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Lipoxygenase in durum wheat: What is the role in pasta colour?

G.M. Borrelli*, A. Troccoli*, C. Fares*, D. Trono*, A.M. De Leonardis*, L. Padalino*, D. Pastore, L. Del Giudice*** and N. Di Fonzo***

*Istituto Sperimentale per la Cerealicoltura, Sezione Operativa di Foggia, S.S. 16 km 675, 71100 Foggia, Italy

**Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Facoltà di Agraria, Università del Molise, Via De Sanctis, 86100 Campobasso, Italy

***Istituto Internazionale di Genetica e Biofisica, CNR, Via G. Marconi 12, I-80125 Napoli, Italy

SUMMARY – Bright yellow colour of durum wheat products is the result of natural carotenoid pigment content and of their oxidative degradation by lipoxygenase (LOX) activity. Such a colour depends on several factors among which intrinsic quality of semolina and processing conditions are considered to be the most important. Although pigment content and LOX level are mainly varietal characteristics, they can be affected by environmental factors. In particular, variations of transcriptional level of LOX gene/s were observed in different cultivars. The phase mainly responsible for pigment loss is pasta processing when a substantial decrease in pigment content with respect to milling occurs. The extent of this decrease was found to be highly correlated with semolina LOX activity. Therefore, a reduction of LOX activity during pasta processing is of interest for technological, nutritional, and commercial purposes. To this regard, it is shown that β-carotene as well as the antioxidant α-tocopherol and L-ascorbate, inhibit semolina LOX activity. Thus, it suggests the use of these inhibitors in order to prevent the semolina bleaching and to improve pasta quality. The analysis at the molecular level of the interactions between carotenoid pigments and LOX enzymes may be helpful to assist durum wheat breeding programs in the selection of cultivars either with low LOX level and/or high antioxidant content for superior manufacturing pasta products.

Key words: Durum wheat, lipoxygenase, carotenoid pigments, pasta colour, inhibitors.

RESUME – *"La lipoxygénase chez le blé dur : Quel rôle dans la couleur des pâtes". La couleur jaune ambré de produits dérivés de blé dur est le résultat du contenu en pigments caroténoïdes et de leur oxydation par la lipoxygénase (LOX). Celle-ci dépend de plusieurs facteurs parmi lesquels la qualité intrinsèque de la semoule et le processus de pastification sont très importants. Le contenu en pigments et le niveau de la LOX sont considérés comme caractéristiques variétales, bien qu'ils soient influencés par les facteurs abiotiques. Des variations du niveau transcriptionnel des gène(s) LOX sont observées sur différentes variétés. La pastification est principalement responsable de la dépigmentation. Ceci est démontré par la diminution du contenu en pigments en comparaison avec la mouture. Le taux de cette diminution est corrélé positivement avec l'activité de la LOX de la semoule. La réduction de cette activité est donc intéressante pour les finalités et les besoins technologiques, nutritionnels et commerciaux. Par contre, les anti-oxydants β-carotène et α-tocophérol et l'acide ascorbique inhibent l'activité de la LOX. Par conséquent, l'emploi de ces inhibiteurs peut prévenir le blanchissement de la semoule et améliorer la qualité de la pâte. L'analyse moléculaire de l'interaction des pigments caroténoïdes et la LOX pourraît être utile pour les programmes d'amélioration génétique du blé dur afin de sélectioner les variétés avec des niveaux bas de LOX et/ou des contenus hauts en composants anti-oxydants pour la production des pâtes de qualité supérieure.*

Mots-clés : *Blé dur, lipoxygénase, pigments caroténoïdes, couleur de la pâte, inhibiteurs.*

Introduction

Pasta is by far the more appreciated durum wheat product in the world. Pasta made from durum wheat cultivars of superior quality results in a bright yellow colour and it retains, after cooking, firmness and absence of stickiness. Bright yellow colour of pasta is an important prerequisite for customers. This colour is the result of a lot of parameters such as: (i) the natural carotenoid pigments present in the seeds; (ii) their residual content after milling and after the storage of either grain or semolina; (iii) their oxidative degradation by lipoxygenase (LOX) during pasta processing; (iv) the oxidative balance among different compounds implied in this reaction; and (v) the processing conditions. These complicated interactions must be considered when programs to select cultivars with higher colour are carried out.

Biochemical aspect

The LOXs are non-heme iron containing dioxygenase that catalyse the oxidation of polyunsaturated fatty acids containing a *cis,cis*-1,4-pentadiene system, producing conjugate *cis,trans*-diene hydroperoxides (Siedow, 1991). Radicals produced during the intermediate steps of this reaction are responsible for oxidative degradation of carotenoid pigments. A recent study of durum wheat LOX purification (Barone *et al.*, 1999) has revealed a monomeric structure of about 95 kDa molecular mass, whit a high affinity for linoleic acid as a substrate and an optimal pH for hydroperoxidation and bleaching activities near to dough pH. Thus, good conditions for pigment degradation during pasta processing are produced. In other studies, the existence of three (Hsieh and McDonald, 1984) or at least two (Pastore *et al.,* 1999) semolina LOX isoforms was shown. The β-carotene bleaching reaction occurred only in presence of linoleic acid, confirming the cooxidative nature of enzymatic pigment degradation.

Carotenoids are important antioxidant compounds that reduce the oxidative damage to biological membranes implied in many human diseases and in the ageing processes. The carotenoid content is one of the main criteria to assess the commercial and nutritional value of pasta products. Therefore, the prevention of their degradation during pasta making is a necessary goal to attain. It is known that many compounds which act as physiological antioxidants, such as the β-carotene (Lomnitski *et al.*, 1993), in addition to the α-tocopherol and the L-ascorbate (Packer, 1992; Buettner, 1993), are able to inhibit the LOX from various systems. Similarly, it has been demonstrated that β-carotene (Trono *et al.*, 1999), α-tocopherol (Pastore *et al.*, 1999) and L-ascorbate (McDonald, 1979; Pastore *et al.*, 1999) inhibit both the linoleate hydroperoxidation and the β-carotene bleaching in durum wheat semolina. Particularly, it is shown that externally added β-carotene can act as inhibitor of the LOX-catalysed linoleate hydroperoxidation and an inverse relation between the % carotenoid loss and the initial carotenoid content in semolina from durum varieties showing similar LOX activity (Trono *et al.*, 1999) was found. So, an increase of the amount of β-carotene, α-tocopherol and L-ascorbate compounds by external addition or breeding could be helpful to reduce pigment loss during pasta processing and to improve pasta quality.

Genetical and molecular aspects

LOX and β-carotene levels in durum wheat are cultivar-related and depend on the environmental conditions (Borrelli *et al.,* 1999). Molecular analysis of durum wheat LOX expression has demonstrated that genotypic variation is due to different transcriptional levels of the gene(s). In fact, Manna *et al.* (1998) isolated a genomic segment of the LOX gene and used it as a probe in order to measure transcriptional levels of LOX gene in the etiolated shoots of different durum wheat cultivars. The different values obtained confirm the genotypic nature of this character.

Technological aspect

The concurrent polyunsaturated fatty acids and pigment oxidation by LOX during durum wheat storage or processing is a complex phenomenon where the availability of the substrates and the balance between antioxidative and prooxidative factors play an important role in determining the quality of final products. Furthermore, the milling determines a mechanical loss of pigments that are mainly located in the more external layer of kernel, in a lower quantity respect to pasta processing in which the maximal pigment degradation by LOX activity occurs (Borrelli *et al.*, 1999). The reaction rate of LOX is particularly high at the beginning of dough mixing when its primary substrates, oxygen and lipid, are present in the highest amounts, and the mixing itself helps their contact and enhances the incorporation of oxygen in the dough (Delcros *et al.*, 1998). The involvement of LOX in colour loss is demonstrated by positive correlation between the decrease of β-carotene content after pastification and LOX activities in semolina. In addition to this, the hydroperoxidation and bleaching activities of LOX are highly correlated demonstrating that the bleaching might be ascribable to a co-oxidative action by LOX. However, it should be reported that the loss of yellow index, another measure of the colour, is highly correlated with the β-carotene loss but not with the LOX activities. This can be explained by the fact that the physical expression of colour is also affected by factors different from LOX activity, such as peroxidase and polyphenoloxidase activities, as well as ash content, that are involved in brownness of products and increase the colour loss after pastification (Taha and Sagi, 1987).

Future perspective

Future work points towards the reduction of LOX levels in order to moderate its negative effect on durum product quality. This can be obtained either by setting up breeding programmes targeted to this objective or by innovative technology. The induction of lines lacking of one or more LOX isoenzymes which can be employed in breeding programs could be one of these as well as the use of transgenic durum wheat lines in which the LOX activity is down-regulated by antisense or co-suppression.

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