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Development and characterization of a coloured sweet corn line as a new functional food

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Abstract

The standard sugary sweet corn (*Zea mays saccharata* Sturt.) is a maize variety grown for the fresh, frozen and canned markets, traditionally appreciated. Its kernels are characterized by the presence of some antioxidant substances suggested to be beneficial for cancer prevention. For this reason an interesting challenge for breeders is the development of sweet corn genotypes with naturally high antioxidant levels, starting from flavonoids. In fact important sources of antioxidants in maize are anthocyanins, considered as nutraceuticals because they have been proven to lower the risk of many chronic diseases.
In this paper we report the development of a new coloured sugary line and the results of some analyses concerning flavonoid content before and after two different cooking treatments are discussed. Attention was mainly focused on the anthocyanins, the molecules suggested as being responsible for the nutraceutical properties of the new coloured sugary line. The results show that the presence of the anthocyanins also pushes up the flavonol and the phenolic acid amounts and gives the new coloured sugary line a higher scavenging power compared to the uncoloured control. The mild cooking seems not to significantly change the metabolites analyzed in the coloured kernels, while the stronger treatment seems to drastically decrease the amounts of pigments, without changing the structure of the leftover molecules. All these findings suggest that the new colored sugary line can be considered a new functional food, able to introduce healthy compounds into the diet of many people.

Keywords: maize, *Sugary*, anthocyanins, *Purple plant1*, *Booster1*, functional food

Introduction

According to the data for the years 2012-2013, the corn global yield overtook 850 million metric tonnes, so that maize can now be considered as the most produced cereal in the world (USDA data). Corn is characterized by a high versatility: it is used for food, forage and for industrial purposes. In USA the amount of corn used as food is about 1.4 billion bushels (35.56 million metric tonnes), to produce high-fructose corn syrup, starch, corn oil and various other food products. (Brester, 2012; http://www.agmrc.org/commodities__products/grains__oilseeds/corn_grain/). Among the different varieties of maize used for different purposes an important one is sweet corn. Sweet corn (Zea mays saccharata Sturt) is a corn type grown for fresh, frozen and canned markets (Bülent Coşkun et al, 2006). In USA the fresh market accounts for nearly 70% of the total production of the sweet corn crop, and it is the second largest processing crop, surpassed only by tomatoes (Hansen R, content specialist, AgMRC, Iowa State University, Sweet corn profile http://www.agmrc.org/commodities__products/grains__oilseeds/corn_grain/sweet-corn-profile/; Haynes et al, Sweet Corn, Iowa State University Horticulture Guide). It differs from starchy field corn by a single recessive naturally-occurring genetic mutation causing a higher sugar content in the kernels. As a consequence sweet corn is harvested during the milk stage, before physiological maturation, approximately 15 to 23 days after the silks emerge, when it retains the highest amount of sugar and its maximum sweetness (Hansen R, content specialist, AgMRC, Iowa State University, Sweet corn profile http://www.agmrc.org/commodities__products/grains__oilseeds/corn_grain/sweet-corn-profile/).

There are three different mutations resulting in the three most widely diffused genetic varieties of sweet corn: *sugary* (*su*), *sugarenhanced* (*se*), and *shrunken-2* (*sh2*): they vary in sweetness, shelf life and cold soil vigour. The most diffused and ancient sweet corn variety is the sugary. This variety has a harvest, storage and shelf life slightly shorter than the others, the sugar content is not so high compared to *se* and *sh2*, but it is characterized by a flavour and a texture traditionally appreciated by consumers (Juvik et al, 2003).

The sweet corn kernels are characterized by a high starch and sugar content, important energy sources, by cellulose and β-glucan which are important dietary fibre for the enteric flora (Topping and

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Clifton 2001; Tokuji et al, 2009) and by the presence of zinc, an essential mineral to assure the functioning of many enzymes and transcription factors (Haase et al, 2008; Tokuji et al, 2009). Some antioxidant substances can also be found in sweet corn kernels, such as the β-carotene and the lutein carotenoids (Kurilich and Juvik, 1999; Tokuji et al, 2009) and above all the phenolic compound ferulic acid (Balasubashini et al, 2003; Tokuji et al, 2009). This molecule seems to be very important for health, in fact Tokuji and colleagues collected data indicating that this compound found in dietary sweet corn can be beneficial for cancer prevention (Tokuji et al, 2009). The antioxidant power seems to be the mechanism through which the molecules carry out their preventive function against human chronic diseases (Virgili and Marino, 2008), and cancer. Therefore vegetable foods containing high levels of antioxidant compounds are now entering the human diet as essential constituents, endowed with the added value of the functional food. So developing sweet corn genotypes with naturally high antioxidant level could be an interesting challenge for breeders. Important sources of antioxidants in maize are the anthocyanins. Anthocyanins are a class of flavonoids: they are water-soluble glycosides of simple or acylated polyhydroxy and polymethoxy derivates of flavylium salts and they are responsible for the red, purple, and blue colours of many fruits, vegetables, and cereal kernels (Giusti and Wrolstad, 2003; Žilić et al, 2012). They are very important for human health because they have been proven in animal system to reduce the risk of death from heart disease (Rissanen et al, 2003; Tsuda, 2012), to be able to lower LDL-cholesterol levels (Castilla et al, 2008; Tsuda, 2012) and to fight obesity (Seymour et al, 2009; Titta et al, 2010; Peng et al, 2011; Tsuda, 2012) and diabetes (Tsuda, 2008; Prior et al, 2008; DeFuria et al, 2009; Tsuda, 2012), to improve visual function (Matsumoto et al, 2005; Iwasaki-Kurashige et al, 2006) and to prevent neurodegenerative diseases (Goyarzu et al, 2004; Lau et al, 2007; Shukitt-Hale et al, 2008; Tsuda, 2012).

Corn (*Zea mays* L) contains around 20 structural and regulatory genes that compose the anthocyanin biosynthetic pathway (Chandler et al, 1989; Dooner et al, 1991; Pilu et al, 2003). The regulatory genes concerned belong to two different multigene families: the class of bHLH transcription factors among which are the *r1/b1* genes, and the class of MYB transcription factors, among which are the *c1/pl1/p1* genes (Chandler et al, 1989; Dooner et al, 1991; Pilu et al, 2003). A member of each family must be present and active in the dominant form to activate anthocyanin structural gene expression. According to the combination of these alleles, the pigments will be synthesized in different plant tissues, for example the *B/Pl* genes combination confers purple colour to the pericarp (Chandler et al,1989; Bodeau and Walbot, 1992; Gaut, 2001; Pilu et al, 2003).

In this paper we describe how a new coloured sugary line has been developed and, together with an uncoloured control, was subjected to three different food processing treatments: raw, steam cooked and autoclaved. Some analyses concerning the quantitative and qualitative characterization of the main flavonoid molecules in the uncoloured and coloured samples are presented and the results obtained after the different cooking treatments are discussed. Attention has been centred on the anthocyanins, the molecules that are supposed to be responsible for the nutraceutical properties of the new coloured sugary line, which is therefore proposed as a new functional food.

Materials and Methods

Plant material

A backcrossing breeding scheme was used to develop a sugary maize line rich in anthocyanins, in the experimental field of the University of Milan located in Landriano (PV, Italy). The source of the anthocyanin biosynthesis regulatory genes was a tropical maize line carrying the homozygous form of the *Booster1* (*B1*) and *Purple Plant1* (*Pl1*) genes, that determine the pigmentation in the pericarp and in the plant. This line was crossed with a commercial yellow sugary line, used as the recurrent parent for 5 cycles of backcrossing. Then 3 cycles of self pollination were performed, selecting in each cycle, the plants with the highest content of anthocyanins by Marker Assisted Selection (MAS).

Molecular Marker assay

Two SSR molecular markers were used to select the coloured sugary plants: the nc009 SSR molecular marker (5'CGAAAGTCGATCGAGAGACC3'/5'CCTCTCTTCACCCCTTCCTT3'), that is part of the pl1 gene located on chromosome 6 and the bnlg1064 SSR molecular marker (5'CTGGTCCGAGATGATGGC3'/5'TCCATTTCTGCATCTGCAAC3') located next to the b1 gene on the short arm of chromosome 2 (http://www.maizeg db.org/ssr.php). After the DNA extraction from the leaves of parental (P1 and P2) and progenies' plants (Dellaporta et al, 1983), the Polymerase Chain Reactions (PCR) and gel running were performed as described in the SSR Methods Manual by MaizeGDB (http://www.maizegdb.org/documentation/maizemap/ssr_protocols.php).

Material Sampling

For the genotypes tested (sugary maize line rich in anthocyanins and his colourless control) in the 2012 field season about 300 plants were grown in adjacent rows, under the same agronomic conditions, in the experimental field of the University of Milan, Italy (45°18'N-9°15'E). These plants were selfed (using paper bags) and then harvested at the same time at the end of the season.

About 50 ears were shelled and the seeds obtained mixed to create a single bulk used for the analyses.

Seed treatments

With the aim to mimic the processing treatments used for the sweet corn already available at the supermarket, we decided to test the uncoloured control seeds and the new coloured ones raw and after 2 different cooking treatments (100 seeds each). The steam cooking method involved a mild cooking of 10 minutes during which no contact between the seeds and the boiling water occurred. Other seeds underwent an autoclave cycle, consisting of 20 minutes of a constant pressure of 1 atm and a constant temperature of 120°C. After these treatments the seeds were analysed as described below.

Metabolite quantification (Anthocyanins, flavonols and phenolic acids quantification)

A pool of 10 seeds per treatment – raw, steam cooked and autoclaved - and per line was used to extract flavonoid metabolites. The seeds were ground in a mortar with the extraction buffer (1% HCl, 95% ethanol) in the presence of quartz sand. A sequence of consecutive washing steps of 30 minutes were performed until the extraction buffer turned out to be transparent. Finally the collected supernatants underwent a centrifugation at 13,000 rpm for 30 minutes, and then were used to determine anthocyanins using a spectrophotometer at λ = 530 nm, flavonols at λ=350 nm and phenolic acids at λ = 280 nm.

The amount of anthocyanins was calculated as cyanidin-3-glucoside equivalents (molar extinction coefficient (e) 26,900 L m^{-1} mol^{-1}, MW 449.2), flavonoids as quercitin-3-glucoside equivalents (molar extinction coefficient (e) 21,877 L m^{-1} mol^{-1}, MW 464.38) and phenolic compounds as ferulic acid equivalents (molar extinction coefficient (e) 14,700 L m^{-1} mol^{-1}, MW 194.18) for 100 g of seed weight.

The analyses were conducted on four seeds bulk (10 seeds each) randomly selected for each type. The confidence interval (CI) at 95% was calculated.

Qualitative determination of anthocyanins: TLC

The pericarp layer of 2 kernels per treatment of coloured and uncoloured lines were excised and boiled at 100°C with 2 ml of 2N HCl for 40 minutes. After adding 1 ml of isoamyl alcohol, the upper phase was dried and dissolved in EtOH 95% and HCl 1%. The standards of cyanidin, pelargonidin and delphinidin were loaded on a pre-coated TLC (Thin Layer Chromatography) plate (POLYGRAM CEL 300, Macherey-Nagel) together with the samples to be tested. The solvent used for the TLC running was formic acid:HCl:water 5:2:3. The developed plates were pictured with a digital camera (A430 Canon) using both white and UV illumination.

Antiradical ability assay

The free radical-scavenging activity was tested using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Brand-Williams et al, 1995; Cevallos-Casals and Cisneros-Zevallos, 2003; Leong and Shui, 2002; Hu et al, 2004; Yang and Zhai, 2010). Five coloured sugary seeds per each treatment were excised from the pericarp; the same procedure was also followed for the uncoloured untreated seeds. The pool of 5 pericarps for each treatment was ground with liquid nitrogen. An adequate aliquot was extracted with acetone 70% (acetone:water 70:30 v/v) according to the ratio 1:8 (w/v) for 3 hours. Then the samples were centrifuged for 10 minutes at 13,000 rpm and the colored extracts were equalized with a dilution based on the anthocyanin content of each treatment.

Then a 0.12 mM DPPH ethanolic solution was added to increasing aliquots of each sample extract, conveniently diluted. The final volumes of 2.5 ml of these preparations were left 1 hour in the dark at room temperature before the discoloration absorbance was spectrophotometrically recorded at 516 nm. The percentage of the scavenged DPPH was calculated as: % DPPH = (Ac–As) × 100 / Ac

where Ac is the absorbance of the control, and As is the absorbance of each increasing aliquot of the sample (Leong and Shui, 2002; Hu et al, 2004; Yang and Zhai, 2010). Finally the amounts of the increasing aliquots of each extract were interpolated with the corresponding DPPH scavenged percentage, tracing the reported curve.

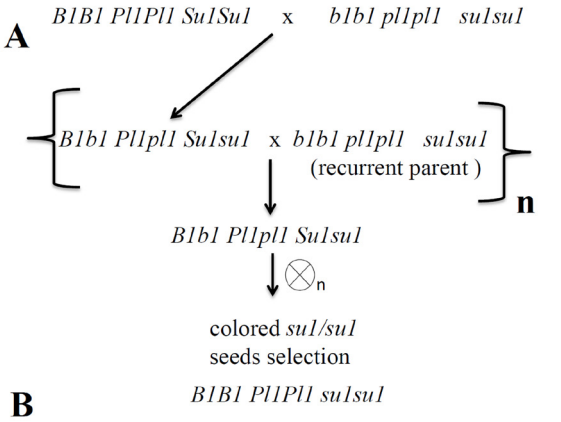
Panel Test

To test the acceptability of the new coloured sugary line, 12 blinded subjects were randomly recruited and asked to taste 4 kernels of both the uncoloured and coloured line. No cooking treatment, nor salt nor dressing were added to the kernels. Each subject expressed his judgment about his appreciation according to a scale from 1, the worst, to 10, the best. The mean, the median and the mode of the judgments relative to the two different kinds of kernels were calculated.

Results

Development of a coloured sugary line

Sugary corn is a well-established product in the market and a very popular ingredient in the diet especially in the USA. Some reports showed that dietary consumption of sweet corn seems to be able to inhibit tumour growth in mice (Tokuji et al, 2009), probably because of the presence of phenolic compounds, particularly ferulic acid (Tokuji et al, 2009). The ability of some molecules to prevent several chronic diseases such as cancer is supposed to originate from their antioxidant potential (Virgili and Marino, 2008). In maize the antioxidant potential could be increased thanks to its capacity to accumulate anthocyanins in the kernels. In fact anthocyanins are antioxidant molecules whose regular consumption is associated with a high number of health benefits. Therefore improving sweet corn by increasing the anthocyanins content could lead it to being considered as a functional food. For this purpose a recurrent breeding scheme was



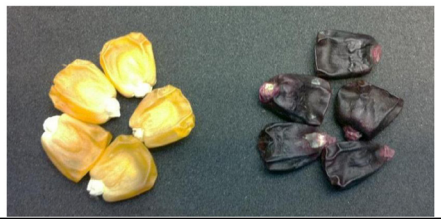


Figure 1 - Breeding scheme. **A**: Recurrent Selection Scheme: the cross between the *B1Pl1* line, source of the regulatory biosynthetic genes and the commercial uncoloured sugary corn gave rise to heterozygous plants for the *B- Pl-* genes. Among them, the highest anthocyanin content plants were selected for the backcrossing with the recurrent parent. Then the best plants underwent some cycles of self-pollination. **B**: Phenotype of uncoloured (left) and coloured (right) sweet corn kernels.

planned (Figure 1A). A tropical black corn plant bearing the *Pl* and *B* regulatory genes, required to activate the anthocyanin accumulation in the seed pericarp, was used as source of the genes for the pigment biosynthesis, while a commercial sugary yellow line was used as the recurrent parent (Figure 1A). The selection of the heterozygous plants used for crossing and the homozygous plants during the self-pollination cycles was based on the use of 2 molecular markers, nc009 and bnlg1064, polymorphic for the *Pl* and *B* genes between the uncoloured and coloured parents of the cross (Figure 2). This breeding scheme allowed us to obtain a sugary plant with a pigmented ear, harvested 21 days after pollination (DAP) (Figure 1B). Simultaneously, in the same field in Landriano (PV, Italy), the yellow commercial sugary line was grown and harvested 22 DAP to be used as the control (Figure 1B).

The breeding scheme provided good results: both the fresh and dry mean seed weights did not show significant differences on comparing coloured with non-coloured raw seeds (Supplementary Figure 1).

The coloured and the uncoloured sweet corn lines are near-isogenic lines, and as a consequence a near-isogenic food, that differs only in the content of specific phytonutrients and thus appears to be an useful tool to reduce the complexity of the studies

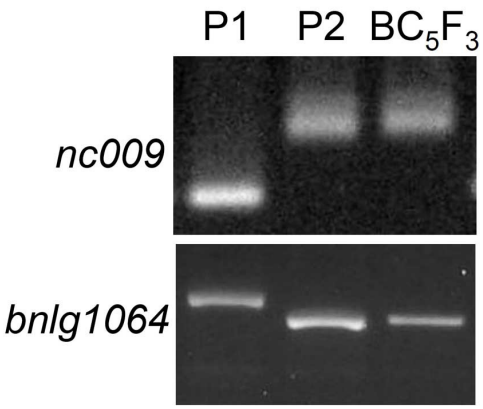


Figure 2 - Molecular Assisted Selection. The nc009 SSR, part of the *Pl1* gene and the bnlg1064 SSR, next to the *B1* gene, were found to be polymorphic between the coloured and the colourless parents. The heterozygous individuals were easily detected and selected to carry on the breeding selection scheme. P1, sugary corn line; P2 *B1Pl1* line; BC_5F_3, coloured sugary corn line developed.

about the diet-health relationship (Martin et al, 2011).

Then for both the coloured and uncoloured sugary lines the seed anthocyanin, flavonol and phenolic acid compounds were spectrophotometrically quantified (Table 1).

The introgression of the colour genes allowed us to obtain a red sugary line able to accumulate 118.92 ± 14.97 mg 100 g^{-1} of anthocyanins in the fresh kernels (Table 1), while no pigment was detected in control sugary kernels (Table 1). This appeared to be a good amount in comparison with berries that accumulate 25 to 698 mg 100 g^{-1} (Mazza and Miniati, 1993; Wang and Lin, 2000; Wu et al, 2006; Koponen et al, 2007), black rice, that accumulates 10 - 493 mg 100 g^{-1} (Ryu et al, 1998) and coloured popcorn, that accumulates around 36 - 66.44 mg 100 g^{-1} (Lago et al, 2013). Also for each of the other metabolite classes, the red line scored a significantly higher value compared to the yellow line: 81.04 mg 100 g^{-1} vs 31.23 mg 100 g^{-1} of flavonols and 121.67 mg 100 g^{-1} vs 52.49 mg 100 g^{-1} of phenolic acids. In addition to anthocyanins, sweet corn is also able to synthesize phenolics compounds, particularly ferulic acid (Balasubashini et al, 2003; Tokuji et al, 2009). So we quantified the amount of phenolic acids and flavonols in both coloured and uncoloured sweet corn lines. The results showed a significantly higher amount of both in the new coloured sugary line, than in the control uncoloured one (Table 1): 81.04 mg 100 g^{-1} vs 31.23 mg 100 g^{-1} of flavonols and 121.67 mg 100 g^{-1} vs 52.49 mg 100 g^{-1} of phenolic acids.

This could be expected because these classes of molecules share the first part of the biosynthesis pathway with the anthocyanin pathway, so that the active alleles of the anthocyanin regulatory genes

Table 1 - Spectrophotometric quantification of anthocyanins, flavonols and phenolic acids content in the raw, steam cooked and autoclaved kernels of uncoloured and coloured lines, The confidence intervals at 95% are shown.

		raw	steam cooked	autoclaved
anthocyanins	uncoloured	0.23±0.24	0.57±0.70	0.22±0.15
	coloured	118.92±14.97	96.82±2.21	19.6±1.75
flavonols	uncoloured	31.23±5.24	39.51±4.8	60.73±9.25
	coloured	81.04±14.54	115.28±2.61	88.94±9.11
phenolic acids	uncoloured	52.49±5.68	64.07±2.68	89.97±2.63
	coloured	121.67±25.67	156.66±3.34	130.72±3.97

could have pushed up the quantities of all the structural genes of the flavonoids biosynthesis. Therefore the presence of the anthocyanin pigments in the new coloured sugary line is a nodal point because they also seem to boost the amounts of other flavonoids and health-promoting compounds too: the anthocyanin presence makes the new sugary coloured line a good candidate as an everyday functional food in the diet of many people.

Effects of the cooking treatments on anthocyanins, flavonols and phenolic acids content.

The steam treatment caused a small decrease in the anthocyanins content of the new coloured line: the 118.92 mg 100 g^{-1} amount in the fresh seeds fell to 96.82 mg 100 g^{-1} (Table 1). The effect of the autoclave cycle was dramatic, destroying a large part of the anthocyanins, which reached the final amount of 19.6 mg 100 g^{-1} (Table 1). Strikingly, neither the flavonols nor the phenolic acids were degraded by the steaming procedure, on the contrary this treatment caused a significant increase in the flavonol amounts (Table 1). This increase seems to be higher for the coloured seeds (81.04 mg 100 g^{-1} before the treatment and 115.28 mg 100 g^{-1} after) than for the uncoloured ones (31.32 mg 100 g^{-1} before the treatment and 39.51 mg 100 g^{-1} after). The same pattern was found for the phenolic acids, with the red line scoring 81.043 mg 100 g^{-1} before and 156.66 mg 100 g^{-1}after the treatment and the yellow line 31.23 mg 100 g^{-1} before and 64.07 mg/100g after the steam treatment (Table 1).

The autoclave cycle led to a marked decrease in flavonols and phenolic acids content in the red line, while this decrease was less evident in the yellow line (Table 1).

Qualitative analysis of anthocyanins.

To understand whether the cooking treatments modified the chemical structure of the leftover anthocyanins, a TLC was performed comparing the extracts of raw and treated seeds uncoloured and coloured. The plate in Figure 3A shows the spots corresponding to the 3 standards delphinidin, cyanidin and pelargonidin (lanes 1-3), then the 3 coloured samples -raw, steam cooked and autoclaved- (lanes 4-6) and finally the 3 uncoloured samples (lanes 7-9). Cyanidin was the most abundant anthocyanin in the 3 coloured samples, while no spots corresponding to the standards were identified in the uncoloured samples. The extract obtained from the raw seeds (lane 4), also revealed the presence of pelargonidin, less abundant than cyanidin, and another spot, not identified by the standards. The same pattern even if less intense, is shown by the coloured steam cooked sample (lane 5). The spots relative to the coloured autoclaved sample were very weak (lane 6), so that only the cyanidin is visible. The UV picture of the TLC plate revealed another unidentified spot (Figure 3B), not detected in visible light, and present in both the coloured autoclaved sample (lane 6) and the uncoloured untreated sample (lane 7). This spot was also present, even if weaker, in the uncoloured steam cooked and autoclaved samples too (lanes 8-9).

DPPH Scavenging ability

The diagram representing the DPPH scavenging ability clearly showed that the new coloured sugary

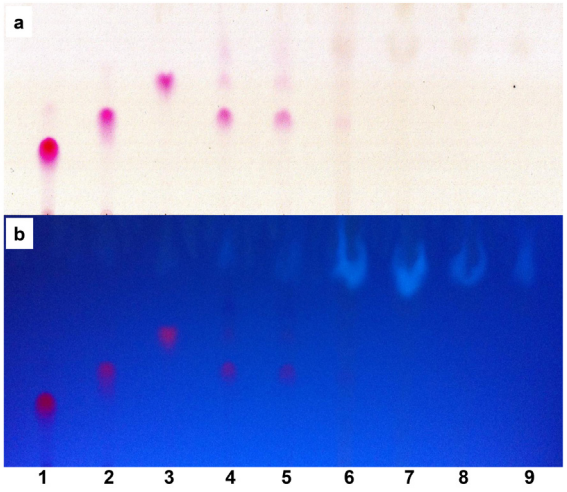


Figure 3 - Pictures of the TLC plate taken under visible (A) or UV (B) light. The spots represent: lane 1 to 3, the delphinidin, cyanidin and pelargonidin standards; lane 4 to 6, the anthocyanin extracts coming from the coloured, raw steamed cooked, and autoclaved kernels; lanes 7 to 9, uncoloured controls.

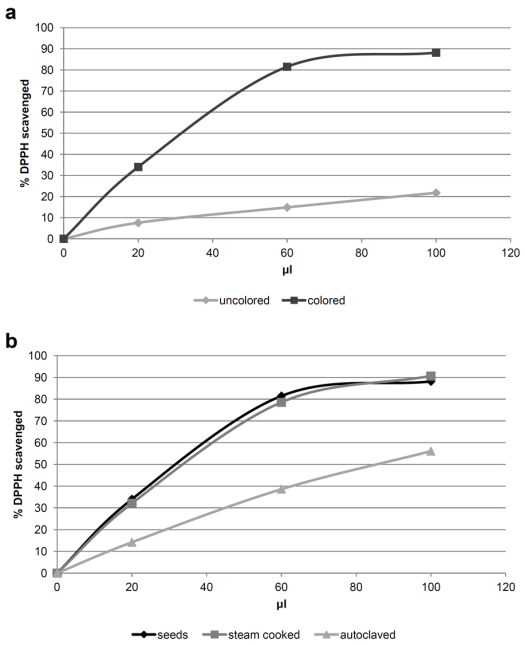


Figure 4 - Comparison of the antioxidant ability in the DPPH radical scavenging assay of the coloured raw vs the uncoloured raw seed extracts (A) and of the raw vs steam cooked vs autoclaved coloured seed extracts (B), equalized and diluted according to the anthocyanins concentration.

line has a much higher antioxidant activity compared to the uncoloured sample (Figure 4A). After the equalization of the extracts among the three different treatments, through suitable dilutions based on the anthocyanins content, the curves of the raw and the steam cooked coloured samples are characterized by a similar tendency, while the autoclaved coloured extract showed a lower radical scavenging ability (Figure 4B).

Panel Test - consumer test

Twelve blinded subjects, randomly chosen, were asked to express a judgment about the acceptability of the new coloured sugary corn and of the respective control (Table 2). Both lines were tested without cooking, salt and dressing.

The acceptability mean scores were 6.75 for both lines, attesting no significant differences between the acceptability for taste alone of the traditionally uncoloured and the new coloured sugary products (Table 2).

Discussion

Sugary corn is a well-established product in the market and a very popular ingredient in the diet especially in the USA. Some reports showed that dietary consumption of sweet corn seems to be able to inhibit tumour growth in mice (Tokuji et al, 2009), probably because of the presence of phenolic compounds, particularly ferulic acid (Tokuji et al, 2009). The ability of some molecules to prevent several chronic diseases such as cancer is supposed to originate from their antioxidant potential (Virgili and Marino, 2008). In maize the antioxidant potential could be increased thanks to its capacity to accumulate anthocyanins in the kernels. In fact anthocyanins are antioxidant molecules whose regular consumption is associated with a high number of health benefits. Therefore improving sweet corn by increasing the anthocyanins content could lead it to being considered as a functional food. For this purpose a recurrent breeding scheme was planned (Figure 1A). A tropical black corn plant bearing the Pl and B regulatory genes, required to activate the anthocyanin accumulation in the seed pericarp, was used as source of the genes for the pigment biosynthesis, while a commercial sugary yellow line was used as the recurrent parent (Figure 1A). The selection procedure was based on the use of 2 molecular markers, nc009 and bnlg1064, polymorphic for the Pl and B genes between the parents of the cross (Figure 2). The result of this breeding scheme is a coloured sugary plant, characterized by the genetic background of the commercial uncoloured sugary line with the exception of the presence of the anthocyanin regulatory genes in the dominant form (Figure 1B). The new coloured sugary line was then analysed using the uncoloured commercial sugary isogenic line as control.

The coloured and the uncoloured sweet corn lines are near-isogenic lines, and as a consequence a near-isogenic food, that differs only in the content of specific phytonutrients and thus appears to be an useful tool to reduce the complexity of the studies about the diet-health relationship (Martin et al, 2011).

Sweet corn is harvested before the time of field maize physiological maturity, at about 20-21 DAP The fresh and dry seed weights did not show significant differences between the two isogenic lines (Supplementary Figure 1), attesting to the good result coming from the breeding work. The introgression of the colour genes allowed us to obtain a red sugary line able to accumulate 118.92 ± 14.97 mg 100 g^{-1} of anthocyanins in the fresh kernels (Table 1), while no pigment was detected in control sugary kernels (Table 1). This appeared to be a good amount in comparison with berries that accumulate 25 to 698 mg 100 g^{-1} (Mazza and Miniati, 1993; Wang and Lin, 2000; Wu et al, 2006; Koponen et al, 2007), black rice, that accumulates 10 - 493 mg 100 g^{-1} (Ryu et al, 1998) and coloured popcorn, that accumulates around 36

Table 2 - Mean, mode and median about the scores of the panel test, relative to the degree of acceptability of the new coloured sugary kernels compared to the commercial uncoloured one, in a randomly blinded group of subjects.

	degree of acceptability	
	uncoloured	coloured
mean	6.75	6.75
mode	7	7
median	7	7

- 66.44 mg 100 g^{-1} (Lago et al, 2013). In addition to anthocyanins, sweet corn is also able to synthesize phenolics compounds, particularly ferulic acid (Balasubashini et al, 2003; Tokuji et al, 2009). Ferulic acid is synthesized starting from phenylalanine following the phenylpropanoids pathway. Therefore phenolic acids share a part of the biosynthetic way with anthocyanins and with flavonols, the most abundant group of flavonoids among plants, proven to have many human health beneficial effects (Formica and Regelson, 1995; Duthie et al, 2000). So we quantified the amount of phenolic acids and flavonols in both coloured and uncoloured sweet corn lines. The results showed a significantly higher amount of both in the new coloured sugary line, than in the control uncoloured one (Table 1). This could be expected because these classes of molecules share the first part of the biosynthesis pathway with the anthocyanin pathway, so that the active alleles of the anthocyanin regulatory genes could have pushed up the quantities of all the structural genes of the flavonoids biosynthesis. Therefore the presence of the anthocyanin pigments in the new coloured sugary line is a nodal point because they also seem to boost the amounts of other flavonoids and health-promoting compounds too: the anthocyanin presence makes the new sugary coloured line a good candidate as an everyday functional food in the diet of many people. The DPPH scavenging ability test seems to strengthen this hypothesis (Figure 4A): the raw uncoloured commercial sugary seed extract showed a much lower antioxidant ability compared to the raw coloured one, attesting the anthocyanins' remarkable power (Figure 4A). This is in agreement with previously reported data about a coloured popcorn line (Lago et al, 2013), consequently the coloured sweet corn can be considered a new functional food.

However while part of the sweet corn crop is consumed as fresh grains or fresh ears, most of it is consumed as processed canned sweet corn (Dewanto et al, 2002). Some of the thermal procedures required for sweet corn processing are known to lower the nutritional level of grains and vegetables in comparison with the fresh ones (Lathrop et al, 1980; Rao et al, 1981; Burge et al, 1995; Murcia et al, 2000; Dewanto et al, 2002). Therefore it is important to understand the effect of sweet corn processing on the anthocyanin molecules, at both quantitative and qualitative level. Big companies, e.g. Bonduelle and Conserve Italia in Italy and Allens in USA, studied the best methods for thermal processing and conservation of canned food: first of all small amounts of salt water and sugars were added to the kernels inside the can, where the vacuum is imposed. Then the product underwent a steam cooking, but the presence of the vacuum allowed a lowering and shortening of the heating procedure, so that the kernels are subjected only to a sterilization and not to a proper cooking. As a consequence the vegetable can keep its flavour and its nutritional properties. The correct balance between vacuum and temperature is often held as a trade secret by the companies (http://www.bonduelle.it/la-cottura-al-vapore-secondo-bonduelle/ accessed 26 august 2013). For this reason we decided to subject the 2 sweet corn lines to different cooking processes: a mild cooking with steam and a severe one with the autoclave. The steam cooking treatment seems to only slightly decrease the anthocyanins amount (Table 1), as already found by Vallejo et al (2003). The autoclave cooking on the other hand resulted in a more dramatic effect causing the reduction of the anthocyanins level by about 83% in comparison with the untreated kernels. This result was in agreement with previous data reporting that the stability of anthocyanins in cooked foods is dependent on the temperature and on the heating time of the thermal process (Abdel-Aal et al, 2003; Cabrita et al, 2000; Hiemori et al, 2009). The big difference in the degrading ability of the cooking processes used could be explained by the fact that the steam cooking was not only milder but also shorter than the autoclave treatment so that it was able only to inactivate some oxidative enzymes and not to destroy the pigments that are present in the edible part of the vegetable (Howard et al, 1994; Vallejo et al, 2003).

Moreover steam cooking seems to increase flavonols and phenolic acids in both the coloured (+42.25% and +28.76%, respectively) and the uncoloured line (+26.51% and +22.06%, respectively) (Table 1).

The autoclave cooking caused an increase of 9.75% for the flavonols and 7.44% for the phenolic acids in the coloured kernels and of 94.46% and of 71.40% respectively in the uncoloured ones (Table 1).

This was in agreement with the results of Dewanto et al (2002) who found that the free phenolic portion in their sweet corn significantly increased after the thermal process. This can be explained by the fact that the heating causing the breakdown of the cellular constituents, allowed the release of the bound phenolic acids portion (Dewanto et al, 2002).

At this point it was important to understand whether the cooking was able to change the structure of the pigments and consequently the antioxidant ability of the leftover anthocyanins, not degraded by the heating. With this purpose the DPPH assay was also performed on the extracts coming from the raw, the steam cooked and from the autoclaved coloured kernels, equalized through proper dilutions on the basis of the anthocyanin amount. Anthocyanin amounts being equal among them, the raw and steamed kernels showed the same scavenging ability (Figure 4B), attesting that no structural changes occurred in the leftover pigment molecules after the steam cooking. On the contrary the extract obtained from the autoclaved kernels had a much lower antioxidant power (Figure 4B). This could be caused by the strong treatment of the autoclave, in contrast to the lighter one

of the steam treatment: probably one hour of heating coupled with the high pressure was able to degrade not only the anthocyanin molecules but also some other antioxidant compound., such as for example vitamin C (Burge and Fraile, 1995; Murcia et al, 2000; Dewanto et al, 2002) or β-carotene and lutein carotenoids (Kurilich and Juvik 1999; Tokuji et al, 2009).

To confirm that no structural or chemical changes in anthocyanin molecules occur after the thermal processes, Thin Layer Chromatography was performed (Figure 3). The spots of the coloured samples clearly showed that the anthocyanin aglycons did not change their structure following cooking, only their amount decreased (Figure 3A). We noticed the presence of a little spot above the pelargonidin one (Figure 3A): it is not present in other B/Pl maize genotypes, such as the coloured popcorn (Lago et al, 2013). This could be explained by the fact that sweet corn is a fresh product, composed by developing kernels that are still accumulating pigments in the pericarp; therefore the metabolite profile is not definitive as in the dry maize kernels. Deeper and more precise analyses are needed to finely characterize the metabolite profile of this coloured line. In the meantime the acceptability of the new product in comparison to the uncoloured one was tested on 12 blinded subjects, randomly chosen (Table 2). The kernels were tested with no cooking, no salt and no dressing in order to level the taste. The appreciation scores did not show significant differences between the uncoloured and coloured sugary kernels (Table 2), suggesting that the healthier properties due to the pigment presence could persuade the consumer to prefer the coloured sweet corn to the uncoloured one.

Conclusion

This study suggested that the new coloured sugary line is a good source of anthocyanins, of other beneficial flavonoids and of antioxidant potential, and thus it can be considered a good functional food. Our results also suggest that to preserve the healthy properties of the coloured sweet corn it is better to consume it fresh but if processing is needed it would be better to use a mild process, such as the steam treatment, in order to benefit from the best nutritional composition. It could be likely that consumers will choose this new product for its healthy properties given that no differences in appreciability was scored.

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