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Heat damage and *in vitro* starch digestibility of puffed wheat kernels

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ABSTRACT

The effect of processing conditions on heat damage, starch digestibility, release of advanced glycation end products (AGEs) and antioxidant capacity of puffed cereals was studied. The determination of several markers arising from Maillard reaction proved pyrraline (PYR) and hydroxymethylfurfural (HMF) as the most reliable indices of heat load applied during puffing. The considerable heat load was evidenced by the high levels of both PYR (57.6–153.4 mg kg^{-1} dry matter) and HMF (13–51.2 mg kg^{-1} dry matter). For cost and simplicity, HMF looked like the most appropriate index in puffed cereals. Puffing influenced starch *in vitro* digestibility, being most of the starch (81–93%) hydrolyzed to maltotriose, maltose and glucose whereas only limited amounts of AGEs were released. The relevant antioxidant capacity revealed by digested puffed kernels can be ascribed to both the new formed Maillard reaction products and the conditions adopted during *in vitro* digestion.

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1. Introduction

Puffed cereals are widely used as ready-to-eat products or as ingredients in snack formulations, and their demand is currently increasing because of changing life styles. They are generally obtained from rice, wheat, maize, oat and barley (Fast, 1993; Hoke et al., 2007), most often from whole kernels. Thus, puffed cereals are particularly nutritious and healthful because the outer layers of the seeds (aleurone and pericarp) are rich in antioxidants (Hidalgo & Brandolini, 2008), proteins (Hidalgo & Brandolini, 2008), minerals (Erba, Hidalgo, Bresciani, & Brandolini, 2011) and fiber (Hidalgo & Brandolini, 2014). Increasing attention to the nutritional quality of foods has fostered renewed interest in the hulled wheat einkorn (*Triticum monococcum* L. subsp. *monococcum*). Einkorn whole meal is poor in dietary fiber but is rich in proteins, lipids (mostly unsaturated fatty acids), fructans, trace elements (including zinc and iron), as well as antioxidant compounds such as carotenoids, tocols, conjugated polyphenols (Hidalgo & Brandolini, 2014).

Two methods are widely used for puffing: (1) oven-puffing, which relies on the sudden application of heat at atmospheric pressure to a pre-wetted cereal; the internal water is quickly vaporized, expanding (puffing) the product; (2) gun-puffing, which consists of the sudden transfer of the kernel containing super-heated water to a low-pressure chamber, thus allowing the instantaneous vaporization of the water. Physical, structural and chemical modifications occur during the puffing process. Puffed grains undergo dehydration, starch gelatinization, increase of volume and textural changes (Hoke et al., 2007). Nonetheless, few studies are available in literature about puffing and its effects on kernel characteristics. Mariotti, Alamprese, Pagani, and Lucisano (2006) studied the effect of puffing on ultrastructure and physical characteristics of bread wheat, emmer wheat, rye, barley, rice and buckwheat seeds. Other Authors (Rufián-Henares, Delgado-Andrade, & Morales, 2006a) evaluated the heat damage in breakfast cereals and the

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relationship with their physical form (puffed or flaked). Hoke et al. (2007) optimized the puffing of naked barley, considering volume expansion as the main parameter, while Castro-Giráldez, Fito, Prieto, Andrés, and Fito (2012) studied the puffing process of amaranth seed by dielectric spectroscopy. Lee and Lee (2009) investigated the influence of soybean puffing on isoflavone distribution.

The processing steps can influence the interactions among kernel components and processing conditions (pH, temperature and a_w), promoting the occurrence of both Maillard reaction (MR) and caramelization, which are largely responsible for the flavor and color of puffed products. Nevertheless, the progress of MR can lead to formation of advanced glycation end-products (AGEs), loss of nutritional properties and production of molecules with pathogenic significance in some chronic diseases (Uribarri et al., 2010). Among the newly formed molecules some MR products (MRP) are classical indicators of the thermal treatment, adopted as target molecules for different cereal products (Rufián-Henares, Delgado-Andrade, & Morales, 2006b). Traditionally the extent of the early MR stages in food is evaluated through furosine (FUR) determination. A survey realized in Spain on breakfast cereals of different physical form evidenced in puffed cereals levels of FUR that doubled those of flaked products and attributed this difference to higher thermal input during processing (Rufián-Henares et al., 2006b; Delgado-Andrade, Rufián-Henares, & Morales, 2007).

Some intermediates of MR, like glucosylisomaltol (GLI) and hydroxymethylfurfural (HMF) (this last being also formed by caramelization at high temperatures) have also been investigated in breakfast cereals (Rufián-Henares et al., 2006a). In the initial stage of MR colorless compounds are formed, while in the successive steps molecules characterized by chromophore moieties can be globally monitored by fluorescence or UV–Vis measurements. Traditionally to assess the extent of MR in foods color measurement at 420 nm is adopted (Martins, Jongen, & van Boekel, 2001).

Starch digestibility in unprocessed grains depends on intrinsic characteristics such as dimension of starch granules, degree of crystallinity and amylose to amylopectin ratio. The modifications induced by processing deeply affect starch susceptibility to degradation by digestive amylases (Alsaffar, 2010). Although *in vivo* studies provide the optimal solution for evaluating the fate of food components upon digestion, the *in vitro* approach represents an easier solution to get information about macronutrients digestibility.

Aims of this research were to study the evolution of several indices of heat damage in puffed wheat samples, and to assess starch degradation and release of selected AGEs during *in vitro* static digestion. The antioxidant capacity of digested products was also evaluated.

2. Materials and methods

2.1. Samples

As a preliminary survey, the heat damage was evaluated in commercial samples of seven puffed cereals (bread wheat, spelt, Kamut®, barley, rice, millet and buckwheat) and one puffed-einkorn *galletta* (water biscuit) from the market. No other ingredients were present in these products.

Two einkorn accessions (cv. Monlis and Monarca) and two bread wheats (cv. Blasco and Bramante) were cropped in 2010–2011 at Sant'Angelo Lodigiano (Po Plain, Lodi, Italy) in 10 m^2 plots arranged in a Randomized Complete Block Design with three replications, following standard cultural practices. After cropping, the kernels from the three replications were put together and kept at 5 °C until processing. The seeds of Monlis and Monarca were de-hulled with an Otake FC4S thresher (Satake, Japan) before puffing.

2.2. Puffing treatments

The kernels were treated in a lab gun-puffing system of a private food industry under two different processing conditions: (a) standard treatment: the seeds were fed into a cooker, heated to 380 °C and held at that temperature for 4 min. Puffing took place in an expansion chamber where the precooked grains came into contact with steam injected under pressure (8.5 bar) for 2.5 min; (b) optimal treatment: the cooking was performed at a temperature between 360 and 390 °C, depending on the genotype, and held at that temperature for 3–5 min while puffing was done under steam pressure between 7 and 10 bar for 1–4 min. This treatment was considered "optimal" because led to the maximum expansion.

2.3. Physical and chemical determinations

The 1000 kernels' weight of untreated seed was determined as described by Brandolini, Hidalgo, and Moscaritolo (2008). Immediately before the analyses, untreated seeds were ground to whole meal flours with a Cyclotec 1093 lab mill (FOSS Tecator, Hillerød, Denmark), while puffed seeds were ground using a Waring Commercial Model 24cb9ec (Waring Commercial, Torrington, CT, USA) at minimum grinding speed (16,800 RPM) for 20 s. The whole meal flours were kept under vacuum at −20 °C until analysis. Dry matter (DM) content was determined as described by Hidalgo and Brandolini (2008). Water activity was evaluated using the Aqua Lab model Series 3TE instrument (Decagon Devices, Pullman, WA, USA). Fructose, glucose, and maltose were analyzed by HPLC as reported by Resmini, Pagani, Pellegrino, and De Noni (1993). Total starch content was determined with a specific Megazyme assay kit (Megazyme International Ireland, Bray, Ireland).

2.4. Determination of heat damage indices

FUR was determined by HPLC according to the conditions described in Standard ISO 18,329:IDF 193 (ISO-IDF, 2004), as detailed in Hidalgo and Brandolini (2011b): 400 mg of sample were hydrolised with 8 mL of 8 N HCl under nitrogen at 110 °C for 23 h and purified by solid-phase extraction (SPE) with a C18 cartridge (Sep-pak, Millipore, Ballerica, MA, USA) and injected in a HPLC apparatus consisting of two 510 HPLC pumps, a 680 automated gradient controller, and a 490 programmable multiwavelength detector (Millipore Waters, Milford, MA). Furosine was quantified using furosine dihydrochloride (NeoMPS, PolyPeptide Laboratories, Strasbourg, France) as external standard. The results are expressed as milligrams of furosine/100 g of protein. For HMF and GLI determination, the samples were prepared as proposed by Rufián-Henares et al. (2006a), and the HPLC analysis was performed as described by Hidalgo and Brandolini (2011a). The supernatant from the extraction of 0.5 g sample was filtered and injected in a Elite LaChrom HPLC system equipped with a L-2130 pump, a L-2300 column oven, a L2450 Diode Array Detector (VWR, Hitachi, Japan) controlled by the software EZChrom Client/Server version 3.1.7. The compounds were separated with a 5 μm, 4.6 mm × 250 mm Prevail C18 column (Grace Davison Discovery Sciences, Deerfield, IL, USA) and quantified using HMF and furfural (Safc, St. Louis, MO, USA) as external standards. GLI quantification was computed considering the response factor of HMF at 280 nm. The results are expressed as mg/kg DM. Pyrraline (PYR) and formyline were measured according to the method of Resmini and Pellegrino (1994). Briefly, 400 mg of ground sample were enzymatically hydrolyzed, purified by SPE on a C18 cartridge (Sep-pak, Millipore, Ballerica, MA, USA) and submitted to HPLC determination by an Alliance 2695 apparatus (Waters, Milford, MA, USA) equipped with a 996-diode array detector (Waters) and

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