Pasta Brownness. A Reappraisal of its Basis and Prediction in Durum Wheat Breeding (*)

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Pasta products have been known in Mediterranean countries for many centuries and are now enjoyed everywhere. Cooking quality and appearance are the two most important factors in assessing pasta quality.

Brownness, Brightness, Yellowness and Redness of Pasta

The appearance of pasta is determined by three groups of parameters: color, specks (brown specks from the grain pericarp, black point) and surface texture (checking, smoothness, white spots, streaks, air bubbles). Pasta color results from a desirable yellow component (bt, an undesirable brown component (100-L) and, under some drying conditions, a red component (a) resulting from Maillard reactions.

In contrast to the yellow component of the pasta color, which has been well explained through carotenoid content and lipoxygenase activity, the brown component remains poorly known as its physico-chemical bases are still controversial; it is therefore difficult to take it into account in durum wheat breeding.

Color appearance of a spaghetti

To the observer, the color appearance of a spaghetti (curve A, with dominant wavelength (DWL) at 576 nm ± 2 nm) is the result of the interactions between the curve B of eye visibility (i.e. the relative luminance of spectra colors emitted at an identical level of energy) and the spaghetti reflectance (curve C). The human judgement is based on this interaction.

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A slight indentation can be observed at 480 nm, which corresponds to an absorption by carotenoids, that is clearly related to pasta yellowness. In contrast, the study of brownness (or more exactly greyness, or its counterpart brightness) is more complicated because brownness is not linked to any specific peak of the curve, as it correspond to a global flattening of the pasta reflectance curve.

Curve A differentiates from curve B by two main characteristics:
- a slight indentation at 480 nm, not far from a characteristic absorption peak of carotenoid. This indentation is related to the spaghetti yellowness.
- a global flattening of curve B, which is easily determined by the spaghetti reflectance at 550 nm. As important is the flattening, and low the reflectance at 550 nm, as low is the brightness of the sample and high the «greyness». In pasta industry, and because addition of greyness to yellowness gives brownness (in this case, DWL becomes greater than 578 nm), it is usual to refer to brownness (100-L), or its complement to 100 (L), brightness rather than to greyness.
- In fact, pasta brownness (or pasta brightness, its more positive counterpart attribute) is an indicator of the overall attenuation of the light reflected by spaghetti samples when illuminated by sunlight or overcast daylight. At the opposite of yellowness, which is related to the amount of specific pigments and corresponds to a well identified modification of the spaghetti reflectance curve, brownness is not linked to any specific change in this curve. That is one of the reasons why its study is more complicated.

**Effect of industrial process on pasta color**

Since the development of new technologies of pasta drying, it has been suggested that pasta brownness could become a limiting factor in the application of very high temperatures. And this concern (which was raised at least among European industries) resulted in a strong request of the industries toward research on the origin of brownness and on how to control it by both industrial processes and genetics, including molecular biology studies of oxidizing enzymes.

In fact, a survey of the literature of the last 50 years showed that the effect of processes on pasta browning was less significant than expected, except that of milling conditions and extraction rate.

In this talk, we will therefore go back on the real effect of processing on pasta color, and then explain our new strategy of research aimed at better understanding and control of pasta color.

**Effect of Pasta-making Processes**

For instance, during semolina hydration, kneading and extrusion, although semolina components undergo several modifications, mainly oxidations, which might contribute to the final color of pasta, most authors (Feillet, Abecassis, Medvedev, Debbouz,...) found that experimental parameters (hydration, temperature, shearing) have (contrary to frequent accepted ideas) little influence of pasta color (whereas they did affect its cooking quality).
Effect of Drying Conditions on Pasta Redness and Brownness

It was also reported in early studies that spaghetti dried at high temperature showed a tendency to «browning». In fact, even at high drying temperature, the brown index does not significantly increase. It is only under extreme conditions that colorimetric measurements of brownness dramatically increase. However, it must be considered that under such conditions (which are not applied in practice, as red indices over 6 or 8 correspond to pasta that are no longer marketable), there is a sharp increase of the redness hue, that does interfere with both expert panel’s judgements and readings of photometers. We state that the factors which are involved in the intensification of the Maillard reaction have been sometimes misunderstood as contributing to the brownness of spaghetti while they are independent parameters.

We therefore incline towards a non significant effect of drying temperatures on brown index in the range of conditions presently used by the pasta-making industry.

Effect of Milling Conditions

Milling yields and milling conditions (tempering, break system, efficiency of purifiers, extraction rate) are well known to have marked effects on semolina and pasta. Brown color of pasta rises sharply with ash content of semolina streams. Here is the Matsuo and Dexter’s equation.

The main industrial concern comes from brownness inherent to the semolina (purity of semolina), that is essentially dependent of milling, but there should be only little concern from the pasta-making process itself.

Brownness and Yellowness of Durum Wheat Milling Streams

Semolina ash and extraction rate have a pronounced effect on spaghetti brightness and dominant wavelength: reflectance measurements show a progressive tendency toward greater brownness in the spaghetti as extraction rate increases due to increasing amount of non-endosperm material in the semolina; at high extraction, spaghetti becomes browner and duller.

Correlation Between Semolina and Pasta Brownness in Durum Progenies

This assumption of a non significant effect of the pasta process itself is strengthened by the significant correlations found between the values of brown index respectively measured on:
- processed and dried spaghetti,
- on pasta disks, either fresh or dried
- on disks of compacted semolina (without any hydration and dough development).
Brightness of Endosperm (BE) and Brownness Resulting from Extraction Rate of Semolina (BRER)

Accordingly, the facts bring us to confirm a former hypothesis of Irvine (1971) who distinguished between two types of brownness:
- inherent brownness of the endosperm
- brownness resulting from high extraction rates

And we suggest the following equation:

Pasta brightness (a more positive counterpart than brownness) is a function of brightness of endosperm, BE, minus brownness related to semolina extraction rate, BRER.

And we will discuss in our second part the factors likely to influence the values of BE and BRER.

Physico-chemical Basis of Brownness

In the literature, pasta brownness has been attributed to:
- enzymatic reactions (catalysed by peroxidases? or polyphenol oxidases?)
- and/or specific grey or brown naturally colored molecules present in the endosperm or in the outer layers of the kernel.

Peroxidases

Peroxidases are known to catalyse the oxidation of hydrogen donors, including a large number of phenols of plant tissues, at the expense of hydrogen peroxide.

Already in the 1970's, our research group found a close relation between pasta brown index and peroxidase activity, which allowed to suggest breeding of durum wheat cultivars on the basis of low peroxidase level in semolina.

Role of peroxidases in pasta brownness?

This appears from both relation between Brown Index / PO activity and PAGE patterns.
Two widely different levels of activity correspond to two main types of patterns.
For instance PO activity was found 10 times higher in Bidi types cvs. than in Lakota type cvs.

When brownness became a concern among pasta makers (THT) the new researches on pasta brownness were therefore stimulated, but on the basis of these old peroxidases results obtained from a collection of cultivars belonging in fact to either the North-African genetic background, or the North-American one.
**Peroxidase Activity and Pasta Brownness: New Results (Feillet et al., 1999)**

Recent results, however, did not confirm the above-mentioned correlations. When we analysed a set of recently registered European cultivars (90 samples consisting of 15 cultivars grown in 6 locations), the relation between peroxidase activity and pasta brownness was found very poor ($r = 0.37$).

In fact there are still two very different families of durum cultivars, one (A) with low PO activities (between 100 and 500 units - $r$ within this subgroup, 0.6), and a second one (B), with very high PO activities (from 1000 to 4000 - $r$ within this subgroup 0.4).

But there is a discrepancy with results obtained in the 1970s results as, today, both classes contain cultivars ranging from a high bright color to a poor brown color.

Possibly, former conclusions were valid because analyses were carried out on the only genetic backgrounds available, either North-African or North-American, and possibly (as suggested by Jim Kruger) this genetic linkage, has been broken by recent breeding.

**Against Role of Peroxidases**

In fact, several arguments go against a role of peroxidases in pasta brownness:
- In pasta, the formation of hydrogen peroxide, the primary substrate of PO, is questionable
- PO activity is essentially dependent of genetic factors, whereas brownness is largely influenced by environnement
- No or very weak effect of processing conditions of pasta (time or temperatureof resting, mixing and drying) (which would be expected if browning resulted from classical oxidative reactions)
- The above-mentioned high correlation between brownness respectively measured on semolina and on processed pasta.

**Polyphenoloxidases**

Polyphenol oxidases catalyse the oxidation of phenolic compounds in the absence of molecular oxygen. They occur widely in plants and cause enzymatic browning in food material through an initial oxidation of phenols into quinones, which readily undergo condensation with amino acids or proteins via their amino groups to form brown polymers.

In soft wheats, they cause discoloration of chappaties and Oriental noodles. They might also cause formation of brown components in pasta through partial oxidation of flavones.

PPO appears early during kernel growth and could attack phenolic compounds that are abundant in immature kernel.
However...

However, PPO activities are very low in semolina, and so far they did not allow discrimination according to pasta brownness.

Also, no correlation was found between PPO isozyme composition and brown index.

Also, PPO activity depends upon the way the wheat is milled and might be involved in brownness related to extraction rate.

Brown-Colored Component?

Matsuo and Irvine (1967) and Feillet (1971) investigated the water extracts of semolina and found a highly significant correlation between pasta brownness and absorption at 400 nm of an aqueous extract of semolina.

The component responsible for the brownish color is a copper protein. The reaction of this protein with a reducing agent in presence of copper was claimed to be responsible for inherent brownness.

In the literature, the amino acid compositions of Matsuo’s Brown Protein (MBP) and wheat o-diphenolase are extremely similar.

Summary

Today, I think that we have no possibility to conclude in favour of one or the other explanation of pasta brownness. Instead, I will suggest a summary of the points on which there seems to be an agreement, and then some new working hypotheses for future investigations.

- Pasta brownness must be distinguished from redness (the product of Maillard reaction) has been attributed to enzymatic reactions, bran contamination, and to a brown-colored endosperm molecule.
- The variation in pasta brownness can be attributed mainly to semolina properties and to milling conditions (the effect of mixing, extrusion and drying being insignificant, at least with well purified semolina).
- There are two types of brownness: inherent brownness of the endosperm and brownness resulting from high extraction rates, the latter being likely to be due to enzyme actions, at least partially. $PB = f [EB - BER]$
- Endosperm brownness, a varietal characteristic, mainly depends on the conditions of grain development: it increases when protein content (and mineral content) increase.
New Working Hypotheses for Inherent Endosperm Brownness (EB)

Inherent endosperm Brownness would be related to the amount of a "brown pigment" synthesized in the endosperm during maturation. According to us, this pigment might be either phenolic compounds oxidised by polyphenol oxidases (PPO) during maturation and/or the Matsuo's brown protein, of which it would have to be checked that it is or not a complex formed during grain maturation between PPO and their substrates; in these conditions, endosperm PPO would no longer be in an active form (but the level of brownish color would be already determined).

The main arguments in favour of this hypothesis are the following:

Main Arguments

- PPO activities in endosperm are substantial during grain maturation before to decrease to near zero in the mature endosperm;
- grain maturation is accompanied by a marked decline in phenolics and flavanols, presumably due to the breakdown of cellular structure which would allow oxidizing reactions to take place (McCallum and Walker, 1995);
- the amino-acid composition of wheat PPO (Interesse et al., 1983) and the Matsuo's protein are almost identical;
- bran pigmentation was considered to be produced by the action of o-diphenolase on flavanols (Gordon, 1979): a similar phenomena could occur in the endosperm during grain maturation.

Further Studies

In further studies, it would be thus interesting:
1) to confirm the existence of relationships between semolina brownness and Mbp content of semolina (endosperm);
2) to explain why an increase in protein content (and in mineral matters) of the grains results in a reduction in brightness;
3) to follow the evolution of PPO activity and Mbp content during grain maturation (to check for a possible genetic variation, at early stages - as we know that there is no variation in mature endosperm) between browning and not browning varieties;
4) to better know the physicochemical properties of Mbp and to specify the varietal and environmental effects on Mbp content of semolina.

If the role of Mbp was not confirmed, the origin of the inherent endosperm brownness should still to be identified.

Pasta Brownness Related to Extraction Rate (PBER)

Pasta brownness related to the rate of extraction (PBER) increases with the extraction rate of semolina. It might be related to the natural brown colour of kernel peripheral tissues (brans) and to semolina PPO activity, which
increases as semolina are contaminated by rich in PPO histological layers of the grain (mainly the aleurone layer).

With identical extraction rate, semolina PPO activity, and thus PBRE, would depend on the amount of PPO in the grain (which is a varietal characteristic influenced by the growing conditions); on the distribution of the kernel PPO activity amongst the various histological areas of the grain (it could be a genetic characteristic); and on the easiness to dissociate and separate the various histological outer layers of the grain from the endosperm during milling, mainly the aleurone layer. Brownness would also depends on the colour, amount and size of contaminant bran particles.

PBRE which results from an enzymatic activity should depend on the pasta manufacturing conditions (i.e. evolution of water activity and temperature values in the products; but also the pH and the oxygen concentration). Differences in PBRE values could be also explained by differences in semolina phenolic compounds composition.

In our knowledge, there are no experimental data to support this assumption and the statement by McCallum and Walker (1995) that common wheat grains contain relatively low levels of oxidable phenolics and that few, in any, o-diphenols are present deserves consideration.

It would be thus interesting a) to specify the relations between PBRE values and PPO activities of the semolina at variable extraction rate; b) to specify the evolution of PPO activity of the milling streams according to their histological origin and to determine the genetic and environmental effects on this distribution; c) to examine the influence of the conditions of pasta manufacturing on PBRE values at different level of PPO activity.

A message to breeders...

To improve the pasta brightness, the breeder's contribution would be primarily to decrease the intrinsic semolina brownness and to break the relation between brownness and protein content (aiming to produce durum wheat varieties with high colour and high cooking quality scores); and as a second aim, to decrease the polyphenol oxidase activity of the kernel peripheral part (aleurone and branny layer) and the intensity of bran colour to avoid pasta discoloration when the semolina purification is insufficient. The paucity of knowledge on wheat phenolic composition and the possible greater variability in o-diphenolase than in phenolic content (McCallum and Walker, 1995) make more difficult any breeding work aiming to modify usefully the phenolic composition of durum wheat endosperm.

...and to industrials

Millers should avoid bran and aleurone layer contamination of semolina by optimal setting of the mill machineries, mainly purifiers.

Pasta manufacturers have no real possibilities to improve the brightness of their product by selecting specific extrusion or drying processes parameters; nevertheless, when processing poorly purified and rich in polyphenol oxidases semolina, they have to carefully select the best drying, and possibly pasta forming, conditions to avoid pasta discoloration. Further studies are necessary to define these conditions.
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Brownness, Brightness, Yellowness and Redness of Pasta

- Appearance of pasta: color, specks, surface texture
- Color: desirable yellow component and undesirable brown component
- In contrast to the yellow component that is well explained and well controlled by breeders, the origin of the brown component is controversial
- Red component, under certain drying conditions
**Color Appearance of a Spaghetti**

Physiological response to spaghetti reflectance (from Alause and Feillet, 1970)

**Effect of Industrial Process on Pasta Color**

- Pasta brownness, a limiting factor in the application of new technologies of pasta drying?
- New impetus of research on the origin of brownness and on how to control it by both industrial processes and genetics.
- In fact, the effect of processes on pasta browning was less significant than expected, except that of milling conditions.
Effect of Pasta-making Processes

✦ During semolina hydration, kneading and extrusion, semolina components undergo several modifications, mainly oxidations, which might contribute to the final color of pasta.

✦ However, experimental parameters (hydration, temperature, shearing) have little influence of pasta color (whereas they did affect its cooking quality)

Effect of Drying Conditions on Pasta Redness and Brownness

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>70</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td>30</td>
<td>60</td>
<td>120</td>
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<tr>
<td>Red index</td>
<td>6.1</td>
<td>6.5</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>6.6</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>10.1</td>
<td>21.2</td>
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<tr>
<td>Brown index</td>
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<td>33.5</td>
<td>32.6</td>
</tr>
<tr>
<td></td>
<td>32.1</td>
<td>32.5</td>
<td>34.3</td>
</tr>
<tr>
<td></td>
<td>32.7</td>
<td>36.8</td>
<td>52.8</td>
</tr>
</tbody>
</table>

From Abecassis et al., 1989
**Effect of Milling Conditions**

- Milling yields and milling conditions (tempering, break system, efficiency of purifiers, extraction rate,...) have marked effects on semolina and pasta
- Brown color of pasta rises sharply with ash content of semolina streams
  
  \[ \text{Spaghetti brightness} = -19.55 \times \text{ash content} + 59.95 \]  
  (Matsuo and Dexter, 1980)
- Industrial concern chiefly comes from brownness inherent to the semolina

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**Correlation Between Semolina and Pasta Brownness in Durum Progenies**

<table>
<thead>
<tr>
<th>Cross</th>
<th>N</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ixos x Lloyd</td>
<td>43</td>
<td>0.874**</td>
</tr>
<tr>
<td>Néodur x Lloyd</td>
<td>41</td>
<td>0.843**</td>
</tr>
<tr>
<td>Néodur x Ixos</td>
<td>40</td>
<td>0.748**</td>
</tr>
<tr>
<td>All samples</td>
<td>124</td>
<td>0.837**</td>
</tr>
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</table>

Semolina disks

Spaghetti
Brownness and Yellowness of Durum Milling Streams

<table>
<thead>
<tr>
<th>Color</th>
<th>Brownness</th>
<th>Yellowness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety</td>
<td>Agathé</td>
<td>Durtal</td>
</tr>
<tr>
<td>S1 (central)</td>
<td>17.3</td>
<td>18.1</td>
</tr>
<tr>
<td>S2 (medium)</td>
<td>19.1</td>
<td>17.9</td>
</tr>
<tr>
<td>S3 (central)</td>
<td>16.8</td>
<td>17.0</td>
</tr>
<tr>
<td>S4 (medium)</td>
<td>18.3</td>
<td>17.4</td>
</tr>
<tr>
<td>S5 (peripheral)</td>
<td>25.5</td>
<td>22.5</td>
</tr>
<tr>
<td>S6 (peripheral)</td>
<td>24.9</td>
<td>22.5</td>
</tr>
<tr>
<td>SRF1 (1st roll)</td>
<td>25.7</td>
<td>21.2</td>
</tr>
<tr>
<td>SRF2 (2nd roll)</td>
<td>30.4</td>
<td>24.9</td>
</tr>
<tr>
<td>SRF3 (3rd roll)</td>
<td>26.7</td>
<td>21.5</td>
</tr>
<tr>
<td>SRF4 (4th roll)</td>
<td>34.3</td>
<td>28.8</td>
</tr>
</tbody>
</table>

(From Houliaropoulos et al., 1981)
Brightness of Endosperm (BE) and Brownness Resulting from Extraction Rate of Semolina (BRER)

- Results support the hypothesis of Irvine (1971) who distinguished between inherent brightness of endosperm and brownness resulting from high extraction rates
- Pasta Brightness = f (BE - BRER)
- Factors likely to influence the values of BE and BRER?

Physico-chemical Basis of Brownness

- Pasta brownness may result from:
  - enzymatic reactions (peroxidases? or polyphenol oxidases?)
  - specific gray or brown naturally colored molecules present in the endosperm or in the outer layers of the kernel
**Peroxidases**

- Enzymes whose primary function is to oxidize hydrogen donors (e.g. a large number of polyphenols of plant tissues) at the expense of hydrogen peroxide.
- In the 1970's a relationship was found between brown index and PO activity, which allowed to suggest breeding of durum cultivars on the basis of peroxidase level.

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**Role of peroxidases in pasta brownness?**

**Relation**

Brown Index / peroxidase activity

**PAGE (pH 8.6)**

of peroxidase isozymes

Durum wheat varieties: B, Bidi 17; M, Montferrier; A, Agathé; La, Lakota; We, Wells; Le, Leeds (Kobrehel and Feillet, 1975).
Against Role of Peroxidases

In pasta, the formation of hydrogen peroxide, the primary substrate of PO, is questionable.

PO activity is mainly dependent of genetic factors, whereas brownness is largely influenced by environment.

No or very weak effect of drying conditions.

High correlation between semolina brownness and brownness measured on processed pasta.
Polyphenoloxidases?

- PPO occur widely in plants and cause enzymatic browning
- In soft wheat, they cause discoloration of chappaties and Oriental noodles (Kruger, 1994)
- PPO might cause formation of brown components in pasta (Menger 1969)
- PPO appears early during kernel growth and could attack phenolic compounds that are abundant in immature kernel (Kruger, 1976)

However...

- PPO activities are very low in semolina, and do not allow discrimination according to pasta brownness (Dexter, 1994)
- No correlation was found between PPO isozyme composition and brown index (Kruger, 1976)
- PPO activity also depends upon the way the wheat is milled
Brown-Colored Component?

- Pasta brownness was found highly correlated with absorption at 400 nm of aqueous extracts of semolina (Matsuo and Irvine, 1967; Feillet, 1971).
- Inherent brownness could result from the reaction of this protein with a reducing agent in presence of copper (Matsuo and Irvine, 1967)
- The amino acid compositions of Matsuo's Brown Protein (MBP) and wheat o-diphenolase (Interesse, 1983) are extremely similar.

Summary

- Pasta brownness (that must be distinguished from redness, the product of Maillard reaction) has been attributed to enzymatic reactions, bran contamination, and to a brown-colored endosperm molecule.
- The variation in pasta brownness can be attributed mainly to semolina properties and to milling conditions.
- There are two types of brownness: inherent brownness of the endosperm (EB) and brownness resulting from high extraction rates (BRER).
- EB, a varietal characteristic, mainly depends on the conditions of grain development: it increases when protein content (and mineral content) increase.
**New Working Hypotheses for Inherent Endosperm Brownness (EB)**

- EB would be related to the amount of a "brown pigment" synthesized in the endosperm during maturation:
  - either phenolic compounds oxidised by polyphenol oxidases (PPO) during maturation
  - and/or the Matsuo’s brown protein (MBP)

- It would have to be checked whether MBP might be or not a complex formed during grain maturation between PPO and their substrates
  - (In these conditions, PPO would no longer be in an active form in the mature endosperm, but the level of brownish color would be already determined).

**Main Arguments**

- PPO activities in endosperm are substantial during grain maturation before to decrease to near zero in the mature endosperm

- Grain maturation is accompanied by a marked decline in phenolics and flavanols, presumably due to the breakdown of cellular structure which would allow oxidizing reactions to take place
  - The amino-acid composition of wheat PPO and the Matsuo’s Brown Protein are almost identical
  - Bran pigmentation was considered to be produced by the action of o-diphenolase on flavanols (Gordon, 1979): a similar phenomena could occur in the endosperm during grain maturation
Further Studies

- Relationships between semolina brownness and MBP content of semolina (endosperm)?
- Why an increase in protein content (and in minerals) of the grains results in an increase in brownness (a reduction in brightness)?
- Evolution of PPO activity and MBP content during grain maturation (comparison between browning and not browning varieties)
- Physicochemical properties of MBP and their variety and environment dependence

Pasta Brownness Related to Extraction Rate (PBER)

- It would be interesting:
  - to determine the relations between PBRE and PPO activities of the semolina at variable extraction rates
  - to determine the evolution of PPO activity of the milling streams according to their histological origin and to specify the genetic and environmental effects on this distribution;
  - to examine the influence of the conditions of pasta manufacturing on PBRE values at different levels of PPO activity.
A Message to Breeders...

To improve pasta brightness, the breeder’s contribution would be:
- (i) to decrease the intrinsic semolina brownness and to break the relation between brownness and protein content
- (ii) to decrease the PPO activity of the peripheral parts of the kernel and the intensity of bran color to avoid pasta discoloration when the semolina purification is insufficient.
- (iii) in a longer term, to modify usefully the phenolic composition of durum wheat endosperm.

... and to Industrials

- Millers should avoid bran and aleurone layer contamination of semolina by optimal setting of the mill machineries, mainly purifiers.
- Pasta manufacturers have no real possibilities to improve brightness through specific extrusion or drying parameters.
- Nevertheless, when processing semolina poorly purified (i.e. rich in PPO), they have to select the best drying, and possibly pasta forming, conditions to avoid pasta discoloration. Further studies are necessary to specify such conditions.