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O EFEITO DA MALTAGEM SOBRE OS CRISTALITOS E MICROESTRUTURA NO CULTIVO DE CEVADA GREGA USANDO DIFRAÇÃO DE RAIOS-X E ANÁLISE MICROSCÓPICA.

THE EFFECT OF MALTING ON THE CRYSTALLITES AND MICROSTRUCTURE IN GREEK BARLEY CULTIVAR USING X-RAY DIFFRACTION AND MICROSCOPIC ANALYSIS

EL EFECTO DEL MALTEADO SOBRE LOS CRISTALITOS Y LA MICROESTRUCTURA EN EL CULTIVO DE UNA CEBADA GRIEGA USANDO DIFRACCIÓN DE RAYOS X Y ANÁLISIS MICROSCÓPICO.

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RESUMO

Introdução: A maltagem era geralmente usada para produzir açúcares a partir do amido e consequentemente permitir a fermentação alcoólica. A variação na microestrutura e cristalinidade do amido afeta sua solubilização de água e a sua hidrólise pelas enzimas.
Objetivos: A microestrutura do amido é explorada para entender e possivelmente modificar o processo de maltagem, se necessário, de uma variedade de cevada grega. Para resolver o problema, foram utilizados uma combinação de métodos de microscopia e de inspeção de difração de raios-X para avaliar a variação da morfologia dos grânulos e o grau de cristalinidade do amido.
Métodos: O efeito da maltagem na microestrutura do amido de cevada foi investigado para uma cultivar de cevada grega (Seirios). As sementes de cevada foram fornecidas pelo ELGO-DEMETER, Instituto de Criação de Plantas e Recursos Genéticos (Thessaloniki, Grécia). A maceração foi realizada a 14 ° C (vários círculos de imersão e arejamento) até a cevada atingir o teor de humidade desejado. Então a cevada mergulhada foi deixada a germinar a 15 ° C. A germinação foi interrompida por secagem a 40-45 ° C, durante pelo menos 20 h (malte seco). O malte foi obtido por secagem durante 6 horas a 100 ° C. A cevada, malte seco e malte foram moídos para passar por uma tela de 0,8 mm antes de serem analisados. As fotografias de microscopia confocal com coloração vermelha do Congo foram usadas para identificar a variação na morfologia dos grânulos de amido. Além disso, foi realizada a análise de difração de raios-x da farinha de cevada, malte seco e moagem de malte. Finalmente, a análise dos difractogramas de raios-X foi realizada para calcular a cristalinidade relativa e o tamanho dos cristalitos de amido.
Resultados: Os resultados demonstraram variações na morfologia dos grânulos de amido. As fotografias de microscopia confocal revelaram que os grânulos de amido na cevada eram redondos, maiores, lisos e granulares, e que o malte seco e o malte pareciam menores e mais alongados com arranhões na superfície. As fotografias coradas da microscopia confocal mostraram o início dos danos na superfície dos grânulos do amido. Os difractogramas de Raios-X revelaram que a cristalinidade do amido de cevada aumentou durante a produção de malte seco e depois diminuiu durante a secagem, mas permaneceu maior do que o amido de cevada nativa.
Conclusões: A maltagem sob as condições especificadas afetou a morfologia dos grânulos de amido da variedade Seirios, bem como a cristalinidade do mesmo, sugerindo a degradação parcial do amido durante o período de maceração e germinação, bem como durante a secagem.

Palavras-chave: cevada; amido; malte; XRD; microscopia;

ABSTRACT

Introduction: Malting was generally used to produce fermentable sugars from starch in order to permit alcoholic fermentation. Variation in the starch microstructure and crystallinity affects its water solubilisation as well as and its breakdown from the enzymes
Objectives: The starch microstructure is explored in order to understand and possibly modify the malting process of a Greek barley variety if needed. To address the issue, a combination of microscopy and X-ray diffraction inspection methods were used to evaluate the variation of the starch granule morphology and the degree of crystallinity of starch.
Methods: The effect of malting on the microstructure of barley starch was investigated for one Greek barley cultivar (Seirios). The barley seeds were provided from ELGO-DEMETER, Institute of Plant Breeding and Genetic Resources (Thessaloniki, Greece). The steeping was performed at 14°C (several soaking and aerating circles) until barley reached the desired moisture content. Then the steeped barley was allowed to germinate at 15°C. The germination was stopped by drying at 40-45°C for at least 20h (dry malt). The malt was obtained by kilning for 6 hours at 100°C. The barley, dry malt and malt were milled to pass through a 0.8mm screen before being analysed. Light microscopy photographs with Congo red staining were used to identify variation in the starch granule morphology. Moreover, the x-ray diffraction analysis of the barley meal, dry malt and malt millings were obtained. Finally, the analysis of X-ray diffractograms was performed in order to calculate relative crystallinity and the crystallite size of the starch.
Results: The results demonstrated variations in the morphology of the starch granules. The stained light microscopy photographs revealed that the starch granules in barley were round, greater, smooth, and granular, and that of the dry malt and the malt appeared smaller and more elongated with scratches on the surface. The stained confocal microscopy photographs showed the beginning of the starch damage on the surface of the granules. X-Ray diffractographs revealed that the crystallinity of the barley starch was increased during dry malt production and then decreased during kilning but still remained greater than the native barley starch.

Conclusions: Malting under the specified conditions, affected the morphology of the starch granules of Seirios variety as well as the crystallinity of the starch suggesting the partial degradation of the starch during steeping and germination as well as during kilning.

Keywords: barley; starch; malting; XRD; microscopy;

RESUMEN

Introducción: El malteado se usaba, en general, para producir azúcares fermentables a partir del almidón a fin de permitir la fermentación alcohólica. La variación en la microestructura y cristalinidad del almidón afecta su solubilidad en agua y su degradación de las enzimas.
Objetivos: La microestructura del almidón se explora para comprender y posiblemente modificar el proceso de malteado de una cebada griega en caso de necesidad. Para abordar el problema, se utilizó una combinación de métodos de inspección por microscopía y por difracción de rayos X para evaluar la variación de la morfología de los gránulos de almidón y el grado de cristalinidad del almidón.
Métodos: Se investigó el efecto del malteado sobre la microestructura del almidón de cebada para un tipo de variedad de cebada griega (Seirios). Las semillas de cebada fueron provistas por ELGO-DEMETER, Instituto de Mejora Vegetal y Recursos Genéticos (Thessaloniki, Grecia). El remojo se realizó a 14 ° C (varios círculos de remojo y aireación) hasta que la cebada alcanzó el contenido de humedad deseado. A continuación, la cebada remojada se dejó germinar a 15 ° C. La germinación fue detenida aplicando una temperatura de secado a 40-45° C durante, por lo menos, 20 h (malta seca). Se obtuvo la malta horneándola durante 6 horas a 100 ° C. La cebada, la malta seca y la malta se molieron para pasar a través de una malla de 0,8 mm antes de analizarse. Para identificar la variación en la morfología de los gránulos de almidón, se utilizaron fotografías de microscopía óptica con tinción con rojo Congo. Además, se obtuvieron los análisis de difracción de rayos X de la harina de cebada, la malta seca y la molienda de la malta. Finalmente, se realizó el análisis de los difractogramas de rayos X para calcular la cristalinidad relativa y el tamaño del cristalito del almidón.
Resultados: Los resultados demostraron variaciones en la morfología de los gránulos de almidón. Las imágenes de microscopía óptica teñida revelaron que los gránulos de almidón en la cebada eran redondos, más grandes, lisos y granulares, y los de la malta seca y de la malta parecían más pequeños y alargados con arañazos en la superficie. Las imágenes teñidas de la microscopía confocal mostraron el comienzo del daño del almidón en la superficie de los gránulos. Los difractogramas de rayos X revelaron que la cristalinidad del almidón de cebada se incrementó durante la producción de malta seca y luego disminuyó durante la cocción, pero aún permaneció mayor que el almidón de la cebada nativa.
Conclusiones: El malteado bajo las condiciones especificadas, afectó la morfología de los gránulos de almidón de la variedad de Seirios, así como la cristalinidad del almidón, lo que sugiere la degradación parcial del almidón durante la maceración y la germinación, así como durante la cocción.

Palabras Clave: cebada, almidón, DRX, microscopía

INTRODUCTION

Starch is composed of amylose and amylopectin packed into granules. There exists a great variation in the granule size distribution among the genotypes (Jaiswal, Båga, Ahuja, Rossnagel, & Chibbar, 2014; Naguleswaran, Vasanthan, Hoover, & Bressler, 2013; Zhu, 2017). Barley starch exists in two types of granules; the large with 10-40μm length and disc-shaped and the small, under 10μm, with irregular spherical shape. Naguleswaran et al. (2013) reported that the weight-based percentages of granules with mean size of 15-16 μm in waxy, normal, and high amylose starches were 88.8, 86.9, and 53.5%, respectively.
According to Chmelík, Krumlová et al. (2001) small starch granules tend to be deeply embedded in the protein matrix of endosperm and gelatinise at higher temperatures and over wider temperature range than large granules. Moreover, the small granules are less susceptible to enzymic degradation during mashing producing hazes that cause technological problems during brewery.
Congo Red (sodium salt of benzidinediazo-biz-1-naphthylamine-4-sulfonic acid) with empirical formula C32H22N6O6S2Na2 and MW 696.66 g/mol is a water-soluble dye which depends on linear hydrogen bonding for staining (Gilbertson, 2018). Congo Red appears to react with the amylose in starch, which is exposed when grains are damaged by structure loss and swollen with water, as when they have been cooked. Both cellulose, and starch grains that have broken bonds, will stain with Congo Red, but their appearance and structure are visibly different, so they can be differentiated under the light as well as confocal microscope. Confocal microscopy is a powerful technique that allows enhanced optical resolution and contrasted images allowing the visualization of morphological structures being very useful for structural carbohydrate analysis (Dürrenberger, Handschin, Conde-Petit, & Escher, 2001).

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According to Bathgate the malted-barley starch had higher apparent amylose content, higher gelatinization temperature, and smaller granules than that from the native barley (Bathgate, 2016). Moreover, the malt amylose has smaller molecular size than that of the barley whereas the amylopectin fractions were of comparable molecular size but differed in the average chain length. The internal chain-lengths of amylopectins are similar, whereas the external chain-lengths are smaller in malted-barley suggesting that the malted barley amylopectin had been degraded by β-amylase to a limited extent (Bathgate, 2016; Chu, Hasjim, Hickey, Fox, & Gilbert, 2014).
Barley starch has A-type polymorph regardless of the granule size (Ao & Jane, 2007; Gao, Vasanthan, & Hoover, 2009; Källman et al., 2015; Kong et al., 2016; Li et al., 2014; Yangcheng, Gong, Zhang, & Jane, 2016) but some genotypes showed a C-type polymorph (Waduge, Hoover, Vasanthan, Gao, & Li, 2006). In the literature the presence of V-type polymorph was also reported and was related to the amylose–lipid inclusion complexes (Gao et al., 2009; Källman et al., 2015).
X-ray diffractometry revealed diversity in the degree of crystallinity of starch granules (Ao & Jane, 2007; Kong et al., 2016; Li et al., 2014). The literature reported that the degree of crystallinity of starches ranged from 10.7% to 44.3% (Källman et al., 2015; Kong et al., 2016; Li et al., 2014; Waduge et al., 2006) with the waxy starch showing highest degree of crystallinity followed by normal and high amylose starches (Naguleswaran et al., 2013).
Barley is widely used cereal for malting. During malting the conversion of native starch into fermentable sugars depends on starch composition, morphology, and molecular and granular structural features (Naguleswaran et al., 2013). Endoamylases, hydrolyse native starch in several steps which consist of diffusion to the solid surface, adsorption and finally catalysis (Oates, 1997). Malted barley starch has higher percentage of amylose and an amylopectin component with degraded external chains (Bathgate, 2016).
Malting of barley induces extensive physical, chemical and structural changes of endosperm (Brennan et al., 1997). The starch granules are released from the protein matrix are gelatinised and became accessible to hydrolytic enzymes during mashing. Thus, it is desirable to know both the variation and development of the structure of barley starch endosperm in order to determine the malting quality of barley. Application of light and confocal microscopy as well as X-ray diffraction analysis will help in the investigation of the surface structure of the starch granules as well as in the structural changes of starch during malting.
The purpose of this study was therefore, the examination the structural changes that occur within the granule interior of starches (molecular structure characterization) from native and malted Greek barley cultivar using light microscopy and confocal microscopy in combination with X-ray diffractometry.

1. METHODS

1.1 Sample and chemicals

Barley kernels of variety Seirios were obtained from the ELGO-DEMETER, Institute of Plant Breeding and Genetic Resources (Thessaloniki, Greece). The barley kernels (with hulls) were free of any impurity. Congo red was acquired from Merck (Darmstadt, Germany). All chemicals used were of analytical grade.

1.2 Malting procedure

Barley kernels (native barley) were thoroughly cleaned by washing with tap water and then soaked in water for 48 h at 14 °C; the soaking water was changed at 8 h intervals. After soaking, the grains were evenly spread on trays and allowed to germinate in dark chamber with 85% moisture content. The temperature of the kernels during germination step was kept at 15 °C. The germination was terminated when the acrospires (sprouts) were grown up to approximately 75% of the grains length by first drying in an air oven at 40 - 45 °C for 28 h (dry malt was obtained at this step) and then at 100 °C for 6 h (malt). The withered rootlets were gently brushed off from the obtained dry malt and malt. The whole barley kernels, the whole dry malt and the malt kernels were milled using a mill (Falling Number AB Type 120 grinder, S-12611, Stockholm, Sweden) equipped with 0.8 mm sieve. The flours were then packaged in polyethylene bag and stored at 4 °C till use.

1.1. Light microscopy microstructure

The microstructure of barley as well as malt flour were examined by light microscopy. The whole barley, dry malt and malt flours were prepared to suspension on a microslide with distilled water, stained with diluted Congo red solution and covered with a coverslip that had been cleared of bubbles. The Congo red was used in order to visualize starch damage and imaged in brightfield. It is recognized that Congo red stains damaged starch granules red but not undamaged starch. Photographs were acquired by light microscopy (LSM 700, Carl Zeiss Micro Imaging GmbH, Jena, Germany) and camera (AxioCam ERc5s). Red staining was viewed under microscope magnification of 200 times.

1.2. Confocal laser scanning microscopy (CLSM)

Flours were stained with Congo red as previously mentioned in section 2.3 and then the dyed samples were left to dry at ambient temperature. In exciting light (excitation wave length, 488 nm) intact starch granules are unstained and appear black.

Contrary, the penetration of the red colour in the starch granules reveals the damage of the starch granules. A confocal laser scanning microscope (LSM 700, Carl Zeiss Micro Imaging GmbH, Jena, Germany) was used to examine the samples. The whole barley flour was used as a control sample.

1.3. X-ray diffraction (XRD) analysis

For the X-ray diffraction measurement of the flours was used an X-Ray Diffractometer X'PertPRO, model MPD, (PANalytical, the Netherlands) that was operated at 45 kV and 40 mA. A divergence slit of 1°, an antiscatter slit of 2° and a receiving slit of 0.4 mm were used. Flours (~200 mg) were placed in the special holders and scanned in the range 6–45° diffraction (2θ) at a scan speed of 0.008°/min with a step size of 0.04°.

1.4. Analysis of X-ray Diffractogram

The computer peak fitting program (origin pro 2015) has been used to determine precisely the peak positions which are characteristic of A- and B-type polymorphs.
The measurement of the crystallinity from the diffractograms, was performed as followed: in the region of interest, a base line was drawn from starting of the curve to the end of the curve as well as at the base of peaks. The relative crystallinity was calculated between 13 and 25° using the formula:
%crystallinity = Acr/(Aam+Acr),
where, Acr and (Aam+Acr) are the measured areas of upper region above peak base line, representing X-ray scattering of the crystalline portion and the whole area under diffractogram comprising both crystalline (Acr) and amorphous portion (Aam).

In addition, the crystallite size was calculated using Scherrer formula:

$$D\ (Å)=0.9\ \lambda/FWHM\times\cos\theta.$$

where, λ is the X-ray wavelength (0.15418 Å), FWHM is the full width of the peak at half maximum in radians and θ is the peak, in radians.
The X-ray pattern of the crystalline portion (13-25°) was analysed with software OriginPro. First were deconvoluted peak profiles and found the respective θ degree of barley, dry malt and malt flour. Then was calculated the total crystallinity as well as the percentage that each peak (15, 17-18, 20 and 23°) contributes to the total crystallinity.

2. RESULTS

2.1. Light microscopy

The Congo red has been used to identify starch since it has a strong interaction with polysaccharides through noncovalent affinity and synthesizing a red complex (Lamb & Loy, 2005). In addition, the Congo red has the ability to stain red the cellulose fibres (Samim, Sandkuijl, Tretyakov, Cisek, & Barzda, 2013). Therefore, both cellulose-like components, and starch granules that have broken bonds, will be stained with Congo Red. They can be easily differentiated under the microscope since their appearance and structure are visibly different.
Starch granules observed in the Figure 1A-C were of two types, both big and small, characteristic for the barley. A decrease in the size of the granules and a change in their shape with malting were observed. The granules became more elongated. The cellulose fibres contained in the flours were stained red and one can distinguish them from the starch granules (Figure 1A–C). In native barley (Figure 1A), it was observed only a few number of starch granules stained red suggesting a low level of damaged/degraded starch. On the other hand, on the surface of the big and small starch granules of the dry malt (Figure 1B) and malt (Figure 1C) were observed fissures and cracks. More fissures and cracks were observed on the surface of the starch granules of malt.

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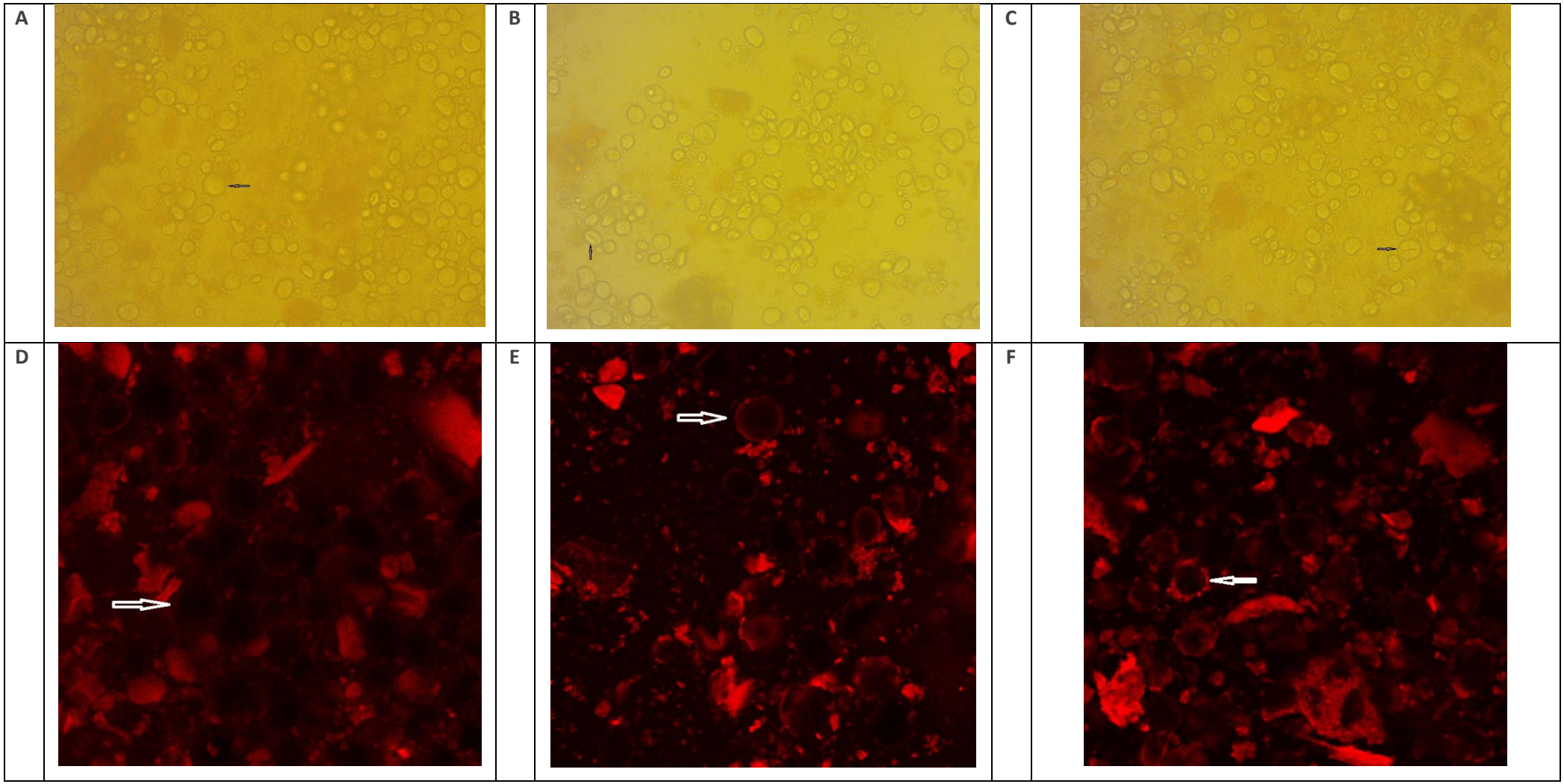

Figure 1. Light (Up) and Confocal (Down) microscopy photographs of Congo red staining : (A, D) whole barley flour (B, E) whole dry malt flour (C, F) whole malt flour

2.2 Confocal microscopy

The confocal photographs of the flours from barley, dry malt and malt are shown in the Figure 1D, E and F, respectively. Stained with red are observed amorphous cellulose-like components of the flour whereas the intact starch granules are visible as black round circles. It is generally known that the damaged starch granules appear red under the confocal microscope. In Figure 1D it is observed that only few granules are coloured red showing the low percentage of damaged starch in the native barley as well as during milling. Contrary, in the dry malt it was observed that the number of intact granules (black circles) was significantly reduced and almost all starch granules showed a thin red aureole. In the malt, the starch granules showed a thicker aureole than that observed in the dry malt. On the other hand, there is not observed any change in the core of the starch granules.

2.3 XRD analysis

The X-ray diffractograms of whole grain flours from barley, and dry malt and malt are presented in Figure 2. As for cereals the malted samples, showed the typical "A" type peak assignments.

Barley, dry malt and malt flours presented similar diffraction patterns with strong peaks at 2θ ≈ 15°, one unresolved doublet at ~17-18°, ~23°, indicating an A-type crystalline pattern (Figure 3 a-c). In addition, the lower peak at 2θ = 20° (Figure 2) was attributed to the formation of a well-formed V-structure which was a consequence of the inserting of bulky group (carboxyl (COOH) and carbonyl (CO)) into starch chains (Pérez & Bertoft, 2010). There were observed no significant changes in the X-ray diffraction patterns between the barley, dry malt and malt.

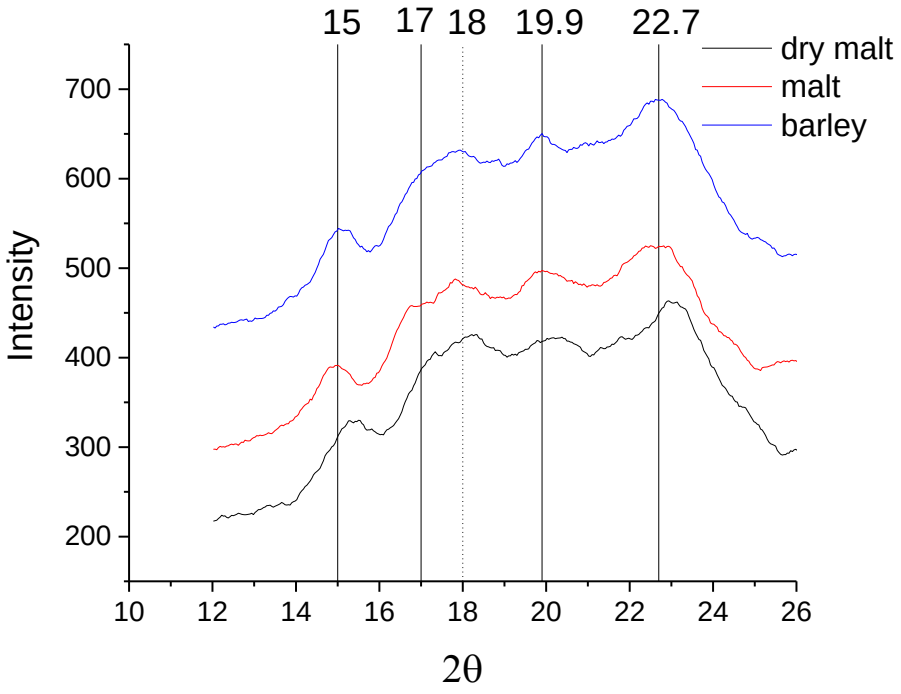

Figure 2. Smoothed XRD patterns for barley, dry malt and malt flours.

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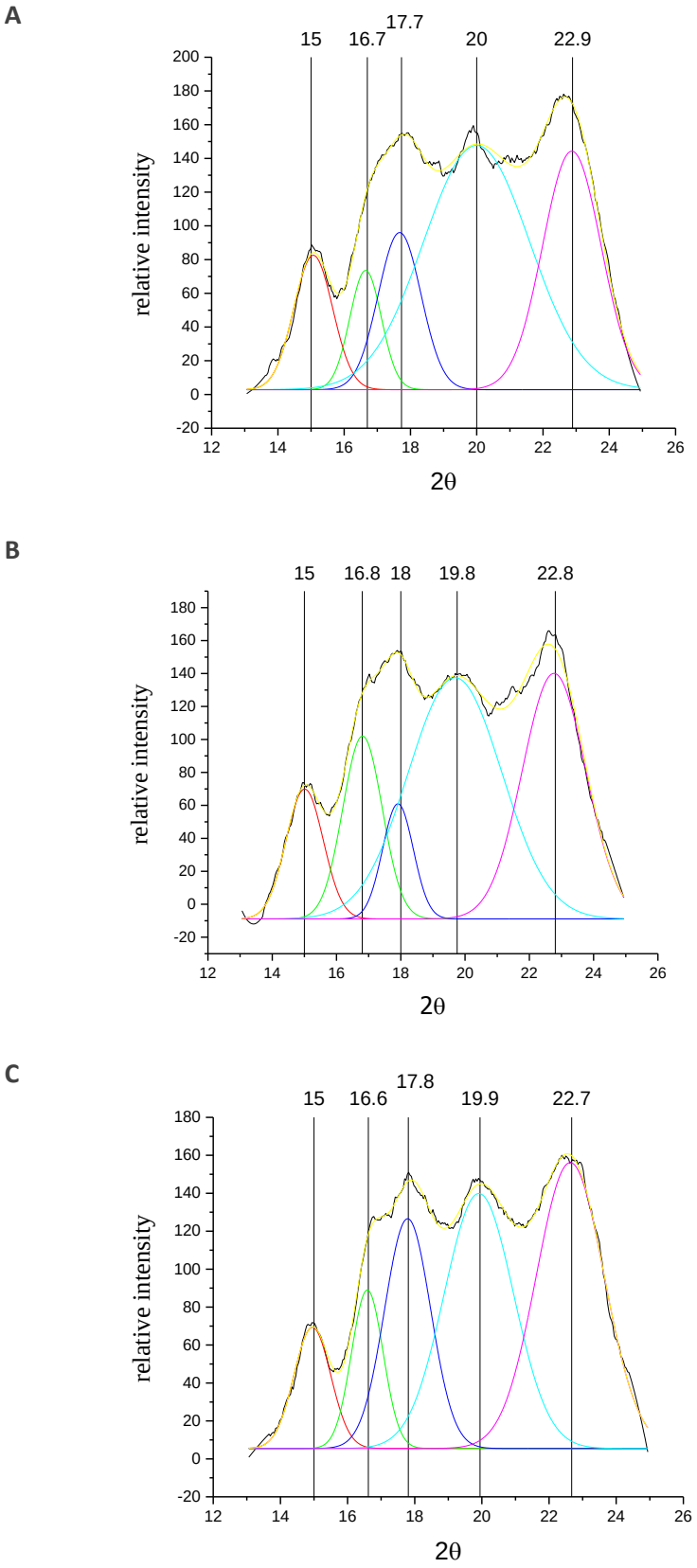

Figure 3. Deconvoluted peak profiles for the X-ray pattern of the crystalline portion of barley flour (A), dry malt (B) and malt (C).

On the other hand, the relative crystallinity varied among the samples (22.7, 24.5, and 22.9% for barley, dry malt, and malt, respectively). It was observed an increase of the overall relative crystallinity value of the dry malt compared to native barley and malt (24.5 vs 22.7 and 22.9%, respectively) (Figure 4).
The increase in the intensity of the peak at 20° during malting is attributed to the formation of a V-type complex favoured when a fatty lipid is grafted into starch chains (Figure 4). The relative crystallinity at the angle 15° is decreased with malting. On the other hand, the crystallinity at 23° was increased in dry malt but decreased in the malt. The opposite was observed for the crystallinity at 17-18°.

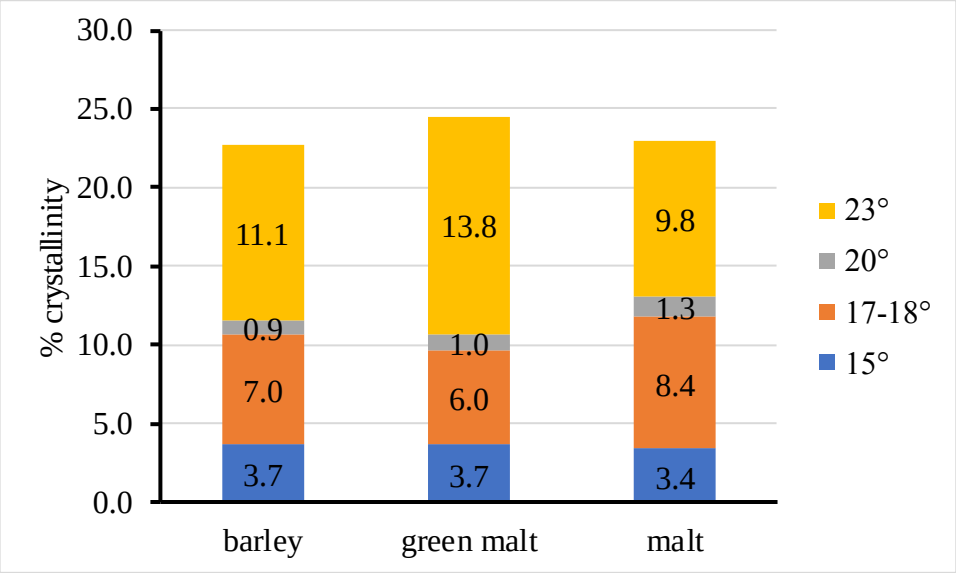

Figure 4. Relative crystallinity of barley, dry malt, and malt flour calculated from the crystalline peaks of the deconvoluted X-ray diffractograms.

Table 1 - Microstructural parameters for barley, dry malt and malt using deconvoluted x-ray data

Sample	2θ	D (nm) deconvoluted
barley	15	8.91
	16.8	10.05
	17.7	6.19
	19.9	16.14
	22.9	4.77
dry malt	14.9	8.02
	16.8	10.05
	17.8	8.05
	19.9	8.97
	22.8	4.51
malt	14.8	8.91
	16.7	11.48
	17.7	6.71
	19.9	11.53
	22.8	5.07

The average sizes of crystallites in the crystalline regions (peaks) of barley, dry malt and malted barley starch are calculated by Scherrer equation (Table 1). The D values observed on different peaks ranging from 4.77 to 16.14 Å for barley, from 4.51 to 10.05 Å for dry malt and from 5.07 to 11.53 Å for malt indicate the variation in the crystallinity of starch during malting. For the

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barley, dry malt and malt, the smallest crystallite size of the starch is 4.77Å, 4.51Å and 5.07Å observed at 22.9°. It was observed that malting leads to changes in crystallite size - it decreases at the angle 20° from 16.14 in barley to 8.97 in dry malt and then increases to 11.53 in malt.

3. DISCUSSION

Distribution of starch granular size observed in the Greek barley is a bimodal distribution of large and small granules, referred to as A- and B-granules, respectively being in accordance with the reported data in the literature (MacGregor & Ballance, 1980). The morphology of these starch granules changed during malting process, both A and B granules become slightly smaller and more elongated (Figure 1A-C). This is in accordance with the data reported in the literature (Greenwood & Thomson, 1959). These changes in the size and shape are possibly due to the decrease of the moisture content (reduced degree of swelling) as well as to the enzyme attack. On the other hand, the creation of the fissures and cracks on the surface of the starch granule suggests that enzymes produced during germination step attack the surface of the starch granules without being inserted inside the granule. The increased number of scratches during kilning suggest that enzymes attack the granules even after first drying. These cracks will represent the location of initial enzyme attack during wort preparation where enzymes will have direct access to the granule interior. In the literature is reported that amylases affect the starch granules in two ways; exocorrosion and endocorrosion (Oates, 1997; Sujka & Jamroz, 2007). During the exocorrosion the erosion of the entire granule surface or sections of it happens resulting in fissures and pits. On the other hand, during endocorrosion hydrolysis of the channels from selected points on the surface toward the granule centre takes place resulting in granule fragmentation. It seems that during malting there was observed only exocorrosion. Possibly the enzymic degradation of the surface of the starch granules happened in the presence of high moisture during steeping and germination. Enzymes begin to hydrolyse the starch chains attacking mainly amylopectin responsible for the amorphous region (Colonna, Buléon, & Lemarié, 1988) during steeping process to a certain straight-chain length, and then the new free chains can be ordered during drying (dry malt) forming double helix and increasing crystallinity (from 24.5 to 22.7%). In the literature it was reported that hydrated starches are known to give well-defined peaks since the added moisture permits the already longitudinal molecules present in the amorphous regions to organize into crystalline units (Singh, Ali, Somashekar, & Mukherjee, 2006). Thus, partial destruction of the amorphous region of starch following by a subsequent alignment of crystallites may be responsible for the observed increase in the crystallinity.
The increase observed in the crystallinity at the angle 20° could be related with the swelling of starch granules during steeping that in their turn may increase the possibility for the lipids (mainly fatty acid and monoglycerides) present in the flour matrix to be inserted into the amylose chain released. Moreover, the fatty lipids present in the flour may be inserted to some of the linear broken chains during enzymic attack. The non-alignment of these chains may be responsible for the observed decrease in the crystallite size at angle 20° during dry malt production. The increase of the crystallinity at angle 20° from dry malt to malt may be due to enhanced ordering of lipid molecules that were present as V-amylose–lipid complexes within granules of the native starches. This suggestion is in agreement with the increase (from 8.97 to 11.53 Å) in the crystallite size of the V-amylose–lipid complexes.
The degree of crystallinity is reported to be one of the several factors that determine starch digestibility (Benmoussa, Suhendra, Aboubacar, & Hamaker, 2006) whereas channels present in the starch granules are the main route of enzyme penetration and the central cavity area represents the starting point of enzyme digestion.

CONCLUSIONS

Malting affected the starch granule morphology as well its crystallinity. The endogenous enzymes seemed to hydrolyse the external surface of starch granules during production of dry malt as well as during kilning producing fissures and cracks. These fissures and cracks will facilitate the introduction of enzymes within the starch granule and fast hydrolyzation during mashing process. During production of the dry malt there was increased crystallinity of starch whereas during kilning (in malt) the crystallinity was decreased. On the other hand, there was observed and increase in the crystallite size in certain crystalline regions and a decrease in others. However, the mean crystallite size in the crystalline region at 20°, first decrease and then increase but were always smaller than that in the native barley.

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REFERENCES

Ao, Z., & Jane, J.-l. (2007). Characterization and modeling of the A- and B-granule starches of wheat, triticale, and barley. Carbohydrate Polymers, 67(1), 46-55. https://doi.org/10.1016/j.carbpol.2006.04.013

Bathgate, G. N. (2016). A review of malting and malt processing for whisky distillation. 122(2), 197-211. https://doi.org/10.1002/jib.332

Benmoussa, M., Suhendra, B., Aboubacar, A., & Hamaker, B. R. (2006). Distinctive Sorghum Starch Granule Morphologies Appear to Improve Raw Starch Digestibility. Starch - Stärke, 58(2), 92-99. https://doi.org/10.1002/star.200400344

Brennan, C. S., Amor, M. A., Harris, N., Smith, D., Cantrell, I., Griggs, D., & Shewry, P. R. (1997). Cultivar Differences in Modification Patterns of Protein and Carbohydrate Reserves during Malting of Barley. Journal of Cereal Science, 26(1), 83-93. https://doi.org/10.1006/jcrs.1996.0103

Chmelík, J., Krumlová, A., Budinská, M., Kruml, T., Psota, V., Bohačenko, I., . . . Vydrová, H. (2001). Comparison of Size Characterization of Barley Starch Granules Determined by Electron and Optical Microscopy, Low Angle Laser Light Scattering and Gravitational Field-Flow Fractionation. Journal of the Institute of Brewing, 107(1), 11-17. https://doi.org/10.1002/j.2050-0416.2001.tb00074.x

Chu, S., Hasjim, J., Hickey, L. T., Fox, G., & Gilbert, R. G. (2014). Structural Changes of Starch Molecules in Barley Grains During Germination. 91(5), 431-437. https://doi.org/10.1094/CCHEM-09-13-0174-R

Colonna, P., Buléon, A., & Lemarié, F. (1988). Action of Bacillus subtilis α-amylase on native wheat starch. Biotechnology and Bioengineering, 31(9), 895-904. https://doi.org/10.1002/bit.260310902

Dürrenberger, M. B., Handschin, S., Conde-Petit, B., & Escher, F. (2001). Visualization of Food Structure by Confocal Laser Scanning Microscopy (CLSM). LWT - Food Science and Technology, 34(1), 11-17. https://doi.org/10.1006/fstl.2000.0739

Gao, J., Vasanthan, T., & Hoover, R. (2009). Isolation and Characterization of High-Purity Starch Isolates from Regular, Waxy, and High-Amylose Hulless Barley Grains. Cereal Chemistry, 86(2), 157-163. https://doi.org/10.1094/CCHEM-86-2-0157

Gilbertson, J. A. (2018). Amyloid. In K. S. Suvarna, C. Layton, & J. D. Bancroft (Eds.), Bancroft's Theory and Practice of Histological Techniques (8th ed.) (pp. 231-253): Elsevier Limited.

Greenwood, C. T., & Thomson, J. (1959). A comparison of the starches from barley and malted barley. Journal of the Institute of Brewing, 65(4), 346-353. https://doi.org/10.1002/j.2050-0416.1959.tb01470.x

Jaiswal, S., Båga, M., Ahuja, G., Rossnagel, B. G., & Chibbar, R. N. (2014). Development of Barley (Hordeum vulgare L.) Lines with Altered Starch Granule Size Distribution. Journal of Agricultural and Food Chemistry, 62(10), 2289-2296. https://doi.org/10.1021/jf405424x

Källman, A., Vamadevan, V., Bertoft, E., Koch, K., Seetharaman, K., Åman, P., & Andersson, R. (2015). Thermal properties of barley starch and its relation to starch characteristics. International Journal of Biological Macromolecules, 81, 692-700. https://doi.org/10.1016/j.ijbiomac.2015.08.068

Kong, X., Kasapis, S., Zhu, P., Sui, Z., Bao, J., & Corke, H. (2016). Physicochemical and structural characteristics of starches from Chinese hull-less barley cultivars. International Journal of Food Science & Technology, 51(2), 509-518. https://doi.org/10.1111/ijfs.12984

Lamb, J., & Loy, T. (2005). Seeing red: the use of Congo Red dye to identify cooked and damaged starch grains in archaeological residues. Journal of Archaeological Science, 32(10), 1433-1440. https://doi.org/10.1016/j.jas.2005.03.020

Li, W., Xiao, X., Zhang, W., Zheng, J., Luo, Q., Ouyang, S., & Zhang, G. (2014). Compositional, morphological, structural and physicochemical properties of starches from seven naked barley cultivars grown in China. Food Research International, 58, 7-14. https://doi.org/10.1016/j.foodres.2014.01.053

MacGregor, A. W., & Ballance, D. L. (1980). Hydrolysis of large and small starch granules from normal and waxy barley cultivars by alpha-amylases from barley malt. Cereal Chemistry., 57(6), 397-402. Retrieved from: https://www.aaccnet.org/publications/cc/backissues/1980/Pages/Number6.aspx

Naguleswaran, S., Vasanthan, T., Hoover, R., & Bressler, D. (2013). The susceptibility of large and small granules of waxy, normal and high-amylose genotypes of barley and corn starches toward amylolysis at sub-gelatinization temperatures. Food Research International, 51(2), 771-782. https://doi.org/10.1016/j.foodres.2013.01.057

Oates, C. G. (1997). Towards an understanding of starch granule structure and hydrolysis. Trends in Food Science & Technology, 8(11), 375-382. https://doi.org/10.1016/S0924-2244(97)01090-X

Pérez, S., & Bertoft, E. (2010). The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. Starch - Stärke, 62(8), 389-420. https://doi.org/10.1002/star.201000013

Adriana Skendi, A., Papageorgiou, M. & Papastergiadis, E. (2018). The effect of malting on the crystallites and microstructure in Greek barley cultivar using X-ray diffraction and microscopic analysis. *Millenium*, 2(7), 67-78.
DOI: https://doi.org/10.29352/mill0207.06.00203

Samim, M., Sandkuijl, D., Tretyakov, I., Cisek, R., & Barzda, V. (2013). Differential Polarization Nonlinear Optical Microscopy with Adaptive Optics Controlled Multiplexed Beams. International Journal of Molecular Sciences, 14(9), 18520. https://doi.org/10.3390/ijms140918520

Singh, V., Ali, S. Z., Somashekar, R., & Mukherjee, P. S. (2006). Nature of Crystallinity in Native and Acid Modified Starches. International Journal of Food Properties, 9(4), 845-854. https://doi.org/10.1002/star.20100001310.1080/10942910600698922

Sujka, M., & Jamroz, J. (2007). Starch granule porosity and its changes by means of amylolysis. International Agrophysics, 21(1), 107-113.

Waduge, R. N., Hoover, R., Vasanthan, T., Gao, J., & Li, J. (2006). Effect of annealing on the structure and physicochemical properties of barley starches of varying amylose content. Food Research International, 39(1), 59-77. https://doi.org/10.1016/j.foodres.2005.05.008

Yangcheng, H., Gong, L., Zhang, Y., & Jane, J.-l. (2016). Pysicochemical properties of Tibetan hull-less barley starch. Carbohydrate Polymers, 137, 525-531. https://doi.org/10.1016/j.carbpol.2015.10.061

Zhu, F. (2017). Barley Starch: Composition, Structure, Properties, and Modifications. Comprehensive Reviews in Food Science and Food Safety, 16(4), 558-579. https://doi.org/10.1111/1541-4337.12265