International Journal of Food Properties, 15:1054–1068, 2012

ISSN: 1094-2912 print / 1532-2386 online
DOI: 10.1080/10942912.2010.513215

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COLOUR ASSESSMENT ON BREAD WHEAT AND TRITICALE FRESH PASTA

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Although durum wheat is the cereal of choice for pasta production, in many areas of Argentina pasta is made from bread wheat, since durum wheat is cultivated only in a small region of the country. The purpose of this work was to determine the influence of different bread flours on the colour of laminated fresh pasta. Triticale flour was also studied. In addition, ash, protein, and protein fractions of each flour were measured. Also, the formulation was modified using different gluten, starch, and water concentrations. Pasta lightness and redness were affected mainly by the ash content, while yellowness was affected by the protein content of different flours. A similar effect was found when the formulation was substituted for starch and gluten, due to protein dilution and concentration, respectively. Albumins and globulins correlated with a* component, while gliadins, soluble and insoluble glutenins correlated with b* component; however, only glutenins presented correlation with colour score ($CS = [L^* + (b^* \times 2)]/20$). The greatest amount of water added to the dough produced a decrease of lightness and an increase of redness and yellowness of pasta samples. The overall ANOVA revealed that the greatest sources of variation for pasta colour were the different flours used, in comparison with the effect of starch and gluten substitution, and with the addition of different amounts of water.

Keywords: ***Color, Fresh pasta, Bread wheat flour, Starch, Gluten, Water.***

INTRODUCTION

There is an enormous variety of pasta as a result of traditional and regional preferences, which vary markedly in texture and appearance.[1] Depending on the kind of wheat used and on the production process, two large groups can be identified: noodles and pasta itself. Noodles are generally made from bread wheat (*Triticum aestivum* L.) using a process of lamination and cutting, while pasta is processed by extrusion using semolina from durum wheat (*Triticum durum* Desf.). The differences between raw materials and production process result in two products easy to differentiate in appearance and texture.

In Asian countries, noodles, a staple of their diet, are generally softer[2] and more elastic[3] and the best noodle colour is a bright creamy white with the absence of any discoloration.[1] But in western countries, dense-textured, hard,[3] yellow, and bright pasta

Received 8 February 2010; accepted 28 July 2010.
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are preferred, since these colour characteristics are associated with the presence of a good egg content. Even when pasta has no egg content, and is only prepared with semolina, water, and salt, the yellow colour will be given by the naturally occurring yellow pigments. These are predominantly hydroxylated carotenoid lutein,[4] the content of which will depend on variety; on the environment; on lipoxygenase activity;[5,6] on polyphenol oxidase and peroxidase;[7] and to a lesser extent, on other enzymes, such as α-amylase, oxidase, isoperoxidase;[8] and on processing conditions.[9]

As a result of the influence of European immigration, Argentine preference is pasta made with durum wheat semolina. However, in Argentine areas where durum wheat is not produced, hard wheat (*Triticum aestivum* L.) is used for pasta making in local industries. In that pasta, which does not include egg in their formulation and are made with flours that have not been selected for their high pigment contents or for their low lipoxygenase activity, colour has to be determined by other factors. On the other hand, Triticale (*Triticosecale* Wittmack) is a hybrid between wheat and rye that provides very good quality flours for products that require weak flours, such as cookies[10] and crackers.[11] But in recent years, there has been an improvement in the production of triticale with different degrees of hardness,[12] which opens up possibilities for their use in other kinds of products, such as pasta. Colour evaluation can be either subjective or objective. In the first case, the human eye is the instrument of measurement. In objective determinations, an instrument is used to provide a specific colour value based on the amount of light reflected off the product surface or the light transmitted through it. In studies performed with commercial pasta, excellent correlations were found between objective and sensorial measurements.[13]

The most extensive studies on pasta colour were on dry pasta, but on fresh pasta have received only some considerations. Besides, there are many reports on the contribution of the protein fractions to pasta texture and cooking quality, but few researches have been carried out on the influence of protein fractions on fresh pasta colour. Therefore, in order to study the factors that influence the colour of fresh pasta, different kinds of flours were studied, evaluating the effect of ash, protein, and protein fractions. At the same time, the role of the main components was studied, modifying the formulation with the addition of starch and gluten and using different amounts of water. In this work, it has been called "pasta" to the samples, although they were made from bread wheat flour or triticale flour using a process of lamination and cutting.

MATERIALS AND METHODS

Materials

Wheat grains from Baguette (BAG) and Buk Guapo (BUK) cultivars were ground with AQC 109-Laboratoy Mill-Agromatic AG (Laupen, Switzerland). Also, commercial wheat flour, without additives, provided by Molino Campodónico (La Plata, Argentina), corresponding to the 2006 campaign (MCA), was used. Besides, flour from milling Tatú triticale (TRI) grains was employed. Wheat starch, food grade (Montreal, Canada) (S), and gluten vital Abeve (USA) (G) also were utilized.

Characterisation of the Flour

Water, ash, and protein content were determined according to Approved Methods 44-15A, 08-01, and 46-30,[14] respectively. Sedimentation index in sodium dodecil sulfate

(SDS) was performed according to Dick and Quick,[15] and water retention capacity was evaluated according to Approved Method 56-10,[15] and modified according to Park and Baik[16] using distilled water instead of the sodium bicarbonate solution. All determinations were made at least in duplicate, and results were expressed as the mean of replications ± SD.

Colour measurements of each flour, and also starch and gluten, were made on a 1.5-cm-thick layer cover with a low reflectance glass. A spectrophotometer (508d; Minolta, Ramsey, NJ, USA), with eight-millimetre measurement aperture, A D65 illuminant, 10° angle of observer, and specular component included was used. Measured parameters were expressed as other works[17] in terms of lightness (L^*), red-green chromaticity (a^*), and yellow-blue chromaticity (b^*) values. The average of five different points of sample surface was taken in triplicate.

Characterisation of Flour Proteins

Around 660 mg of flour were subjected to three sequential extractions to obtain proteins on the basis of the affinity for the different solvents; the first one with 10 mL of NaCl 5% (750214; Cicarelli, Santa Fe, Argentina), for 2 h, to obtain albumins and globulins; then with 10 mL of isopropanol 70% (927110; Cicarelli) for 3 h to obtain gliadins; and lastly with 10 mL of SDS 1.5% (L4509; Sigma, Argentina) for 8 h to obtain soluble glutenins; the remainder were considered insoluble glutenins. The resulting supernatants of each solvent of extraction were divided into two portions, one for analysis by electrophoresis (SDS-PAGE) and the other to determine total proteins by the Kjeldahl method. Electrophoresis was performed in a plaque (70 × 80 mm) over a gel of polyacrylamide, acrylamide (A8887; Sigma)-bisacrylamide (M7279; Sigma), 4–12% with SDS, according to the discontinuous buffer system of Laemmli,[18] using an electrophoretic Mini Protean II Dual Slab Cell camera (Bio-Rad Laboratories®, Hercules, CA, USA), with an approximate run time of 90 min, at a constant voltage of 150 V. Supernatants set aside for electrophoresis, were precipitated with acetone (702110; Cicarelli). After centrifugation (VT 3216; Cavour, Buenos Aires, Argentina) at 3500 rpm, for 15 min, precipitates were recovered, dried at 35°C for 8 h, and finally they were resuspended with 0.5 mL of buffer of sample [TRIS (T6066; Sigma), SDS, Bromophenol Blue (161-0404; BioRad), glycerol (160214; Cicarelli), and beta mercapto ethanol (161-0710; Bio-Rad)], and placed in a boiling water bath for 5 min. The volume of the sample loaded was between 10 and 15 μL. It was used as a molecular weight marker a SDS-PAGE Molecular Weight Standards Broad Range (161-0317; Bio-Rad). The gels were stained with 0.25% Coomassie Brilliant Blue R-250 (161-0436; Bio-Rad) in methanol (D016-03-06; Dorwil)/distilled water/acetic acid (D008-03-03; Dorwil, Buenos Aires, Argentina), (4:5:1 v/v) and were destained in the same solvent. All analyses were done in duplicate.

Pasta Production

Samples were made from 50 g of flour, 500 mg of NaCl (food grade), and 37.5 mL of distilled water/100 g flour. Each flour was substituted with two levels of starch, 5 and 10 g/100 g of flour, and two of gluten, 3 and 6 g/100 g of flour (S5, S10, G3, and G6, respectively). Furthermore, a new formulation water amount was increased to 43.8%. Over the sifted dry ingredients (flour, flour and starch, or flour and gluten), the water with salt was added, and immediately after that, the blend started to be kneaded with a hand mixer (190 W, HR 1495; Philips, Buenos Aires, Argentina) at maximum speed, for 3 min. Using

the hands, the dough was shaped into a roll and rested for 10 min. For lamination, a Drago® (San Andres, Argentina) pasta maker was used, 14 cm sheet width, with adjustable opening of rolling pins from 1 to 7. For lamination, dough was passed through the rolls of a Drago® pasta maker at #7 roll gap, then dough was folded and put through the sheeting rolls. The folding and sheeting were repeated once more through #7, and then, at progressively decreasing roll gaps until it reaches the #3 roll gap. To finish lamination, the dough was passed once again through #3 roll gap, without any previous folding. Pastas were cut $\approx$2.5 mm wide and $\approx$2 mm thick, and immediately after that, they were put in hermetically sealed plastic bags until they were used.

Colour of Pasta Dough

The colour of a single layer of the pasta sheet was measured immediately after lamination process was finished. The layer was covered with a low reflectance glass and was measured using the same conditions as those described for flour colour. Two measurements were performed in each of the five areas of the pasta sheet surface evaluated (placing the spectrophotometer in five different areas of the surface to be measured) resulting in a total of ten measurements per assay. From the values of L^* and b^* measured, a Colour score (CS) was calculated according to Hareland et al.,[19] as $CS = [L^* + (b \times 2)]/20$.

Statistical Analysis

Data were statistically analysed using InfoStat Statistical Software (Facultad de Ciencias Agropecuarias, UNC, Argentina) for computing Fisher's least significant difference (LSD) of multiple comparisons, Pearson's correlation coefficients ($p < 0.05 = {}^*$; $p < 0.01 = {}^{**}$), and analysis of variance (ANOVA). The evaluation of the effect of the different variables studied on pasta colour was performed through the overall ANOVA F-values for individual model components and interaction terms.

RESULTS AND DISCUSSION

Characterization of Raw Materials

Table 1 shows protein, ash, sedimentation index in SDS (I-SDS), water retention capacity (WRC) and colour values of each flour, in terms of L^*, a^*, and b^*. Also starch and gluten were included. Between wheat flours, BUK presented the highest protein and I-SDS values, while BAG showed the lowest ones. In agreement with other studies,[20–22] the behaviour of protein were similar to I-SDS values. BUK also presented the highest ash and WRC values, followed by BAG and MCA. TRI protein values did not show significance differences with BAG; however, I-SDS value was the lowest of all wheat flours. Ash content from TRI flour was the highest value of all samples. As expected, starch presented a very low protein and ash content, while gluten, which is almost all protein, showed a similar ash content than BUK.

With regard to flour colours, MCA presented the highest L^*value, followed by BAG and BUK. Related to a^* measurement, all samples presented negative values (greenness), been MCA sample with the highest one between wheat flours. Regarding yellow blue chromaticity, BAG presented the highest b^* value, followed by BUK and MCA. L^* from the TRI sample did not show a significant difference in respect to BUK, while a^* and b^* values were the highest and the lowest ones respectively, of all flour samples.

Table 1 Characterisation of flours, starch, and gluten used to made pasta.

Sample	Protein[1]	Ash[1]	I-SDS	WRC	L*	a*	b*
MCA	11.2 ± 0.5^{c}	0.64 ± 0.03^{b}	9.8 ± 0.4^{b}	59.7 ± 0.9^{a}	91.6 ± 0.1^{d}	-0.42 ± 0.02^{c}	8.96 ± 0.06^{c}
BAG	10.1 ± 0.5^{b}	0.68 ± 0.01^{b}	9.3 ± 0.1^{b}	69.2 ± 0.0^{b}	90.4 ± 0.1^{c}	-0.40 ± 0.03^{c}	10.69 ± 0.10^{e}
BUK	15.6 ± 0.0^{d}	0.89 ± 0.03^{cd}	17.5 ± 0.1^{c}	84.6 ± 0.0^{d}	89.4 ± 0.2^{b}	-0.21 ± 0.06^{d}	9.96 ± 0.17^{d}
TRI	9.8 ± 0.6^{b}	0.92 ± 0.01^{d}	7.1 ± 0.2^{a}	79.1 ± 0.0^{c}	89.5 ± 0.2^{b}	-0.62 ± 0.05^{b}	8.18 ± 0.10^{b}
S	0.2 ± 0.0^{a}	0.13 ± 0.03^{a}	nd	nd	96.2 ± 0.1^{e}	-0.87 ± 0.05^{a}	1.88 ± 0.07^{a}
G	76.7 ± 0.6^{e}	0.86 ± 0.01^{c}	nd	nd	84.8 ± 0.1^{a}	0.47 ± 0.01^{e}	13.16 ± 0.38^{f}

Values followed by the same letter within a column do not present significant differences ($p \leq 0.05$).
[1]Values determined on flours, expressed in dry base; g/100 g flour.
nd = Not detected under experimental conditions.

Influence of Flour

Colour of pasta dough and CS is shown in Table 2. The resulting colour of pasta made from the different flours was significantly different (ANOVA, $p < 0.05$) for L*, and for a* and b*, as well. In respect to the lightness, it was found that dough samples presented the same profile of values than those measured in the corresponding flours. In this way, a sample prepared with MCA showed the highest L* value, followed by BAG and BUK. A different situation was found for a* and b* values of dough with respect to flour samples. All a* values changed from negative in flours to positive in dough samples. In such way, BUK dough showed the highest a* values, whereas MCA presented the lowest one. With regard to b* values, BUK dough exhibited the highest value followed by the BAG sample, even though the corresponding flours showed an opposite profile. Also, the a* value measured on TRI dough was positive, where as TRI flour showed a negative one. These results could mean that hydration of flour particles and dough development affects the colour of flour components in terms of a* and b* values.

According to the CS estimated, and considering the preference of light yellow colour pasta (positive b* values and high L* values),[22] it was found that the sample made from BUK was the best qualified, followed by those made from BAG and MCA, while pasta made from TRI was the worst qualified. Taking into account that in CS, b* component (yellowness) doubles the weight of L* component, the low values obtained in general for all the samples are not surprising, since CS was proposed for pasta made from semolina where the yellow pigment content is quantitatively higher than the one that may be present in bread wheat and triticale flours.

Using the Pearson correlation analysis, the incidence of protein, ash, I-SDS, and WRC values of the flours on the resulting colour of flour and fresh pasta made from them, were evaluated. In this way, the ash content was found to have a significant negative effect on L* component of flour (r: -0.90**) and dough (r: -0.92**); this observation is in agreement with previous reports,[1,5,23,24] The negative correlation was also found in the production of other bakery products, such as cookies, in which ash concentration is one of the main factors that affect lightness.[25] Besides, ash content showed a positive linear correlation with the a* component of colour ($r = 0.93^{**}$).

With respect to total protein content, a significant correlation with b* component was found in the dough ($r = 0.84^{**}$) in agreement with previous works on noodles,[1] but not in the flour. I-SDS also showed correlation with yellow-blue colour space (b*), ($r = 0.89^{**}$), as was found for protein content, as expected since I-SDS is an indirect measure of the proteins present in the sample. It was also found that WRC correlated negatively with lightness of flour (L*: $r = -0.85^{**}$) and dough (L*: $r = -0.85^{*}$) and positively with redness (a*: $r = 0.97^{**}$) and yellowness (b*: $r = 0.71^{*}$) only of dough pasta.

Table 2 Colour of pasta dough made from different flours.[1]

Dough[1]	L*	a*	b*	Colour score[2]
MCA	82.1 ± 0.5^{d}	1.4 ± 0.1^{a}	13.6 ± 0.3^{a}	5.46 ± 0.01^{b}
BAG	78.1 ± 0.6^{c}	2.1 ± 0.0^{b}	16.0 ± 0.0^{c}	5.50 ± 0.02^{b}
BUK	74.7 ± 0.8^{b}	2.7 ± 0.0^{c}	19.3 ± 0.0^{d}	5.66 ± 0.04^{c}
TRI	70.7 ± 0.5^{a}	2.7 ± 0.0^{c}	14.2 ± 0.2^{b}	4.96 ± 0.01^{a}

[1]Dough prepared with 37.5% of water.

[2]Colour score: $(L^* + (b^* \times 2))/20$; score range: 1–10, with 10 being the best qualification.[14]

Values followed by the same letter within a column do not present significant differences ($p \leq 0.05$).

Table 3 Proteins fractions (Fr) resulting from sequential extraction.

Flour	Fr-NaCl 5% albumins and globulins[1]	Fr-Isopropanol 70% gliadins[1]	Fr-SDS 1.5% soluble glutenins[1]	Fr-Residual insoluble glutenins[1]
MCA	1.8 ± 0.0^{b}	3.5 ± 0.0^{a}	2.2 ± 0.1^{a}	5.6 ± 0.4^{b}
BAG	1.7 ± 0.0^{a}	3.5 ± 0.6^{a}	2.3 ± 0.2^{a}	5.0 ± 0.4^{ab}
BUK	$2.5 \pm 0, 1^{d}$	5.3 ± 0.2^{b}	3.4 ± 0.4^{b}	8.2 ± 0.9^{c}
TRI	2.2 ± 0.0^{c}	3.9 ± 0.7^{a}	2.1 ± 0.0^{a}	4.1 ± 0.2^{a}

[1]Grams of proteins in 100 g of flour, dry basis.
Values followed by the same letter within a column do not present significant differences ($p \leq 0.05$).

Besides evaluating the influence of total protein content on pasta colour, it was also studied how the different protein fractions resulting from the sequential extraction performed with solvents of different polarities could contribute. With respect to the fraction recovered from NaCl 5%, rich mainly in albumins and globulins, it showed correlation with L^* component of flour and with a^* component ($r = 0.74^*$) of dough. Table 3 shows that BUK followed by TRI flour were the ones with higher content of albumins and globulins fraction; this situation corresponds with the highest values of a^* found for fresh pasta made from these flours. In the electrophoretic runs, bands of greater intensity can also be observed in these two flours, principally in the area between 31.0 y 66.0 kDa (Fig. 1-F1).

In the fraction extracted with isopropanol 70%, where gliadins are mainly present, it only was found correlation with b^* component ($r = 0.72^*$). BUK followed by BAG flour presented the higher gliadin fraction content (Table 3), in agreement with the higher values of b^* measured in the corresponding pasta. Likewise, Fig. 1-F2 shows that in these flours, bands have a greater intensity mainly in the area between 31.0–45.0 kDa. Moreover, to recovered and residual fractions from SDS 1.5%, corresponding to soluble and insoluble glutenins respectively, showed positive correlation with a^* ($r = 0.88^{**}$; $r = 0.93^{**}$), with b^* ($r = 0.96^*$; $r = 0.80^{**}$) components of flour, and also with colour score of dough ($r = 0.76^*$ and 0.77^*). In Table 3 can be observed that BUK flour presented a higher amount of both protein fractions. Similarly, in Fig. 1-F3, bands of higher intensity in BUK flour can be observed.

Effect of Starch and Gluten Addition to Each of the Flours Used in the Formulation

In order to evaluate the effect of starch and gluten on dough pasta colour, samples of fresh pasta with 5 and 10 g of starch, and 3 and 6 g of gluten/100 g flour, were prepared. The addition of starch produced an increase in L^* and a decrease in a^* and b^* in all the samples; while the opposite effect was observed when gluten was added, except in the G6-TRI sample, which showed a slight increase of L^*. Figure 2(a–c), show colour differences observed (ΔL^*, Δa^*, and Δb^*) in dough pasta substituted with starch and gluten, with respect to the colour measured in the pasta prepared with the same flour, without the addition of any modifier. Colour differences observed with the addition of starch and gluten can be explained partly because starch granules reflect more light, while gluten affects clarity negatively.[1] Extracted gluten is lower in lightness than isolated wheat starch (Table 1), so to some extent, simple dilution of starch by gluten should be responsible for low lightness in pasta.[26] According to Oh et al.,[27] flour protein may produce a tight pasta structure resulting from a strong adherence between starch and protein. Such a tight structure would

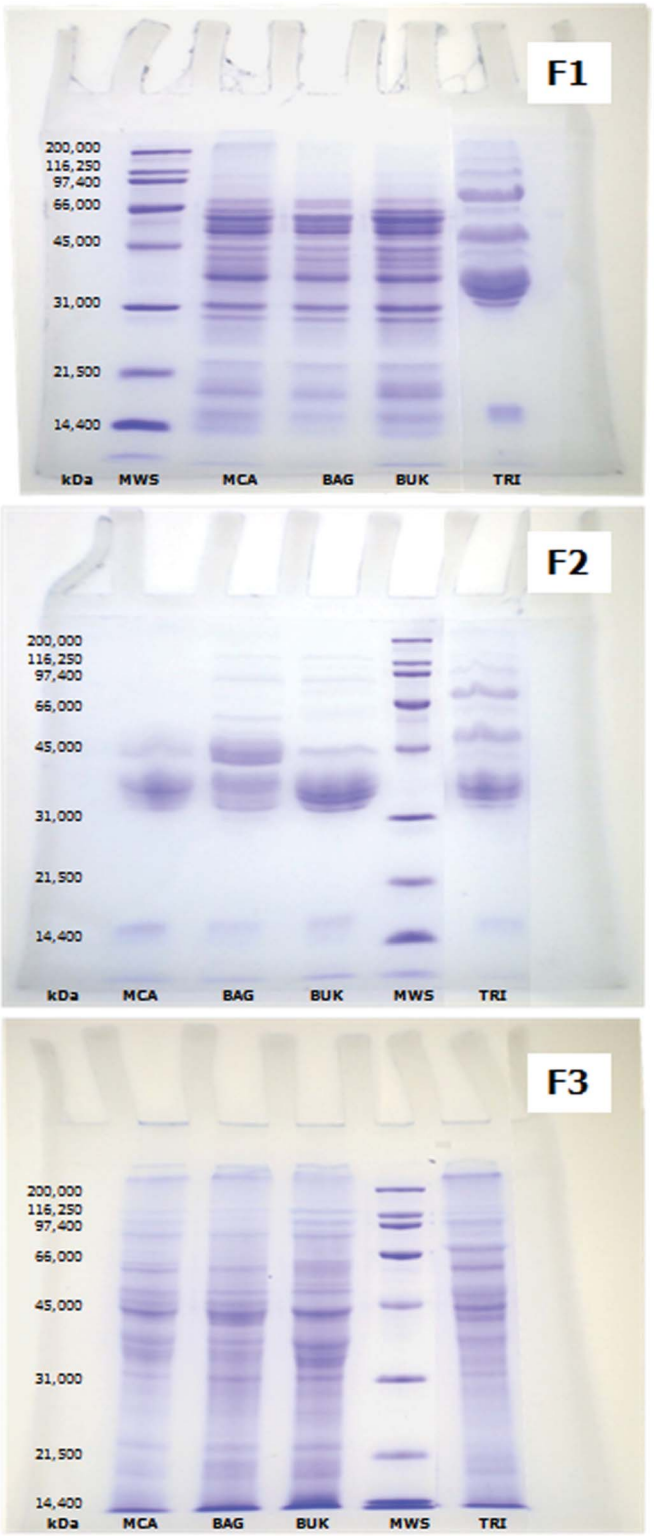

Figure 1 Electrophoretic patters of multistep extractions from flour samples. F1: NaCl 5%; F2: isopropanol 70%; F3: SDS 1.5%. MWS: molecular weight standard. (Color figure available online.)

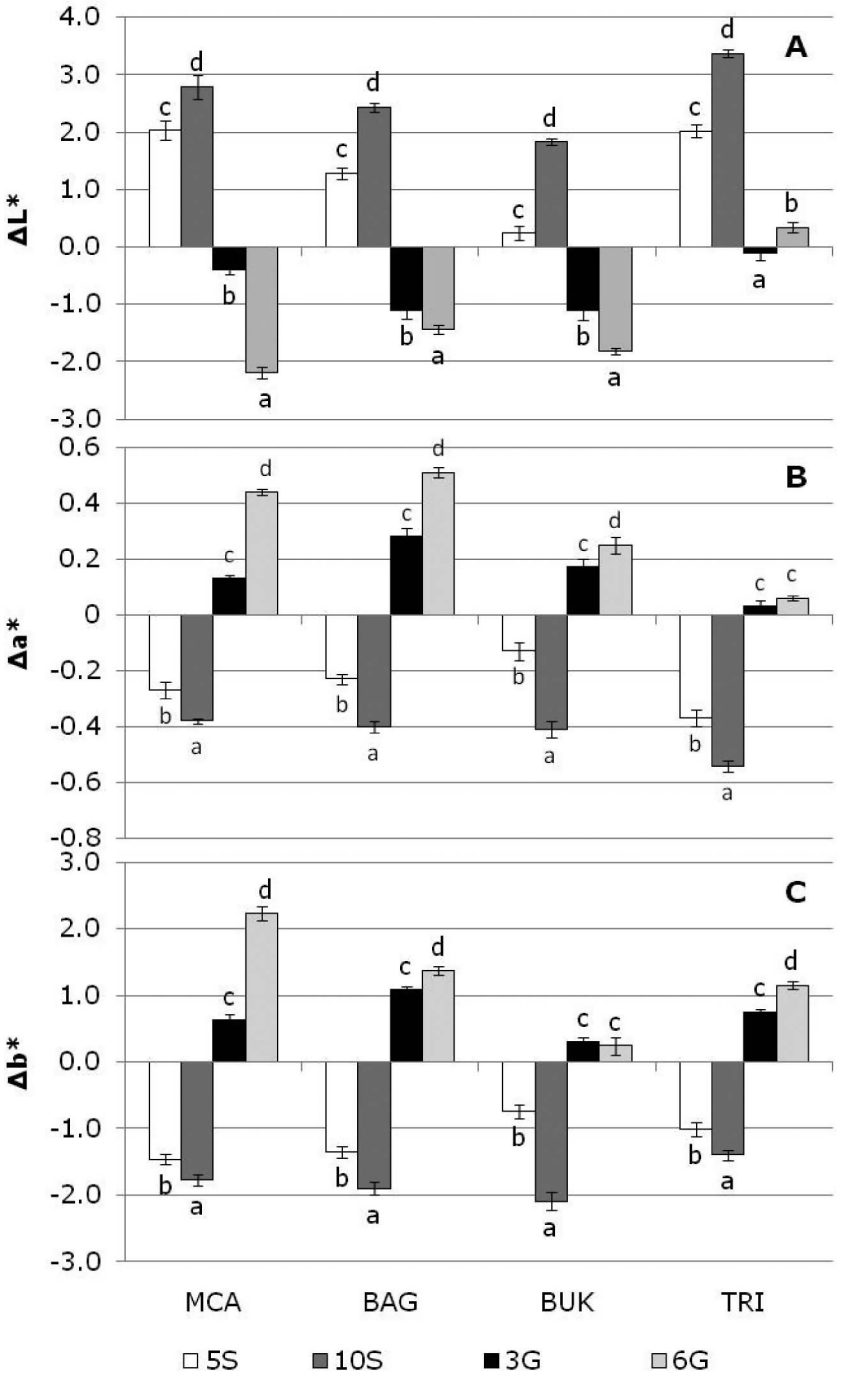

Figure 2 (a) Lightness, (b) redness, and (c) yellowness differences due to starch (5S, 10S) and gluten (3G, 6G) addition with respect to control pasta dough. Error bands correspond to standard error. Values followed by a different letter are significantly different ($p \leq 0.05$) within a sample.

Table 4 I-SDS and WRC of the flours blended with 5 and 10 g of starch, and 3 and 6 g of gluten/100 g flour.

Flour	Modifier	I-SDS	WRC	Colour score
MCA	S5	9.4 ± 0.2^{b}	69.1 ± 0.6^{a}	5.42 ± 0.05^{a}
MCA	S10	7.6 ± 0.3^{a}	68.3 ± 1.0^{a}	5.42 ± 0.07^{a}
MCA	G3	15.1 ± 0.4^{c}	70.5 ± 02^{a}	5.50 ± 0.01^{ab}
MCA	G6	16.1 ± 0.2^{d}	69.0 ± 1.2^{a}	5.57 ± 0.00^{b}
BAG	S5	8.5 ± 0.1^{b}	68.1 ± 0.1^{a}	5.43 ± 0.03^{a}
BAG	S10	7.8 ± 0.1^{a}	68.7 ± 0.4^{a}	5.43 ± 0.03^{a}
BAG	G3	11.0 ± 0.1^{c}	69.1 ± 0.1^{ab}	5.55 ± 0.03^{b}
BAG	G6	13.0 ± 0.0^{d}	70.7 ± 1.3^{b}	5.57 ± 0.01^{b}
BUK	S5	16.5 ± 0.3^{b}	82.6 ± 0.0^{b}	5.60 ± 0.02^{ab}
BUK	S10	13.8 ± 0.4^{a}	81.1 ± 0.5^{a}	5.54 ± 0.02^{a}
BUK	G3	18.0 ± 0.1^{c}	84.4 ± 0.3^{c}	5.64 ± 0.03^{b}
BUK	G6	18.7 ± 0.3^{c}	85.4 ± 0.1^{d}	5.60 ± 0.03^{ab}
TRI	S5	6.4 ± 0.2^{a}	79.2 ± 0.1^{ab}	4.96 ± 0.00^{a}
TRI	S10	6.1 ± 0.2^{a}	76.8 ± 0.1^{a}	4.99 ± 0.01^{ab}
TRI	G3	8.9 ± 0.2^{b}	80.0 ± 1.5^{bc}	5.03 ± 0.02^{b}
TRI	G6	10.1 ± 0.1^{c}	82.2 ± 1.1^{c}	5.09 ± 0.02^{c}

Values followed by a different letter are significantly different ($p < 0.05$) within the same sample.
S: starch; G: gluten.

cause uncooked pasta to appear translucent, resulting in less reflected light in high-protein pasta.

Besides, I-SDS and WRC was determined on the blend of flour and starch and gluten (Table 4). I-SDS values increased due to the addition of gluten and decreased due to the addition of starch; this result was expected because of the simple dilution effect that the latter has on proteins. The results observed with the addition of starch and gluten was less defined on WRC. No differences were found for MCA in any of the substitution levels, while BAG was only significantly greater when it was replaced by 6% gluten; whereas BUK and TRI flours showed a more marked increase of WRC with the addition of gluten.

With Pearson correlation analysis between I-SDS, WRC, and the colour of dough pasta obtained with these blends, it was found that I-SDS presented correlation with yellowness ($r = 0.81^{**}$) and with CS ($r = 0.76^{**}$), while WRC presented correlation with all colour components (lightness: $r = -0.82^{**}$; redness: $r = 0.81^{**}$; yellowness: $r = 0.61^{**}$), as it was found for the different flours used in this work. Considering the significance of b^* component in the estimation of colour score and the positive correlation between I-SDS content and b^*, it could be observed that in general pasta with a greater colour score were those where I-SDS content was higher (Table 4). The exception observed in the samples made from BUK may be because the higher protein content affected L^* component negatively, although it also increased b^* component; this situation is important to consider, since both yellowness and lightness are desired characteristics in pasta colour.

Effect of Different Water Amounts in Formulation

Lightness was affected negatively by the addition of higher amounts of water to the dough, finding significant differences (ANOVA, $p < 0.05$) in all the samples, except in pasta made from BAG (Fig. 3a). In the case of a^* component (Fig. 3b), although a tendency to increase with the amount of water added can be observed, significant differences were

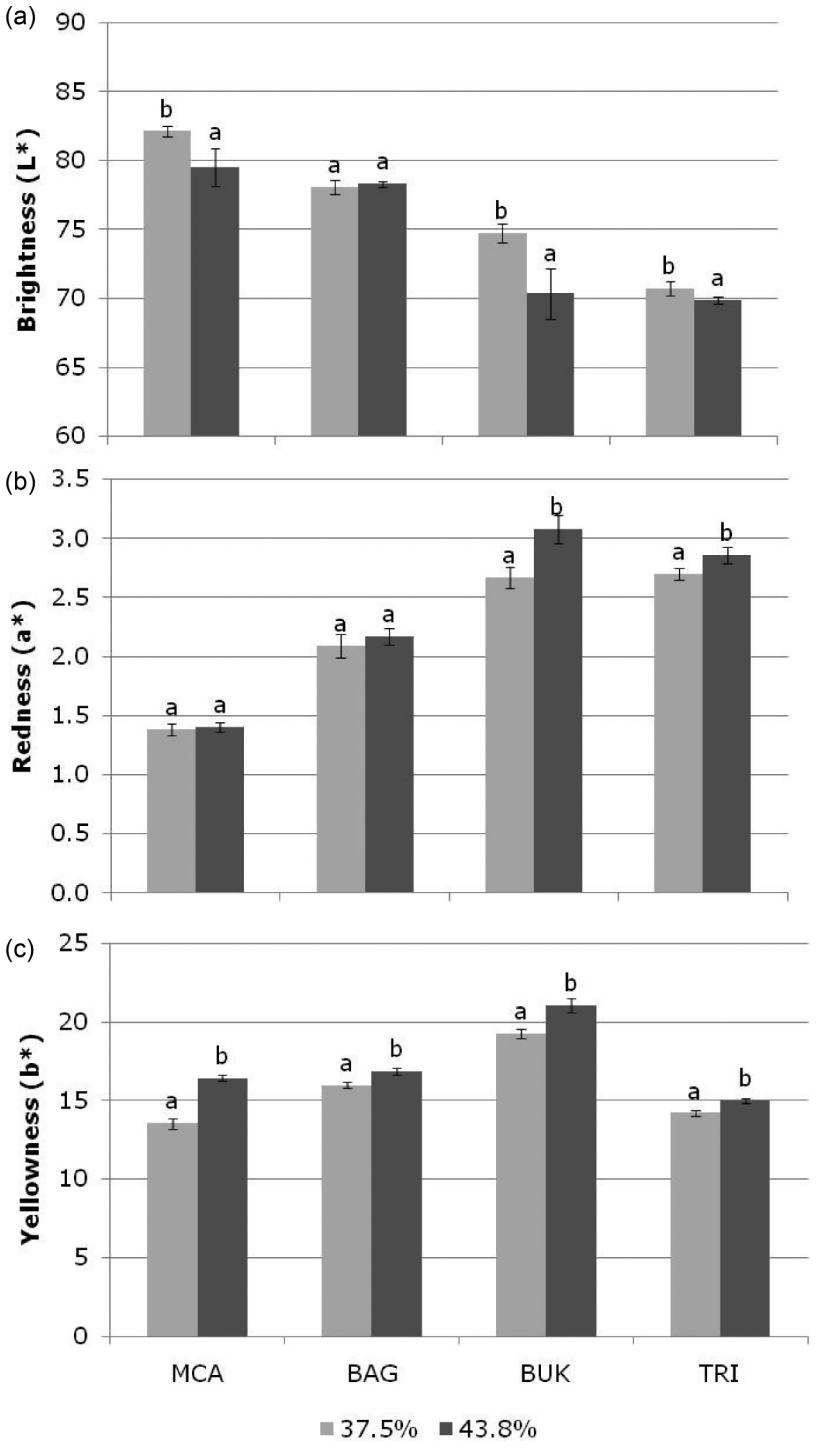

Figure 3 Effect of the addition of different amounts of water on dough pasta colour: (a) lightness, (b) redness, and (c) yellowness. Error bands correspond to standard deviation. Values followed by a different letter are significantly different ($p \leq 0.05$) within a sample.

only found for BUK and TRI. On the contrary, b* component was significantly higher with the addition of higher amounts of water to the dough (Fig. 3c). Similar results were observed in works performed with noodles.[1,4,26,28,29] To quantify the effect that the different flours and the addition of starch and gluten have on pasta colour, an overall ANOVA was performed (Table 5). Also the consequence on colour of different amounts of water added to pasta dough was evaluated.

In the first case, it was found that almost the entire (99%) experimental variation can be explained by the model proposed which includes terms such as flours, modifiers (starch and gluten) and the interaction flour × modifiers. Flours are the ones that provide the greatest variation to the model for b*, followed by a*, while for L*, the modifiers produced the greatest source of variation. The flour × modifiers interaction only showed a minor effect on colour, being more marked on b* component.

In the second case, for the model proposed for flours, the different amounts of water added, and the interaction flour × water added, also experimental variation could be explained at least in a 96%. Once again, flours were clearly the greatest source of variation in pasta colour, especially on b* component, and then on a* component. The effect of the addition of different amounts of water on pasta colour, was particularly important on yellow-blue (b*) colour space, while on the rest of the colour components the effect was much less. The flour × water interaction only showed a small effect, and like for the rest of the terms of the model, it was more pronounced on b* component.

Furthermore, another overall ANOVA was conducted to evaluate which of the two treatments, modifiers (starch and gluten) or amount of water added, affected pasta colour more. Overall ANOVA, with a good adjustment of the model ($\geq$0.89), showed that the effect of the treatments on pasta colour was different in each of the colour components. While for lightness (L*) and yellowness (b*) the greatest source of variation were clearly

Table 5 Model fit and F-values of analysis of variance (ANOVA) of pasta dough sheet colour of different flours, substituted with 5 and 10 g of starch, 3 and 6 g of gluten, and 37.5 and 43.8 ml of water/100 g flour.

Treatments		Source	L*	a*	b*
Flours		Model fit	0.99	0.99	0.99
Modifiers	F-Value[1]				
		Model	1439.43	724.74	726.58
		Flour (F)	6190.6	2627.05	2559.85
		Modifier (M)	947.45	953.4	983.05
		F × M	19.7	14.41	30, 0
Flours		Model fit	0.96	0.99	0.99
Water	F-Value[1]				
		Model	264.49	691.52	963.98
		Flour (F)	568.14	1567.75	1929.28
		Water (W)	74.56	65.29	739.36
		F × M	24.15	24.04	73.57
Water		Model fit	0.89	0.97	0.98
Modifiers	F-Value[1]				
		Model	83.27	388.82	693.93
		Water (W)	420.6	521.12	3614.54
		Modifier	75.74	712.87	647.63
		M × W	6.46	31.7	10.06

[1] All F-values are significant at $p \leq 0.001$.

the different amounts of water added, for red-green colour space (a*), the addition of modifiers had a greater weight as a source of variation, although the addition of water was also important. Although lower F-values to water-by modifier interaction were found, this can be considered a source of secondary variation, especially for a* component.

CONCLUSION

The evaluation of the effect of different flours and the two levels of starch, gluten, and water added to the formulation on the fresh pasta colour, showed that the flours were the ones that affected more the colour of the resulting pasta. Considering that the flours studied came from cereals that did not suffer a selection pressure for a higher pigment content and less oxidase content, it was found that pasta lightness and redness were affected mainly by the ash content, while yellowness was affected mainly by the protein content. Besides, from the CS calculated and being b* the colour component of greater weight in the formula, pasta made from flours with higher amounts of proteins was the best qualified. Curiously, colour flour profile, in terms of a* and b* values, was different from the colour of pasta dough, meaning that flour particle hydration and dough processing modified flour colour. When flours were substituted partially with starch or gluten, it was found that yellowness was affected mainly by the protein content; that is, when the protein content was diluted or concentrated. However, in this case it is necessary to consider a point of equilibrium for the negative effect that proteins had on lightness, as observed for the studied flours, beyond the well-known negative effect of ash on lightness, too. From the study of protein fractions, it was found that albumins and globulins correlate with a* component, while gliadins and soluble and insoluble glutenins correlate with b* component, but only glutenins presented a correlation with colour score. The overall ANOVA showed that the studied flour effect was quantitatively the most important in all colour components with respect to starch, gluten, and water effects. When the influence of water added to the formulation on fresh pasta was evaluated with respect to the incorporation of starch and gluten, it was found that the first one affected mainly lightness and yellowness, while gluten and starch had a bearing on a* component.

ACKNOWLEDGMENTS

This work was sponsored by the YPF Foundation. The authors also express their appreciation to M.Sc. Laura Gonzalez for her helpful advice on statistical analysis during the preparation of this manuscript.

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