Martín-Esparza, M.; Raigón Jiménez, MD.; Raga-Soriano, A.; Albors, A. (2018). Functional, Thermal and Rheological Properties of High Fibre Fresh Pasta: Effect of Tiger Nut Flour and Xanthan Gum Addition. Food and Bioprocess Technology. 11(12):2131-2141. doi:10.1007/s11947-018-2172-8

Functional, thermal and rheological properties of high fibre fresh pasta: effect of tiger nut flour and xanthan gum addition

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ABSTRACT

Tiger nut flour (TNF) is a rich source of dietary fibre with potential to be used in cereal based products. However, research on improving the rheological properties of tiger nut based doughs is limited. In this paper, the significance of TNF and xanthan gum (X) incorporation into fresh egg pasta, in terms of its thermal and dynamic rheological properties, has been investigated. Plain semolina pasta (DWS) was used as control. High fibre doughs (20 and 40% TNF) with or without X (0 and 1 %) were assessed. Both fundamental (dynamic oscillatory and creep tests) and empirical (texture profile analysis) tests were performed to assess the viscoelasticity of TNF-DWS composite blends. Raw solids (TNF, DWS) were characterised in terms of their chemical composition, particle size distribution and functional properties. For both fresh and cooked pasta, water activity, water content and gelatinisation temperatures were estimated. The results from the rheological tests revealed that partial replacement of DWS by TNF lead to less cohesive and weaker structures due to the lower presence of a gluten network. X significantly improved the rheological response of the TNF based doughs. Thermal analysis showed a single endothermic peak in the temperature range between 60 and 78°C during heating, which corresponds to the amylopectin gelatinisation. However, when replacing 40% of DWS by TNF, two-phase transitions were observed, probably associated to the starch tiger nut gelatinisation or the formation of amylose-lipid complexes. The optimum cooking time for the tiger nut pasta was two minutes as determined by calorimetric analysis.

Keywords: fresh pasta; tiger nut flour; fibre; xanthan gum; dynamic rheology; DSC

1. Introduction

Nowadays, it is a fact that consumers increasingly demand healthier foods obtained by using raw ingredients that are naturally high in components with health-promoting effects. Epidemiological studies show a correlation between diets which are moderate to high in fibre and a lower incidence of some chronic disorders such as cardiovascular diseases (Kendall et al. 2010), diabetes (Chandalia et al. 2000; Schulze et al. 2004), colon cancer (WHO 2003), gastrointestinal disorders (Kaczmarczyk et al. 2012) and obesity (Buttriss and Stokes 2008). It is also a fact that the daily fibre intake per capita is still today lower than that recommended by the WHO (25 g / day, Romo et al. 2008). Tiger nut flour is a rich source of dietary fibre (8-15g/100g), minerals such as phosphorus, potassium, iron and calcium, and vitamins E and C (Sánchez-Zapata et al. 2012). It also has a high lipid content (23-31 g/100 g) with a fatty acid profile similar to olive and hazelnut oil (predominance of oleic, linoleic and linolenic acids) (Aguilar et al. 2015; Demirkesen et al. 2013). Tiger nut flour has a moderate amount of proteins with a demonstrated higher amount of essential amino acids (such as lysine, cystine, arginine and histidine) than the protein standard proposed by the FAO/WHO (1985) for satisfying adult needs (Ade-Omowaye et al. 2008). Therefore, tiger nut flour incorporation into pasta products -a staple cereal-based food that is widely produced and consumed across the world, easy to prepare and with a low price for consumers- could help to overcome this deficit whilst providing health benefits (Aravind et al. 2012; Yokoyama et al. 1997). Other recent studies have been assessed the possibility of turning pasta into a functional food with health benefits by adding ingredients rich in dietary fibre, such as teff (Giuberti et al. 2016), kimchi by-product (Kim et al. 2017), bran (la Gatta et al. 2017; Kaur et al. 2012), brewer's spent grains

(Cappa and Alamprese, 2017) or tomato by-product (Padalino et al. 2017). Considering the nutritional value of tiger nut flour, it may be a potential and interesting alternative to improve not only the fibre content of wheat formulations but also to provide a better nutritional quality. Pasta products made from wheat are characterised by proteins that form a viscoelastic gluten network, providing a certain cohesiveness to the dough and contributing to the cooked pasta's water holding capacity (Wieser 2007). Cooked pasta made from durum wheat semolina maintains good texture and resists surface disintegration (Mastromatteo et al. 2011). Replacement of wheat semolina by significant amounts of non-gluten forming flours such as tiger nut flour can seriously constrain dough viscoelasticity and the cooking behaviour of dough matrices that have a weakened mixed protein network. In a previous work, we investigated the effect of incorporating tiger nut flour in up to 30% w/w semolina basis into fresh egg pasta (Albors et al. 2016). The obtained results revealed a need to strengthen the protein network and thus reduce cooking losses and increase the pasta's firmness (Albors et al. 2016). One way to overcome this problem is to use hydrocolloids with high water binding capacity, such as xanthan gum, to limit the swelling of the tiger nut fibre. Xanthan gum has strong viscoelastic properties, adopting in aqueous solutions a double-stranded helix rigidly ordered conformation. It can be used to mimic the properties of gluten to form the elastic texture of pasta (Larrosa et al. 2013). Previous trials (paper under publication) have demonstrated that the use of xanthan gum over 0.8% (DWS basis) may lead to better mechanical properties and cooking behaviour of tiger nut pasta. Cai et al. (2016) found positive correlations between xanthan gum content and tensile strength and texture properties of noodles. And Romero et al. (2017) found an improvement in the network strength of proso millet pasta with xanthan gum. The rheological and

mechanical characterisation of tiger nut dough pasta is important in predicting the processing behaviour and controlling the quality of fresh pasta products. The interactions between dough components (starch, protein, fibre, water and additives) as well as the flour's functional properties and particle size play an important role in the structure development and thus the rheological and thermal properties of fresh pasta (Chillo et al. 2009). To the author's knowledge, no data has been published on fresh pasta affected by tiger nut and xanthan gum incorporation. Therefore, this paper is aimed at: (i) assessing the functional properties, particle size distribution and chemical composition of wheat semolina and tiger nut flour, and their mixtures; (ii) assessing the effect of these ingredients (tiger nut flour and xanthan gum) on the rheological and thermal properties of "high fibre" fresh pasta (>6% w/w, Official Journal of European Commission, 2006). The fundamental (dynamic tests) and empirical (texture profile analysis) rheological properties, as well as the starch-water interactions (differential scanning calorimetry) were assessed in tiger nut wheat doughs and compared to wheat semolina counterparts.

2. Material and methods

2.1 Experimental design

Wheat semolina was replaced with TNF up to 40% (w/w) to obtain pasta with more than 6% (w/w) of fibre content ("high fibre"), according to the Nutritional Claims for Dietary Fiber Foods (Official Journal of European Commission, 2006). The fibre content was estimated considering the chemical composition of the raw materials (Table 1). The obtained values for tiger nut pasta ranged from 7.6 to 8.6 g/100 g pasta. Six formulations were evaluated in total, considering three levels for wheat semolina replacement (0%, 20% and 40% w/w, named henceforth as S, TNF20 and TNF40 respectively) and two for xanthan gum (0%

and 1% w/w, this last one being noted with an X at the end of the code, that is, TNF20X, TNF40X and SX). The percentage of X was chosen taking into account the obtained results in previous trials with different hydrocolloids (paper under publication). TNF and DWS were evaluated in terms of their chemical composition, functional properties and particle size distribution. For fresh pasta doughs, the following analyses were assessed: water content, water activity, fundamental (oscillatory and creep tests) and empirical (textural profile analysis, TPA) rheological properties, and differential scanning calorimetry (DSC). All measurements were carried out in triplicate.

2.2 Raw materials and characterisation

Commercial durum wheat semolina –abbreviated as DWS– (Harinas Villamayor, S.A., Huesca, Spain), tiger nut flour –abbreviated as TNF–(Tigernuts Traders S.L., Valencia, Spain) and xanthan gum 1400 cps –abbreviated as X– (E-415) (Shandong Fufeng Fermentation Co. Ltd., China) were used. Fresh eggs and mineral water were purchased in a local market. 2.2.1 Proximate composition

DWS and TNF were analysed for their water, protein, fat and ash content according to the American Association of Cereal Chemists´ approved methods (AACC, 2000) and for their total, soluble and insoluble fibre according to the Megazyme method K-TDFR (Megazyme Ltd., Ireland). Digestible carbohydrates were determined by difference (100 – percentage of estimated proximate chemical composition). Three replicates were carried out for each analysis.

2.2.2 Particle size analysis

The particle size distribution (PSD) of both DWS and TNF was determined by applying the laser diffraction method and Mie theory, following the ISO13320 normative (AENOR 2009). A Laser diffractometer (Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, U.K.), equipped with a PS 65 (dry sample) or a

wet sample dispersion unit (Malvern Instruments Ltd., Hydro 2000 MU, U.K.) was employed to perform, respectively, dry and wet analyses. The particle refraction and absorption indexes were 1.52 and 0.1, respectively, and the water refraction index was 1.03. Distributions were made in triplicate and for each sample, and 10–20 g of flour was used. Size distribution was quantified as the relative volume of particles in size bands, presented as size distribution curves (Malvern MasterSizer Micro software v 5.40). The PSD parameters recorded included largest particle size d(0.9), mean particle volume d(0.5), smallest particle size d(0.1), and mean particle diameter/volume mean diameter (D[4,3]). The Span value or measurement of the width of the size distribution, calculated from the values of standard percentiles, was complementarily reported. The wider the particle size distribution, the bigger the Span becomes. The fundamental size distribution by laser diffraction method is expressed in terms of equivalent spheres (the results are volume based), so the number distributions should only be considered as a guide of the volume distribution.

2.2.3 Functional properties

The functional properties of both DWS and TNF, and their blends with or without X, were determined as follows. Solvent retention capacity (SRC) was determined according to the AACC method 56-11 to quantify potential contributions to water-holding capacity by flour components having water-uptake capabilities (Paul and Minn 2005). The used solvents were sucrose (50% w/v), sodium bicarbonate (5% w/v) and lactic acid (5% v/v). The water-holding capacity (WHC) was determined by using the modified methods from Heywood et al. (2002) and Lin and Zayas (1987). The fat adsorption capacity (FAC) was determined according to Ahn et al. (2005). Foam capacity (FC) and foam stability (FS) were determined as described by Narayana and Narasinga Rao (1982) and modified by Alu'datt et

al. (2012).

2.3 Pasta preparation

The S formulation, used as the control sample, was obtained by mixing durum wheat semolina (71% w/w), fresh egg (13% w/w) and water (16% w/w). For the other formulations, durum wheat semolina was replaced by TNF and X at different levels, as described in section 2.1. All raw materials were mixed and kneaded in an electric cooking device (Thermomix TM-31, Vorwerk Spain M.S.L., S.C., Madrid). The dried (semolina/tiger nut flour/xanthan gum) and liquid (egg/water) components were separately mixed at low speed (set 2) for 45 s. The resulting blends were then kneaded in two steps for 5 min each (with a rest period of 5 min between them). The resulting doughs were rested for 20 min at 4 ºC inside a plastic bag in order to enable sample relaxation. Afterwards, the fresh pasta sheets (1.0±0.03 mm thick) were formed by using a domestic pasta making machine (Simplex SP150, Imperia, Italy) coupled with a specific motor (A2500, Imperia, Italy).

2.4 Water content and water activity of fresh pasta doughs

The water content (x_w, g/g) was determined according to the AACC-approved gravimetric method 44-40 (AACC 2000). The AquaLab Series 4 TEV equipment (Decagon, CX-1, sensitivity 0.001) was used to measure water activity (a_w).

2.5 Small deformation mechanical test: oscillatory and creep tests

A RheoStress rheometer (RS1-Thermo Haake, Karlsruhe, Germany) with serrated parallel plate geometry surfaces (60 mm diameter, 1 mm gap) was used to perform the oscillatory and creep tests on the pasta doughs. Dough excess was carefully removed and a thin layer of mineral oil was applied to cover the exposed sample surfaces during the measurements. Before the measurements, the dough was rested for 5 min to allow relaxation. Temperature analysis was 20

ºC. The linear viscoelastic region (LVR) was identified and established for each dough by means of stress sweep tests from 5 to 900 Pa at 1Hz. Frequency sweeps were carried out from 10 to 0.1 Hz in the LVR (between 45 y 68 Pa, depending on the pasta sample).

Creep tests were performed by applying an instantaneous and constant shear stress in the LVR for 300 s. Creep data are presented in terms of creep compliance, J, which is defined as the strain divided by the stress applied. Each test was performed in triplicate.

2.6 Empirical rheological test: TPA

A TA.XT2 Texture Analyser (Stable Micro Systems, Godalming, Surrey, UK) was used to perform the texture profile analysis (TPA) of the pasta dough. The TPA analysis provides a significant measurement of the textural characteristics of the product (Szczesniak 2002; Olivera and Salvadori 2006) and the dough machinability (Angioloni and Dalla Rosa 2007). The settings of the experiments were the following: 50% compression, 30 mm diameter probe (flat-end aluminium compression disc), test speed 1 mm/s, 75 s gap between compressions, 5 kg load cell, 5 mm dough thickness. The data was processed using Texture Exponent 6.1.7 (Stable Micro Systems Software).

2.7 Thermal properties of fresh pasta doughs

Starch gelatinisation temperature (T_p) was measured in duplicate for each pasta dough by differential scanning calorimetry (DSC 1, Mettler Toledo, España). The freeze-dried (LyoAlfa 10-85, Telstar, Spain) pasta samples (about 5 mg) were placed into 40 μl aluminium hermetic pans (Mettler Toledo, Spain) and 15 μl of water were added. The pans were placed at 4 ºC for 24 h to equilibrate the water content. The heating rate of the samples in the calorimeter was 5 ºC/min and the analysis was carried out between 20 to 80 ºC. Air was used as reference. Onset

(T_o), peak (T_p) and end (T_e) temperatures, and gelatinisation enthalpy (ΔH_n) were recorded. The same procedure was used for the cooked pasta samples at different cooking times to determine the optimal cooking time (complete starch gelatinisation). The cooking process was carried out as described on Albors et al. (2016).

2.8 Field emission scanning electron microscope (FESEM)

FESEM (ULTRA 55, Carl Zeiss AG, Oberkochen, Germany) was used in order to observe the starch granules of tiger nut flour. The samples (cross-section) were fixed on copper stubs, platinum coated and observed using an accelerating voltage of 2 kV.

2.9 Statistical analysis

Analysis of variance (ANOVA) was carried out by using Statgraphics Centurion software version 16.1.17 (StatPoint Technologies, Inc., Warrenton, VA) in order to evaluate the effects of partial semolina replacement by tiger nut flour and xanthan gum on the measured parameters. The significance level was p= 0.05 in all cases.

3. Results and discussion

3.1 DWS and TNF characterisation: chemical composition, functional properties and particle size distribution

The chemical composition and the particle size distribution (PSD) of the solid raw materials (TNF and DWS) are shown in Table 1. As expected, the TNF fibre and fat contents were much higher than those of DWS, while the protein content was much lower. On the other hand, the amount of digestible carbohydrates in tiger nut flour was lower. All this may affect the various formulations obtained after the partial substitution of DWS by TNF, meaning that pasta with different nutritional

208 properties were attained. As commented on the material and methods' section,
209 tiger nut pasta may be labelled as a "high fibre" product taking into account the
210 obtained proximate composition for TNF and DWS. The particle size distribution
211 of the solids (DWS and TNF) provides information about their water absorption
212 capacity (velocity) and uniformity (dissolution into solid systems or suspensions).
213 Particle size is a factor that will therefore affect the behaviour of the formulations
214 during their processing and the uniformity of the final product. In water (DWSh
215 and TNFh in Table 1), the results correspond with the particle size distribution of
216 the insoluble fibre. It can be observed that the average particle size (expressed
217 as the mean diameter of the equivalent volume) was lower for TNF (203 μm
218 versus 319 μm in DWS) in the dry analysis, but significantly higher in the wet
219 analysis (253 μm versus 150 μm). Furthermore, the uniformity in the particle size
220 distribution (span) was higher in dehydrated semolina, while the opposite
221 occurred in the wet analysis. The greater presence of fibre in tiger nut flour
222 (15.85% compared to 10% in wheat semolina) -with a much higher insoluble /
223 soluble ratio- (13.74/2.10% in TNF compared to 5.25/4.75% in DWS) could be
224 responsible for the greater heterogeneity in TNF (span 2.28). The capacity to
225 absorb and bind water by this fibre (as shown in section 3.1.3, the presence of
226 TNF supposes a higher WHC index) causes –therefore- an increase in the mean
227 particle size (wet analysis), with 10% of particles under 667 μm (d (0.9), Table 3)
228 and having a much lower span (4.8). The amount of starch granules and their
229 morphology also influences water absorption and, therefore, the particle size
230 obtained by wet analysis. The starch granules of tiger nut flour are rounded,
231 smaller than those of wheat, with greater solubility (Fig. 1; Abo-El-Fetoh et al.
232 2010).

233 Table 2 shows the results obtained after analysing the functional properties of

DWS and the mixtures of wheat semolina and TNF. Water holding capacity (WHC) reports the ability of a protein matrix to absorb and retain bound, hydrodynamic, capillary and physically entrapped water against gravity. The results show a significant increase ($p < 0.05$) when increasing the percentage of TNF. This increase can be attributed to higher fibre content, by increasing the percentage of tiger nut in the mix (Ade-Omowaye et al. 2008), fibre with high water retention capacity due to the high proportion of hemicellulose and lignin (Sanchez -Zapata et al. 2009). In addition, as discussed above, the smaller particle size of tiger nut flour implies a larger surface area available for water absorption (Albors et al. 2016). The solvent retention capacity reports the water retention capacity of pentosans (SRCS), damaged starch (SRCB) and glutenin (SRCAL). The values obtained for SRCS were not significantly different, but a decrease in this parameter can be observed when increasing the percentage of TNF. As for SCRB, a significant increase ($p < 0.05$) was registered with the rise in the percentage of TNF in the mixture, which could be due to a greater presence of damaged starch in the tiger nut flour. The same trend was observed between SRCB and WHC, with a positive correlation between both variables ($r = 0.944$). Finally, the value of SRCAL decreased when adding TNF (20% w/w), yet without significant differences ($p < 0.05$) when the amount increased further. This decrease is due to the lower presence of gluten and, therefore, of glutenin when using less amount of wheat in the sample. The fat absorption capacity (FAC) is attributed to the ability of proteins to bind lipids. The greater presence of fat in TNF could be responsible for the significant differences found between DWS and the other analysed samples. Finally, there was a clear decrease in both foam capacity and foam stability with the substitution of wheat semolina by tiger nut flour. The presence of xanthan gum only significantly increased the WHC value,

due to its high water binding capacity.

3.2 Water content and water activity of fresh pasta

The water content and water activity values obtained for each of the resulting dough formulations are shown in Table 3. The analysis of variance (ANOVA) showed significantly higher values ($p < 0.05$) for the 100% wheat semolina formulation. This seems logical if we consider that the water content of wheat semolina (13.67%) is higher than that of tiger nut flour (8.83%). The presence of xanthan gum did not affect the water activity of the doughs.

3.3 Rheological assessment: fundamental and empirical properties

3.3.1 Dynamic oscillatory properties: frequency sweep and creep tests

Table 3 shows that the storage modulus values (G') are significantly higher ($p < 0.05$) -in all cases- than those of the loss modulus (G") ($\tan \delta < 1$), which reveals the more elastic and less viscous nature (greater solid nature) of the studied doughs. Similar results were obtained previously using various hydrocolloids (Lazaridou et al. 2007) and modified starches (Witczak et al. 2012). It is also possible to see that the partial substitution of wheat semolina by TNF resulted -as expected- in a significant decrease in the G' and G" values, exposing the formation of a less cohesive structure in these cases, due to the lower presence of gluten. Similar results were observed in pasta made with rice (Sozer 2009) and maize (Larrosa et al. 2013), where the values of the loss modulus (G") were between 5 and 10 times lower than the values of the storage modulus (G'), and as the frequency increased G" was lower, showing a system that acted more elastically (Sozer 2009). This elastic behaviour is characteristic of a highly structured material, where the storage modulus is always greater than the loss modulus over the entire frequency range studied (Larrosa et al. 2013). As for the

hydrocolloid xanthan gum, it can be observed that the DWSX formulation has the highest G', significantly higher ($p < 0.05$) than the DWS control formulation, reflecting the clear influence of this gum. This hydrocolloid, in the tested concentration, therefore appears to influence the rheological behaviour of the control sample and that of TNF40, bringing the values of the latter closer to those obtained by the 20%. Only for TNF20 no significant differences ($p > 0.05$) were found with the use of X. The dependence of both moduli (G' and G") on the angular frequency (ω) can be explained by the parameters (a and b exponents) obtained by equations 1 and 2 (Georgopoulos et al. 2004; Sivaramakrishnan et al. 2004) (Table 4).

$$G'(\omega) = K' \cdot \omega^{a} \qquad \text{Eq. (1)}$$

$$G''(\omega) = K'' \cdot \omega^{b} \qquad \text{Eq. (2)}$$

The presence of xanthan gum caused a significant decrease in parameter a, which shows a greater independence of G' and, therefore, a more cohesive structure. However, contrary to expectations, there was no greater dependence of G' when substituting the wheat semolina by TNF. Possibly, the greater presence of fat in these formulations "mitigates" the lower structure cohesion. As for the loss modulus (G"), it is possible to see again how the values are clearly lower than those of the storage modulus (G') in the whole spectrum of frequencies. In addition, the values of exponent b are close to 0 in all cases, which demonstrates the gel-type structure (semi-solid nature) of the studied doughs.

In creep tests, a constant stress is applied and the sample is rapidly deformed, inflicting a deformation on the material that increases continuously at a decreasing rate over time (Rao and Skinner 1986). Fig. 2 shows an example of the creep curves of the control dough (DWS) and samples with the addition of

311 tiger nut flour and xanthan gum (DWSX, TNF20, TNF20X, TNF40 and TNF40X).
312 It can be observed that all the curves obtained showed a behaviour that is typical
313 of viscoelastic materials. The creep phenomenon is caused by a reorientation of
314 the bonds in this type of materials (Mariusz et al. 2012). It has been observed
315 that the viscoelastic properties of different wheat (Edwards et al. 2001) and rice
316 doughs (Sozer 2009) analysed in trials of this kind can be adequately described
317 with the Burger's model (Burgers 1935), which corresponds with the serial
318 combination of the Maxwell and Kelvin models. The experimental data from
319 creep tests were fitted by the 8-parameter Burger's model (equation 3), where Jo
320 is the instantaneous creep compliance, J_1, J_2 y J_3 are the retardation creep
321 compliances (the sum of J_o, J_1, J_2 and J_3 is the steady state compliance), λ_1, λ_2
322 and λ_3 are the retardation times of the elastic response (Kelvin component), μ_o
323 provides information about the steady-state viscosity and t shows time (Van
324 Bockstaele et al. 2011).

325 $$J(t) = J_o + J_1\left(1 - e^{\left(\frac{-t}{\lambda_1}\right)}\right) + J_2\left(1 - e^{\left(\frac{-t}{\lambda_2}\right)}\right) + J_3\left(1 - e^{\left(\frac{-t}{\lambda_3}\right)}\right) + \frac{t}{\mu_o} \quad \text{(eq.3)}$$

326 Instantaneous compliance is related to the energy of elastic stretching of the
327 bonds when stress is applied, while retardation compliances are related to the
328 disruption and conversion of the bonds (Witczak et al. 2012). Table 5
329 summarises the values obtained for the model's coefficients. It is possible to
330 observe that when comparing separately the doughs without or with xanthan
331 gum, the addition of TNF caused an increase of instantaneous compliance
332 values respect to control samples (83.8-105.4% higher in TNF20 and TNF40,
333 respectively, and 55.6% higher in the samples with X). This is consistent as the
334 instantaneous compliance is greater in materials with weak structures, whereas
335 small values are characteristic of strong (solid) or hard structures (Sozer 2009).

The incorporation of xanthan gum in the formulation increases the resistance of the dough to deformation, which is shown in Fig. 2 by the reduction of the maximum percentage of creep deformation (deformation at the end of the creep phase) (Lazaridou et al. 2007). This hydrocolloid, once hydrated, may fill up free space in the system, which strengthens dough structure. The elastic retardation or viscoelastic compliances (J_1, J_2 and J_3) increase with the tiger nut substitution percentage, which means that the greater the presence of tiger nut flour, the less the deformation resistance of the doughs. As for the retardation time (λ_1) and steady-state viscosity (μ_o), no significant differences were observed between the different formulations except for the TX formulation, which is also the one with the highest value. Longer retardation times (λ_1, λ_2 and λ_3) indicate a slower retarded elastic response. Therefore, the TX sample was the one with the greatest solid nature (the furthest one down), where the energy absorbed by the material -due to the deformation suffered- is dissipated to a lesser extent. Lazaridou et al. (2007) found that, in general, λ1 values were higher in gluten-free doughs with hydrocolloids.

3.3.2 Texture profile analysis (TPA)

The results in Table 6 show a significant decrease ($p < 0.05$) in the hardness of the doughs as the percentage of TNF increased in the formulation, with a much more pronounced decrease between the control formulation and that with a 20% substitution level. It was also observed that by incorporating the hydrocolloid xanthan gum in the formulation, the decrease in hardness was less pronounced (27.67% versus 52.27% when using 20% of TNF), reflecting the clear influence of this hydrocolloid in the analysed parameter, since it reinforces the structure by increasing the viscosity and imitating viscoelastic gluten properties. Concerning adhesiveness, the most adhesive formulation was TNF20 and the least adhesive

was TNF40X. It can also be seen how the partial substitution of durum wheat semolina and incorporation of xanthan gum influence this parameter -although without showing a visible trend, contrary to what occurs with the hardness. The same is true for the cohesiveness. Finally, the elasticity parameter shows that the most elastic formulation is TNF40X and the least elastic is T.

3.4 Thermal properties: DSC results

It is known that DSC thermal analysis allows studying the gelatinisation, retrogradation and crystallisation phenomena of starch polymers. It is possible to measure the enthalpies (heat fluxes) associated with the amylose and amylopectin fusion (with melting temperatures between 60-100 ºC, depending on the type of starch (Cano et al. 2015)) as well as the dissociation and fusion enthalpy of the amylose-lipid complexes. When the starch is heated in excess of water (water/starch ratio of 2:1) in the DSC analysis, an endothermic peak is obtained. The peak's onset (where it deviates from the baseline, T_o, °C) corresponds with the starch granule's birefringence loss onset. The area below the peak is a measurement of the energy (enthalpy, ΔH) required for the transition from an orderly (crystalline) state to a disordered or amorphous state (fusion) to take place (Biliaderis 2009). The temperature range between the onset and the end of the peak (T_c-T_o) represents the so-called gelatinisation period, and the peak temperature (T_p) is the gelatinisation temperature. This gelatinisation in excess water is believed to involve the primary hydration of the amorphous regions around and above the glass transition temperature, with an associated glassy-gum transition. This in turn facilitates molecular mobility in amorphous regions (with reversible swelling), which causes an irreversible molecular transition. This irreversible step involves the dissociation of double helices (most of which are in crystalline regions) and the expansion of the

388 granules and polymers (and interstices of the granule). After Tc, all the
389 amylopectin double helices have dissociated, although the swollen granular
390 structures have remained (Tester and Debón 2000). Table 7 summarises the
391 values obtained for the thermal properties of the fresh pasta formulations. All the
392 samples, except for those in which wheat semolina was replaced by 40% tiger
393 nut flour (TNF40 and TNF40X), had a single endothermic peak in a temperature
394 range between 61 and 78 °C during the heating process, which corresponds with
395 the gelatinisation of the amylopectin. However, for the TNF40 and TNF40X
396 samples, two phase transitions were observed. This could be due to the different
397 gelatinisation temperature of the tiger nut starch -which may gelatinise at higher
398 temperatures-, or to the fusion of amylose-lipid complexes, due to the
399 incorporation of lipid-rich tiger nut flour. It seems therefore that the incorporation
400 of TNF -from a certain value of substitution onwards (40% in the case of this
401 study) produces the emergence of these complexes, resulting in a dough of fresh
402 pasta with 7.62% fat content and a fat/starch ratio of 0.2).. Consequently, there is
403 a smaller amount of starch available to gelatinise and, therefore, a lower amount
404 of energy is needed for such transition to occur. In these formulations, no
405 significant differences ($p<0.05$) were found in terms of the gelatinisation
406 temperature (T_p value), either with the incorporation of xanthan gum or with 20%
407 of tiger nut flour.

408 When performing the DSC thermal analysis on the samples cooked at different
409 times, it can be observed that as the cooking time increased, the gelatinisation
410 temperature increased as well, while the size of the endotherm area decreased
411 until a point when no transition was observed (completely gelatinised starch).
412 The fraction of starch which remained ungelatinised was calculated -at each
413 cooking time- on the basis of the area involved in the phase transition

414 ($\Delta H/\Delta H_o$,%) and taking as a reference the corresponding value of the uncooked
415 pasta sample (ΔH_o, J/g). The results obtained are summarised in Table 8. In
416 short, the optimal cooking time for the control pasta (DWS formulation) was four
417 minutes. For the rest of the formulations tested, the absence of transition was
418 already observed at two minutes, which may signal a greater ease of hydration of
419 the starch granules during cooking, as a result of the lower cohesiveness of the
420 dough's structure and therefore the greater accessibility of the water molecules
421 to the amorphous regions of the granule.

422 **4. Conclusions**

423 Basing on the obtained results it could be concluded that the presence of TNF
424 and X significantly modifies rheological properties of fresh pasta dough. Changes
425 included: (i) lower values of storage, loss moduli and hardness, and higher
426 values of instantaneous and retardation compliances with TNF addition,
427 revealing the formation of a less cohesive structure with a lower resistance to
428 deformation (less rigid) due to the lower presence of a gluten network. (ii) a
429 greater independence of storage modulus on frequency, a less pronounced
430 hardness and a reduction of the deformation at the end of the creep test when
431 using xanthan gum at 1% (DWS basis), showing the structure strengthen of the
432 doughs. The thermal analysis showed a single endothermic peak in all the
433 samples -in a temperature range between 60 and 78 °C -, corresponding with the
434 gelatinisation of amylopectin. However, in the samples with a higher percentage
435 of tiger nut flour (TNF40 and TNF40X), two phase transitions were observed,
436 associated either to the gelatinisation of the tiger nut starch or the emergence of
437 amylose-lipid complexes. As the cooking time increased, the gelatinisation
438 temperature increased as well and the size of the endotherm area decreased,
439 showing an optimal cooking time of four minutes for the control pasta (DWS),

while for the rest of the formulations the optimal time was found to be two minutes. This indicates a greater ease of hydration of the starch granules during cooking, as a result of the lower cohesiveness of the dough's structure and therefore the greater accessibility of the water molecules to the amorphous regions of the granule.

Acknowledgements

This work was supported by the Conselleria de Empresa, Universidad y Ciencia (Spain) throughout the project AICO/2016/056. Authors are thankful to Harinas Villamayor S.A. for providing raw materials for conducting this study.

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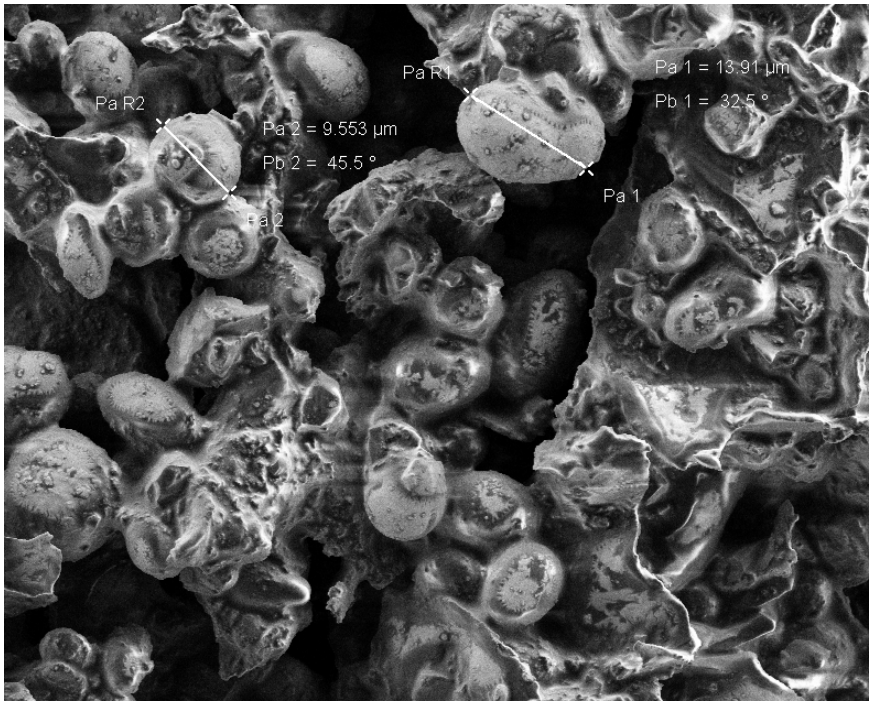

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609 **Fig. 1.** Scanning electron micrograph of cross section of tiger nut flour (1000x)

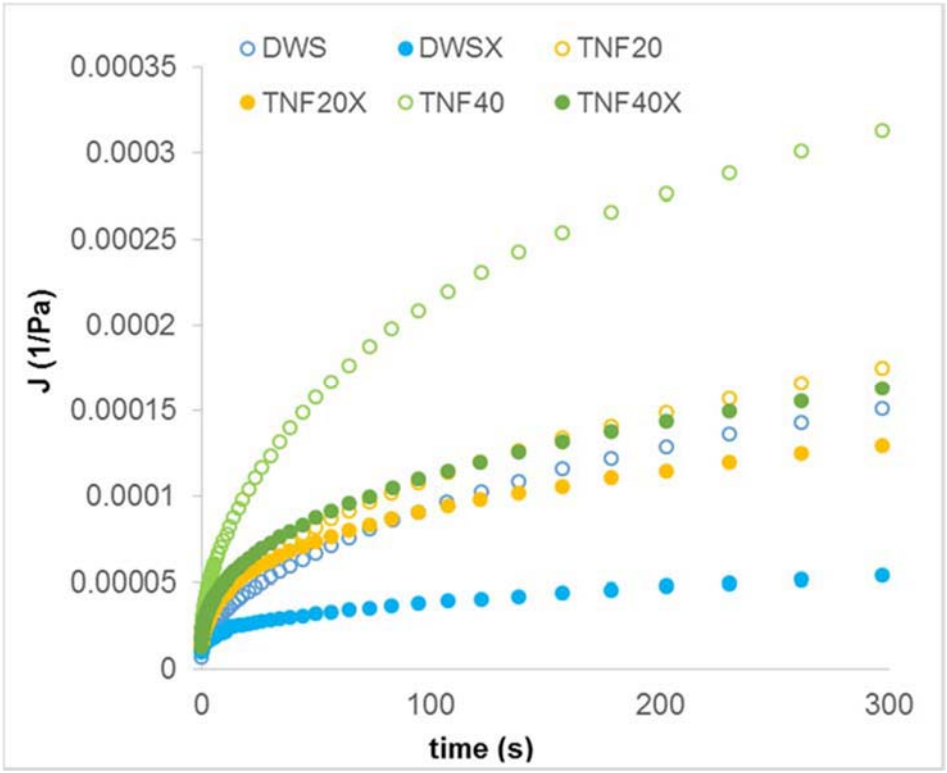

Fig. 2. Effect of tiger nut flour and xanthan gum incorporation on creep test curves of fresh pasta doughs.

Table 1. Proximate chemical composition (PCC) and particle size distribution (PSD) of durum wheat semolina (DWS) and tiger nut flour (TNF) (g/100 g). Mean values of three replicates (standard deviation).

		DWS	TNF	DWS_h	TNF_h
PCC	**Water**	14.03(0.03)[a]	8.03(0.02)[b]		
	Protein	13.81(0.40)[a]	4.80(0.05)[b]		
	Fat	0.92(0.06)[b]	25.30(0.02)[a]		
	Ash	1.67(0.07)[b]	2.02(0.04)[a]		
	Dietary Fibre			-	-
	-Soluble	3.75(0.02)[a]	2.05(0.03)[b]		
	-Insoluble	4.75(0.02)[b]	13.80(0.03)[a]		
	-Total	9.50(0.02)[b]	15.85(0.03)[a]		
	DC[a]	60.08(0.02)[a]	44.00(0.03)[b]		
PSD[b]	**D[4, 3] (μm)**	319(3)[a]	203(4)[c]	150(12)[d]	253(26)[b]
	$d_{(0.1)}$ (μm)	133(4)[a]	50(1)[b]	8.5(0.3)[d]	11.6(0.8)[c]
	$d_{(0.5)}$ (μm)	305(3)[a]	162(4)[b]	50(4)[d]	135(12)[c]
	$d_{(0.9)}$ (μm)	530(4)[b]	419(4)[d]	457(33)[c]	667(68)[a]
	Span	1.30(0.02)[d]	2.28(0.06)[c]	9.00(0.30)[a]	4.81(0.20)[b]

Within rows, values with the same following letter do not differ significantly from each other ($p > 0.05$)

[a]Digestible carbohydrates calculated by difference.

[b]D[3,2] and D[4,3], d(0.1), d(0.5), d(0.9), represent, respectively, Sauter mean diameter, mean particle diameter, 10%, 50% and 90% of all particles finer than this size.

Table 2. Functional properties of durum wheat semolina (DWS), tiger nut flour (TNF) and DWS/TNF blends (TNF20: 80/20, TNF40: 60/40, w/w semolina basis) with or without xanthan gum (X, 1% semolina basis). Mean values of three replicates (standard deviation).

Sample	SRC^{S} (%)	SRC^{B} (%)	SRC^{AL} (%)	WHC (g/g semolina)	FAC (g/g)	FC (mL)	FS (%)
DWS	93(10)^{a}	64.02(0.98)^{c}	108(4)^{a}	0.925(0.007)^{f}	1.210(0.202)^{c}	12.5(0.2)^{a}	74.0(3.0)^{a}
TNF	91(8)^{a}	99(9)c	-	2.050(0.191)^{a}	1.749(0.104)^{a}	-	-
TNF20	87(5)^{a}	88(6)^{b}	83(11)^{b}	1.094(0.011)^{e}	1.300(0.201)^{bc}	1.6(0.1)^{b}	16.0(0.2)^{b}
TNF40	83(2)^{a}	129(9)^{a}	79(5)^{b}	1.125(0.005)^{d}	1.560(0.090)^{b}	1.5(0.1)^{b}	17.0(0.2)^{b}
TNF20X	80(12)^{a}	89(7)^{b}	96(3)^{b}	1.270(0.060)^{c}	1.490(0.040)^{b}	0.7(0.3)^{c}	15.6(0.2)^{b}
TNF40X	89(7)^{a}	124(2)^{a}	82(2)^{b}	1.360(0.050)^{b}	1.600(0.151)^{b}	0.8(0.3)^{c}	17.3(0.2)^{b}

Within columns, values with the same following letter do not differ significantly from each other ($p > 0.05$)
SRC^{S}: water retention capacity by pentosans; SRC^{B}: water retention capacity by damaged starch; SRC^{AL}: water retention capacity by glutenin; WHC: water holding capacity; FAC: fat adsorption capacity; FC: foam capacity; FS: foam stability

Table 4. Mean values (standard deviation) of the obtained parameters for the fitted equation for elastic-G'- and viscous-G''- moduli as affected by frequency (w)

Sample	$G'(\omega) = K' \cdot \omega^a$		$G''(\omega) = K'' \cdot \omega^b$	
	K' [Pa s^n]	**a**	**K'' [Pa s^n]**	**b**
DWS	124049(20712)^b	0.234(0.009)^a	32552(2770)^a	0.14(0.02)^{bc}
DWSX	175362(4708)^a	0.205(0.003)^{cd}	33139(523)^a	0.123(0.010)^c
TNF20	81494(6736)^c	0.226(0.007)^{ab}	23255(1576)^b	0.130(0.004)^c
TNF20X	84365(4895)^c	0.214(0.009)^c	21792(1706)^{bc}	0.157(0.009)^{ab}
TNF40	47246(8849)^d	0.221(0.006)^b	14839(1979)^d	0.161(0.018)^a
TNF40X	82507(4258)^c	0.199(0.002)^d	20266(1028)^c	0.141(0.007)^{abc}

Within columns, values with the same following letter do not differ significantly from each other ($p > 0.05$)

Table 5. Parameters of Burgers model for creep data. Mean values of three replicates (standard deviation)

Sample	**$J_o \cdot 10^{-6}$ (Pa $^{-1}$)**	**$\mu_o \cdot 10^{6}$ (Pa·s)**	**$J_1 \cdot 10^{-5}$ (Pa $^{-1}$)**	**λ_1 (s)**	**$J_2 \cdot 10^{-6}$ (Pa $^{-1}$)**	**λ_2 (s)**	**$J_3 \cdot 10^{-6}$ (Pa $^{-1}$)**	**λ_3 (s)**	**R^2**
DWS	9(1)^{d}	8(2)^{b}	4(3)^{c}	88(19)^{b}	14(1)^{c}	10.0(1.2)^{ab}	6(8)^{bc}	0.94(0.10)^{b}	0.9979
DWSX	9(4)^{cd}	40(30)^{a}	2(1)^{c}	233(86)^{a}	8(5)^{d}	11.9(1.9)^{a}	4(2)^{c}	1.11(0.07)^{a}	0.9989
TNF20	17(3)^{ab}	4(26)^{b}	9(3)^{ab}	113(23)^{b}	19(2)^{b}	10.3(1.4)^{ab}	8(2)^{ab}	0.94(0.07)^{abc}	0.9980
TNF20X	14(3)^{bc}	11(4)^{b}	3(1)^{c}	70(4)^{b}	15(2)^{c}	8.5(1.5)^{bc}	8(1)^{ab}	0.79(0.07)^{cd}	0.9956
TNF40	19(2)^{a}	3(7)^{b}	13(4)^{a}	66.2(0.5)^{b}	27(1)^{a}	6.5(1.2)^{c}	10(4)^{a}	0.64(0.14)^{d}	0.9960
TNF40X	14(2)^{bc}	7(1)^{b}	5(1)^{bc}	79(2)^{b}	16(4)^{bc}	8.3(0.3)^{bc}	9(1)^{ab}	0.83(0.03)^{bc}	0.9964

Within columns, values with the same following letter do not differ significantly from each other ($p > 0.05$)

Table 6. TPA results. Mean values of three replicates (standard deviation).

Sample	Hardness (N)	Adhesiveness (N·s)	Cohesiveness	Springiness
DWS	572(18)^{a}	-4086(276)^{c}	0.53(0.01)^{c}	0.55(0.02)^{d}
DWSX	553(63)^{a}	-1303(637)^{a}	0.76(0.04)^{a}	0.73(0.09)^{bc}
TNF20	273(8)^{c}	-2850(278)^{b}	0.62(0.03)^{b}	0.61(0.05)^{cd}
TNF20X	400(21)^{b}	-6924(951)^{d}	0.65(0.06)^{b}	0.82(0.14)^{b}
TNF40	220(12)^{d}	-6273(244)^{d}	0.46(0.02)^{c}	0.66(0.07)^{cd}
TNF40X	231(3)^{cd}	-10505(369)^{e}	0.68(0.07)^{b}	0.97(0.01)^{a}

Within columns. values with the same following letter do not differ significantly from each other ($p > 0.05$)

Table 7. Thermal properties of doughs (gelatinisation enthalpy (ΔH_n), gelatinisation range (T_o-T_e), and onset (T_o), peak (T_p) and conclusion (T_e) temperatures.

	Sample	ΔH_n(J/g)	T_o (ºC)	T_p (ºC)	T_e (ºC)	T_o-T_e (ºC)
A	**DWS**	1.11(0.21)^{a}	54.6(0.9)^{c}	61.1(0.1)^{c}	66.8(0.5)^{b}	12.2(1.3)^{a}
	DWSX	0.88(0.12)^{a}	55.0(0.2)^{c}	61.2(0.4)^{c}	67.1(0.4)^{b}	12.1(0.1)^{a}
	TNF20	1.02(0.06)^{a}	55.6(0.3)^{bc}	62.4(0.3)^{c}	68.0(0.2)^{b}	12.4(0.2)^{a}
	TNF20X	0.96(0.12)^{a}	55.3(0.3)^{c}	62.1(0.3)^{c}	67.9(0.2)^{b}	12.6(0.3)^{a}
	TNF40	0.72(0.08)^{b}	57.3(0.2)^{bc}	66.0(3.0)^{bc}	68.9(0.2)^{b}	11.6(0.4)^{a}
	TNF40X	0.84(0.13)^{ab}	57.1(0.5)^{ab}	64.0(1.0)^{ab}	69.5(0.8)^{a}	12.4(0.3)^{a}
B	**TNF40**	0.33(0.02)	74.1(0.3)	78.1(0.2)	82.7(0.1)	8.6(0.2)^{b}
	TNF40X	0.30(0.03)	74.55(1.12)	78.6(1.0)	83.1(1.2)	8.5(0.1)^{b}

Within columns, values with the same following letter do not differ significantly from each other ($p > 0.05$). **A:** wheat amylopectin gelatinisation; **B:** associated transition to amylose-lipid complexes or tiger nut starch gelatinisation

Table 8. Gelatinisation temperature (T_p) and percentage of non gelatinised starch (NGS) at different cooking times

		T_p (ºC)				NGS (%)			
	Time (min)	**t=0**	**t=2**	**t=3**	**t=4**	**t=0**	**t=2**	**t=3**	**t=4**
Sample	**DWS**	61.1(0.1)	71.0(1.2)	74.5(0.1)	-	100	15	6	0
	DWSX	61.2(0.4)	-	-	-	100	0	0	0
	TNF20	62.4(0.3)	-	-	-	100	0	0	0
	TNF20X	62.1(0.3)	-	-	-	100	0	0	0
	TNF40	66.0(3.0)	-	-	-	100	0	0	0
	TNF40X	64.0(1.0)	-	-	-	100	0	0	0

Within columns, values with the same following letter do not differ significantly from each other ($p > 0.05$)