because the killing temperature was above 75-80 °C. In accordance with Palmer (2006) report, kilning and roasting temperatures of different malt types were following: Pilsener – 75; Munich – 100; Caramel – 150; Black - 230°C. Thermal stability of vitamin E in the Black malt was destroyed, because kilning temperature was too high, reaching 230 °C.
The results of our experiments proved that more intensive synthesis of TPC was detected in Caramel and Black malts, respectively 6.773 and 6.783 GAE mg g^{-1} DW. The TPC increased as roasting time increased showing that Maillard browning reaction products were generated and were probably responsible for the TPC, which are important with regard to improving beer stability and as a source of antioxidants in the diets of beer drinkers (Palmer, 2006).
For further understanding of interrelationship between biologically active compounds of barley and malted barley, correlation analyses were performed. Strong correlation (r=0.84) was found between vitamin E in barley and malted barley, whereas weak correlation (r=0.53) between TPC in barley and malted barley. These data indicate that the content of vitamin E is mainly influenced by technological operations, but for TPC formation other factors, as grain structure, starch molecule size and also amino acid, reducing sugars content that are involved in synthesis of new polyphenols from Maillard reaction, are significant.

Conclusion

1. The content of total phenolic compounds and the vitamin E content of flaky and hull-less barley varieties increase after steeping, germination and kilning in its corresponding malts.
2. The content of vitamin E in barley significantly increased during malting and was heat stable under kilning temperature.
3. As a result of Maillard reaction at higher malt production temperature provides greater content of phenolics in the products.

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