Storage and preservation of dry pasta into biodegradable packaging made from triticale flour

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Abstract We studied the quality of dry pasta packaged and stored for 45 days in biodegradable bags made from triticale flour films. Results were compared with the quality of pasta packaged in commercial bags. Characterization of films and technologic and microbiological quality of pasta were performed. Biodegradable bags presented adequate properties during storage. There was no microbiological growth or differences in moisture and breaking force of dry pasta within both types of packaging during storage. Cooking quality of pasta was not affected by the type of packaging or storage time. We also studied the antioxidant capacity of pasta enriched with partially-deoiled chia flour during storage in both types of packaging. A decrease in the antioxidant activity measured by ABTS assay was found at 45 days of storage in pasta packaged in biodegradable bags. Nevertheless, the total phenolic content and the antioxidant activity evaluated by FRAP method did not change significantly with time or type of packaging.

Keywords Shelf life · Biomaterial · Pasta quality · Packaging · Triticale · Cooking quality

Introduction

Most food packaging materials are manufactured using petroleum-based non-biodegradable polymers, and their discard is becoming a serious environmental concern. The partial replacement of these materials with biodegradable polymers from renewable sources can reduce the impact that those packaging materials have on the environment (de Camargo Andrade-Molina et al. 2013). Some of the polymers obtained from renewable sources have been proved as materials in the production of new types of sustainable food packaging (Borneo et al. 2016; Martins Sousa et al. 2016). The importance of bio-based materials is increasing all over and their functionality for food packaging is a big challenge for the sector. Previous studies have developed biodegradable films elaborated with triticale flour and have investigated their physiochemical and functional properties (Borneo et al. 2016).

Food pasta is a popular staple food all over the world. Potentially, pasta is a useful carrier for substances acting as nutrition complement or providing specific physiological functions (Li et al. 2014). So, pasta may be a meal-carrier to add antioxidant compounds and enhance the nutrition of human diets (Borneo and Aguirre 2008; Aranibar et al. 2018; Lu et al. 2018). On the other hand, consumer acceptance is influenced by the color, cooking properties, texture and flavor of the pasta. The raw dry pasta should have an attractive appearance, while the cooked pasta should retain a good color, pleasant flavor, minimum cooking losses and good firmness (Borneo and Aguirre 2008; Carini et al. 2014; Brandolini et al. 2018).

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To the best of our knowledge, there are no studies on the use of bio-based packaging materials with adequate properties for dry pasta preservation during storage.

The aim of the present study was to evaluate and determine (i) the shelf life and quality of dry commercial pasta and (ii) the antioxidant capacity of dry enriched pasta, packaged both in bags made from biodegradable triticale flour films and compare with those packaged in commercial bags. The characterization and analysis of films and pasta samples were also performed before and during storage with the main objective to observe the efficiency of the produced packaging biomaterial.

Materials and methods

Materials

Packages of dry commercial pasta of the same brand and lot number were purchased from a local retail market. Commercial wheat flour (*Triticum aestivum*) and chia seeds (*Salvia hispanica* L.) were obtained in a local market. All chemical reagents were of analytical grade.

Biodegradable packaging preparation

To form the bio-based bags used in this study two rectangles of triticale flour films of 12 cm × 9 cm were cut and sealed by heat in three sides. The triticale flour films were obtained by dispersion of triticale flour (4.0 g/100 mL) in water for 15 min, pH 10.7. Glycerol (30 g/100 g flour) was added as plasticizer, the film forming solution was stirred at 75 °C for 15 min and poured onto a flat silicon tray. Films were dried at 30 °C in an oven with air circulation. Dry films were conditioned in an environmental chamber at 25 °C and 52% relative humidity before characterization. Thickness of the films was determined using a micrometer Schwyz SC1. The average value of five measurements at different positions on each film sample was used in all calculations.

Pasta packaging and storage

Samples of dry pasta were placed into triticale flour bags (made with triticale flour films) and sealed completely. Heat seal was used for joining triticale films. Commercial bags (made with polyethylene films) were used as a control pouch (Fig. 1). Bags were stored under light at room temperature for 45 days. Samples of pasta and triticale films (from the bags) were taken at 0, 15, 30 and 45 days of storage and used for analysis.

Biodegradable packaging characterization

Moisture content and water vapor permeability (WVP) of films

Moisture content of triticale flour films was determined according to Aguirre et al. (2013). Triplicate measurements were conducted for each type of film and an average was taken as the result. WVP of triticale flour films taken from biodegradable bags were measured according to Aguirre et al. (2011).

Mechanical properties: puncture strength

The puncture strength (PS) test provides information about the mechanical resistance of the films. In this study the PS was measured using a texture analyzer Instron Texturometer according to Salvucci et al. (2019). Experimental measurements were fitted using the following model:

$$\text{Polynomic model: } PS = ax^2 + bx + c \quad (1)$$

where x is the storage time (days). In previous studies Borneo et al. (2016) fitted experimental values of the puncture test of stored triticale flour films using several models. The polynomial model explained better (than the other models) the variability of puncture test with higher coefficients of determination (R^2) values. Excel was used to fit data to the models.

Color evaluation

Measurements of the color of the triticale flour films were taken on a white background with a Minolta colorimeter (Minolta CM-508d, Tokyo, Japan). The Cielab parameters (L^* , a^* and b^*) were measured in five positions randomly selected for each film. The difference in color (ΔE) was calculated as:

$$\Delta E = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2 \right]^{1/2} \quad (2)$$

where ΔL^* , Δa^* and Δb^* represent the differentials between the color parameter of the stored film at time t (from biodegradable bags) and the control films (at time 0).

Scanning electron microscopy (SEM)

The microstructure of the films samples was obtained using a FE-SEM Sigma scanning electron microscope (Facultad de Matemáticas, Astronomía y Física, Universidad Nacional de Córdoba, Córdoba, Argentina). The samples were prepared accordingly with a fine gold layer before obtaining the micrographs.

Fig. 1 Pasta samples in commercial (C) and biodegradable (B) bags

Pasta characterization

Cooking quality determination

Cooking quality of pasta was evaluated using the official methods of the American Association of Cereal Chemists (AACC 2000). Optimum cooking time (OCT), weight gain (WG), and cooking loss (CL) were evaluated.

Moisture, texture and color

All pasta samples were tested in duplicate for moisture (AACC 2000). Texture of uncooked and cooked pasta was analyzed using a texture analyzer Instron Texturometer equipped with a 500 N cell. Raw pasta was evaluated by the three-point bending test (AACC 2000) carried out at room temperature and the Breaking Force (N) was determined. Firmness (N) of cooked pasta was evaluated using the Application Study Ref N002/P35 (Stable Micro System, Surrey, UK). An AP/35-cylinder probe was used and force was measured in compression mode at fixed 50% strain.

The color was measured using a Minolta colorimeter (Minolta CM-508d, Tokyo, Japan). Three random readings were recorded on the surface of aligned pasta strands. The results were expressed in the CIE LAB space as L^* , a^* and b^* values. Color differential (ΔE) between pasta packaged in biodegradable triticale flour bags (BB) and pasta packaged in commercial bags (CB) was calculated as follows:

$$\Delta E = \left[(L_{BB}^* - L_{CB}^*)^2 + (a_{BB}^* - a_{CB}^*)^2 + (b_{BB}^* - b_{CB}^*)^2 \right]^{1/2} \quad (3)$$

Microbiological count

Counts of aerobic mesophilic bacteria were determined by culturing samples in PCA (Plate Count Agar, Britania Lab). Quantitative enumeration was done using the pour-plated method. Ten grams of each raw pasta sample were blended and homogenized in 90 mL peptone water sterile (0.1 g/100 mL). Plates were incubated for 48 h at 28–30 °C. The results were expressed as colony forming units per g of pasta (CFU/g) (FDA-BAM 2001).

Counting of total yeasts and molds was determined as stated above except that YGC (Yeast Glucose Chloramphenicol Agar, Britania Lab) was used as culture medium by pour-plated method. Plates used for counting were those containing 10–100 colony forming units (CFU). All plates were incubated at 25 °C for 5 days. The results were expressed as colony forming units per g of pasta (ISO method 7954 1987).

Enriched pasta preparation and storage

To study the capacity of biodegradable bags to maintain the antioxidant activity of fortified food, enriched pasta with 5.0% (weight flour basis) of partially-deoiled chia flour (PDCF) was prepared, packaged and stored. PDCF was obtained according to Aranibar et al. (2018). The composition of the PDCF obtained was: 27.70 ± 0.18 protein (% d.b.), 5.62 ± 0.15 ash (% d.b.), 7.06 ± 0.28 lipids (% d.b.) and 221.20 ± 5.49 total polyphenols (mg gallic acid equivalent/100 g). A small-scale standardized laboratory procedure was used for pasta manufacture (Aranibar et al. 2018). Pasta samples were packaged and stored as is explained previously in 2.3.

Antioxidant properties of enriched pasta

Extraction of phenolic compounds

One gram of PDCF enriched pasta powder (milled in a coffee grinder) was extracted with 10 mL of a mixture acetone/water (70:30 v/v), for 7 h at room temperature in darkness. The solution was centrifuged at 5000 g/30 min, the supernatant was removed and stored at -80°C for its subsequent measurement. Samples were prepared in duplicate.

Total polyphenol content (TPC)

The Folin–Ciocalteu method (Orthofer and Lamuelas-Raventos 1999) was followed to measure TPC of extracts. The absorbance was read at 750 nm. TPC was calculated by linear regression using gallic acid as standard. Results are expressed in mg of gallic acid equivalents (GAE) per 100 g of sample. All samples were analyzed in duplicate.

Determination of antioxidant capacity

The antioxidant capacity of enriched pasta was measured by two methods. The ferric reducing ability of plasma assay (FRAP) was performed to evaluate the reducing power and the ABTS^{•+} radical cation decolorization assay, to assess the radical scavenging activity. FRAP assay was done according to Benzie and Strain (1996). Results were expressed in mmol Trolox eq./100 g of pasta. The ABTS^{•+} radical cation storage solution was produced by reacting 7 mM ABTS^{•+} with 2.47 mM potassium persulfate solution (1:1 v/v) and storing in the dark at room temperature for 12–16 h before use. The working solution was prepared by dilution of the storage solution with ultra-pure water at a ratio of 1/80 to obtain an absorbance of 0.70 ± 0.02 at 734 nm. A solution of 300 μL of extract + 200 μL distilled water was prepared. One hundred μL of this solution were added to 1900 μL of working solution. Mixtures were incubated in the dark for 15 min and absorbance measured at 734 nm by using a spectrophotometer (V730 UV–visible, Jasco, Japan) against a reagent blank. Trolox was used as standard. All pasta samples were analyzed in duplicate and results were expressed in mg Trolox eq./100 g of pasta.

Statistical analyses

Analysis of variance (ANOVA) was carried out to test mean differences and Fisher LSD multiple comparison test was used to find out which means were statistically and significantly different ($\alpha = 0.05$). Statistical data were performed using the InfoStat statistical software (Infostat 2019).

Results and discussion

Film characterization

Thickness of triticale flour films was $170 \pm 30 \mu\text{m}$. The functional properties of triticale flour films from biodegradable bags during 45 days of storage were periodically determined and the results are shown in Table 1.

The moisture content (MC) values of triticale flour-based films ranged from 15.00 to 16.42% and as can be observed in Table 1, there was no significant change in the MC of films from stored biodegradable bags. The observed WVP value of $2 \times 10^{-11} \text{ g}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$ agrees with previous results (Borneo et al. 2016) and did not change significantly with the storage time of bags. Values of puncture strength (PS) of films shown in Table 1 are in the range of those described by other studies for triticale flour films (Borneo et al. 2016; Salvucci et al. 2019) and whey protein films (Díaz et al. 2016). Only after 45 days of storage bags showed statistically significantly higher ($p < 0.05$) PS values than films at time 0. Similar changes in mechanical properties of triticale films have been attributed to new chain–chain associations that can be formed during storage (Borneo et al. 2016). The experimental values obtained of PS during storage time studied were adjusted by Eq. 1. The polynomial model explains the variability of PS with the time with a coefficient of determination (R^2) of 0.9242. Packaging color is an important factor in terms of consumer acceptance. Color properties are essential for packaging applications of these films. Color parameters of films from stored bags are shown in Table 1. The values of parameter L^* observed in the bags during storage are close to those reported by Borneo et al. (2016) for triticale films. In this study no significant differences for L^* were found during storage. Regarding the a^* color parameter, it can be observed that the only statistically different value ($p > 0.05$) was obtained at 30 days of storage. Color parameter b^* and total color difference (ΔE) increased their values after 30 days of storage. This variation in b^* values is related to an increase of the yellowness of the films with the time. Similar trend was found by Borneo et al. (2016).

Microstructure of the triticale flour films

Micrographs of triticale flour films (surface and cross section), at a magnification of $1000 \times$ are presented in Fig. 2. Initially (S0 and C0), triticale flour films had compact matrix and structural integrity. After 45 days of storage the films presented a structure with some few discontinuous zones and few cracks or fissures distributed along the network. Change in puncture strength was

Table 1 Properties of triticale flour films from biodegradable bags at different storage times

Storage time (days)	Moisture content (%)	WVP $\times 10^{11}$ ($\text{g}^{-1} \text{s}^{-1} \text{Pa}^{-1}$)	Puncture strength (MPa)	Color values			
				L*	a*	b*	ΔE
0	16.42 $\pm$ 3.74 ^a	2.00 $\pm$ 0.25 ^a	1.07 $\pm$ 0.06 ^a	85.87 $\pm$ 0.86 ^a	− 1.01 $\pm$ 0.01 ^a	5.73 $\pm$ 0.19 ^a	
15	15.47 $\pm$ 4.41 ^a	1.88 $\pm$ 0.25 ^a	0.98 $\pm$ 0.03 ^a	84.94 $\pm$ 2.07 ^a	− 1.04 $\pm$ 0.23 ^a	6.17 $\pm$ 1.52 ^a	1.03 ^a
30	16.41 $\pm$ 3.76 ^a	2.10 $\pm$ 0.20 ^a	1.16 $\pm$ 0.03 ^a	84.43 $\pm$ 3.88 ^a	− 1.49 $\pm$ 0.01 ^b	9.63 $\pm$ 1.15 ^b	4.18 ^b
45	15.00 $\pm$ 3.27 ^a	2.00 $\pm$ 0.16 ^a	1.31 $\pm$ 0.04 ^b	83.90 $\pm$ 1.06 ^a	− 1.04 $\pm$ 0.01 ^a	9.12 $\pm$ 1.19 ^b	3.92 ^b

Reported values are mean $\pm$ standard deviation. Different lowercase letters as superscripts in a column indicate significant differences ($p < 0.05$)

Fig. 2 SEM micrographs of surface (S) and cross-section (C) of triticale flour films at 0 and 45 days of storage

accompanied by small changes in the film structure as observed by SEM (S45 and C45) in Fig. 2.

Packaged pasta characterization

Our first goal was to test the potential of the biodegradable packaging to store dry commercial pasta. So, raw pasta quality and cooking quality of pasta packaged in commercial and biodegradable bags were evaluated and compared.

Properties of uncooked pasta

Dry pasta samples had moistures ranging from 9.04 $\pm$ 0.38% to 9.76 $\pm$ 0.51% when were stored in commercial bags and from 8.98 $\pm$ 0.99% to 9.76 $\pm$ 0.51% when stored in triticale flour bags, with no statistically significant ($p > 0.05$) differences in the values obtained with the both types of packaging (Table 2) during storage.

Food pasta color is a feature of customer's assessment of the pasta quality. The pigments causing the natural coloring belong to the group of xanthophylls (carotenoids). The color difference (ΔE) between pasta samples packaged in commercial and biodegradable bags during storage was calculated. The perception of the ΔE varies according to the sensitivity of the human eye and the observed color. But we can say that the human eye only distinguishes color difference if ΔE is larger than 3 (Bodart et al. 2008). The color difference ΔE between pasta packaged in commercial and biodegradable bags was assessed using Eq. 3. The ΔE values obtained were equal or less than 3 (3, 2.25 and 0.75 for 15, 30 and 45 storage days respectively) and therefore the human eye does not detect any difference in the color of the pasta in both type of packages.

The maximum breaking force is the force at which a pasta sample (e.g. spaghetti strand) fractures under compression. It is an indication of the strength of the pasta and how it will withstand handling and storage. Results of the effects of different packaging bags on the breaking force of raw pasta during storage are shown in Table 2. The breaking force obtained (from 1.48 to 1.86 N) did not differ among storage time or packaging bags.

Microbiological analyses

Microbial contamination should be controlled for the safety and quality of the stored food products. All pasta samples were evaluated between 0 and 45 days to detect levels of total aerobic mesophilic bacteria, yeasts and molds. At time 0 the pasta samples tested negative for aerobic mesophilic

Table 2 Comparison of characteristics of pasta samples packaged in commercial and biodegradable packaging during storage

Parameters	Storage time (days)	Commercial packaging	Triticale flour packaging
<i>Uncooked pasta</i>			
Moisture (%)	0	9.76 ± 0.51 ^{aA}	9.76 ± 0.51 ^{aA}
	15	9.06 ± 0.41 ^{aA}	9.04 ± 0.69 ^{aA}
	30	9.04 ± 0.38 ^{aA}	9.31 ± 0.72 ^{aA}
	45	9.17 ± 0.31 ^{aA}	8.98 ± 0.99 ^{aA}
ΔE	0	–	–
	15	–	3.00
	30	–	2.25
	45	–	0.75
Breaking force (N)	0	1.48 ± 0.10 ^{aA}	1.48 ± 0.10 ^{aA}
	15	1.59 ± 0.35 ^{aA}	1.66 ± 0.21 ^{aA}
	30	1.58 ± 0.31 ^{aA}	1.55 ± 0.20 ^{aA}
	45	1.68 ± 0.09 ^{aA}	1.86 ± 0.20 ^{aA}
<i>Cooked pasta</i>			
Weight gain (%)	0	168.50 ± 12.21 ^{aA}	168.50 ± 12.21 ^{aA}
	15	168.80 ± 27.11 ^{aA}	167.00 ± 21.80 ^{aA}
	30	162.00 ± 20.14 ^{aA}	160.00 ± 16.37 ^{aA}
	45	172.50 ± 16.51 ^{aA}	178.6 ± 21.50 ^{aA}
Cooking loss (%)	0	5.44 ± 0.50 ^{aA}	5.44 ± 0.50 ^{aA}
	15	5.70 ± 0.69 ^{aA}	5.79 ± 1.00 ^{aA}
	30	5.50 ± 0.47 ^{aA}	5.50 ± 0.50 ^{aA}
	45	5.50 ± 0.40 ^{aA}	5.60 ± 0.50 ^{aA}
ΔE	0	–	–
	15	–	3.00
	30	–	1.00
	45	–	1.09
Firmness (N)	0	5.3 ± 1.58 ^{aA}	5.3 ± 1.58 ^{aA}
	15	4.79 ± 1.59 ^{aA}	4.79 ± 1.59 ^{aA}
	30	4.84 ± 1.37 ^{aA}	4.79 ± 1.33 ^{aA}
	45	4.11 ± 0.65 ^{aA}	4.09 ± 0.42 ^{aA}

Reported values are mean ± standard deviation. Different lowercase letters in a column (in each characteristic) indicate significant differences ($p < 0.05$). Different capital letters in the same row (in each characteristic) indicate significant differences ($p < 0.05$)

bacteria and yeast and mold, indicating no contamination and good manufacturing practices. The microbiological analyses of pasta samples (Table 3) evidenced very low values of CFU/g for all studied groups of microorganisms at all storage times. The maximum microbial number reached in all samples was close to zero. The

biodegradable packaging used allowed that all microbial groups remained at very low levels during storage and all samples satisfied the microbial limits imposed by the national law (Cabrera 2013).

Table 3 Microbial evolution in dry pasta packaged in commercial (C) and biodegradable (B) packaging during storage

Storage time (days)	0	15		30		45	
		C	B	C	B	C	B
Aerobic mesophilic bacteria (CFU/g)	1.0×10^1	1.0×10^1	4.5×10^1	3.5×10^1	4.5×10^1	4.0×10^1	4.0×10^1
Yeast and mould (CFU/g)	0	0	0	0	0	1.0×10^1	0

Fig. 3 Total phenolic content and antioxidant capacity by FRAP and ABTS assays of pasta made with 5% PDCF and packaged in commercial (C, dotted bar) and biodegradable (B, cross bar) bags. Bars are the mean $\pm$ SD of 4 values. ns = not significant; * = significant ($p < 0.05$)

Cooking quality and properties of cooked pasta

The cooking quality characteristics of commercial dry pasta packaged in commercial and biodegradable bags were evaluated. There was no statistically significant difference in the optimum cooking time between samples during storage time. Pasta samples had the same value of optimum cooking time: 9.33 ± 0.52 min. Generally, the cooking time of dry pasta is higher than 5 min (Padalino et al. 2013). For all samples, the other cooking characteristics were evaluated at the optimum cooking time obtained. Measurement of cooking loss is an important criterion frequently used as an indicator of pasta quality. During pasta cooking, soluble components wash away and the cooking water becomes dense and thick (Sant'Anna et al. 2014). The weight gain is an indicator of the water absorbed by the starch and the proteins during cooking. The results of cooking loss and weight gain obtained in this work are shown in Table 2. There was no statistically significant ($p > 0.05$) difference in cooking loss or weight gain between pasta packaged in commercial and biodegradable triticale bags during storage time. Reported data evidenced that the cooking quality was maintained in the biodegradable packaging as in the commercial one.

The differences of color ΔE calculated by the Eq. 3 were equal or less than 3 (Table 2) so, these variations were not visually perceptible and the different types of bags did not affect the color of the pasta after the cooking procedure. Data concerning firmness of cooked pasta, the most important textural characteristics of quality, are also shown in Table 2. The values of firmness obtained for pasta packaging in commercial or biodegradable triticale bags did not change significantly during storage. These results showed a comparable performance of both types of packaging regarding these properties.

Antioxidant activity of enriched pasta

Antioxidants and other functional ingredients have been added into food products to enhance their nutritional value (Boroski et al. 2011; Lu et al. 2018). Foods (such as chia seed) with high total phenolic content can contribute to human health because of their antioxidant capability. One of the purposes of food pasta enrichment with partially-deoiled chia flour (PDCF) is the boost of its antioxidant activity (Aranibar et al. 2018). Different methods are used for the measurement of the antioxidant activity of foods. They act through several mechanisms and with the generation of different radicals. Most of the methods generate a redox-active compound or colored radical. The capability of the food sample to scavenge the radical or to reduce the redox-active compound is measured by spectrophotometry.

Some methods are based on an electron transfer and involve the reduction of a colored oxidant, e.g. in ABTS and FRAP assays. The ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) method measures the antioxidant activity of phenolics and other antioxidants determined by the decolorization of the $ABTS^{\cdot+}$, as the inhibition of absorbance at 734 nm. The ferric reducing ability of plasma (FRAP) assay involves the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). When applied to food analysis, the antioxidant capacity measurements may be different depending on the assay used (Floegel et al. 2011). In this work we studied the antioxidant activity of PDCF enriched pasta during storage in biodegradable triticale bags. Figure 3 shows the Total Phenolic Content (TPC) and the antioxidant activity evaluated by FRAP and ABTS methods of PDCF enriched pasta packaged in commercial and biodegradable bags for 45 days. All pasta samples (at different storage time and/or packaging) showed similar values of TPC and antioxidant capacity measured by the FRAP assay. However, a statistically significant decrease in antioxidant activity of pasta in biodegradable triticale bags was observed when measured by the ABTS method at 45 days of storage, compared with those packaged in commercial bags. This is likely due to the possibility of increased interactions between the product (pasta) with the package and/or the environment in biodegradable packaging as compared to the commercial packaging which are known to be good barriers for product protection. Similar protective effect of a defatted peanut flour-based film on sunflower oil during storage was observed by Riveros et al. (2018). The results observed in this study indicate that the triticale flour bags maintain the antioxidant capacity of enriched pasta in a similar way to commercial bags until 45 days of storage.

Conclusion

This study showed that triticale flour film is a suitable material for dry pasta packaging. The biodegradable bags prepared with triticale films showed color, barrier and mechanical properties suitable for use as packaging for dry pasta during 45 days of storage. Pasta could be stored for 45 days with the same quality and characteristics and without microorganism growth. This work illustrates the potential for using biodegradable triticale flour films to packaged dry pasta. For enriched pasta products the protective effects of antioxidant activity were express without the incorporation of any additive in the film forming solution formulation.

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Compliance with ethical standards

Conflict of interest The authors report no conflict of interest.

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