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PhD. In Food Science and Technology

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EFFECT OF FORMULATION ON PHYSICO-CHEMICAL PROPERTIES, WATER STATUS AND STABILITY OF PASTA, TOMATO SAUCE AND READY TO EAT PASTA MEALS

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Dedicata a mio padre (Gerard),

mia sorella (Odette) e

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Summary

Ready to eat pasta meals are an important segment of convenience food, but these products are subjected to significant changes in physico-chemical properties during storage, which reduce their acceptability at the time of consumption. A deep understanding of the properties of the single phases, their dependence upon formulation, and the changes they undergo during storage is very important to intelligently intervene on products properties to improve their quality at the time of consumer's consumption.

This work has focused on the effect of formulation on physico-chemical properties of pasta and tomato sauce with a special focus on mechanical/rheological attributes and water status. Variable considered in pasta formulation were gluten, glycerol and moisture content and their effect was studied in both freshly cooked or shelf-stable cooked pasta. The effect of multiple hydrocolloids (at different levels) was considered in the case of tomato sauce.

In the case of pasta, it was found that water content was indeed a very important variable in defying pasta mechanical properties and water status. Higher moisture contents in pasta resulted in softer samples and reduced the changes in physico-chemical parameters during storage. Glycerol was found to favor water uptake and to soften the pasta matrix, acting as plasticizer and increasing molecular mobility. The addition of gluten hardened pasta but did not affect the water status. The combination of higher amount of gluten (15%, g gluten / 100 g product) with higher moisture content (59-65%, g water / 100 g product) were found to minimize the physico-chemical changes occurring in RTE pasta meals during storage, improving quality at longer storage times.

Hydrocolloids added into tomato sauce modulated its mechanical attributes and water status in very different manner, depending on hydrocolloid type and concentration. This may allow to produce

tomato sauce for different applications and that are expected to have different performance if placed in contact with pasta in a RTE meal.

Future work should include an investigation of how the interaction between the two phases (pasta and sauce) can be modulated and controlled by controlling the properties of the single phases with the goal of obtaining highly acceptable products also at longer storage times.

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INTRODUCTION

Traditional pasta is obtained from semolina and water, that are mixed and kneaded to obtain a viscoelastic doughwhich is forced through die to get a desired geometry, and is then dried to obtain the final product. Dry pasta is cooked in excess boiling water, where the two main pasta components, starch and gluten, undergo very important phase changes that are strictly related to the quality and acceptability of the cooked product. Upon heating and hydrating starch swells and gelatinizes while gluten coagulates and traps gelatinized starch forming a strengthened network (Resmini & Pagani, 1983).

Traditionally cooked pasta is eaten immediately after cooking, but the increased demand of convenience food has led to the development of ready to eat (RTE) pasta-based products. Pasta industry has been introducing in the market many frozen, refrigerated and shelf-stable products that need only to be heated before consumption, as proven by the significant market-share increase (~10%) in the recent 10 years. Among RTE meals, pasta meals are indeed the main category as they represent a very large market segment, especially in Asia, Latin America, Middle and North Africa.

RTE pasta meals are subjected to changes in physico-chemical properties during storage, with consequent quality loss. Many ready to eat pasta meals accessible on the market are made of pasta and tomato sauce, that can be mixed together or lodged in different compartments (they will be mixed only at time of consumption). Acceptability of RTE pasta meals are related to the properties and the changes occurring into the single phase during storage and, in the case of mixed phases to the interactions among them (e.g. water migration). A good understanding of how these changes occur in pasta and tomato sauce (as function of formulation and processing) during storage both as single phases or mixed would help improving the quality of the products and their shelf-life stability.

Only a few reports on the changes occurring in ready to eat pasta during storage are available in the scientific literature. Frozen cooked tagliatelle were studied over a 12 months storage period (Olivera & Salvadori, 2011) and they were reported to loose moisture and become harder in the first 4 and 2 months, respectively, and not to be subject to further changes during the rest of storage. McCarthy et al. (2002) reported the presence of moisture gradient between the surface and the center of lasagna pasta (without sauce) after cooking, but this gradient was equilibrated during the holding time (80 minutes). Carini et al. (2014) focused their study on changes in shelf-stable RTE pasta during two months and they observed an increase in pasta hardness and retrograded amylopectin. They also reported an increased molecular rigidity measured by ^{1}HNMR, correlated to an increase of ^{1}H FID steepness and decreased of ^{1}H T_2 relaxation times.

Other changes in product where both phases were mixed were explored in manner to understand which phase is mostly affected. Olivera & Salvadori (2012) reported pasta hardness decrease and moisture content increase in refrigerated lasagna (pasta with sauce) during 8 days storage**.** Whereas Carini et al. (2013) investigated water status in shelf-stable pasta meals with a tomato based sauce during 34 days of storage, and reported pasta softening and water migration between pasta and sauce phases detected only at a molecular level (^{1}H T_1 and T_2), while moisture content and water activity did not reveal a macroscopic water migration between the pasta and sauce phases.

To overcome / reduce these changes it might be necessary to understand how these occurred in each phase. Considering that the changes into pasta phase might be similar to those occurred in other cereal based products, as bread, which were deeply investigated, it might be helpful to change pasta formulation to investigate its effect on physico-chemical properties. It should be, therefore, important to focus on the role of water, glycerol and gluten on pasta properties and stability, as these components have been proven to have a very important role in bread stability (Baik & Chinachoti, 2001; Lin & Lineback, 1990; Schiraldi & Fessas, 2001; Chen et al., 1997; Sereno et al.,

2007; Vodovotz et al., 2002; Berkowitz & Oleksyk, 1991; Hallberg & Chinachoti, 1992; Taub et al., 1994; Eliasson, 1983a, 1983b; Ottenhof & Farhat, 2004; Callejo et al., 1999; Curti et al., 2014).

The properties of the sauce should, also be carefully considered when used in a multiphasic meal (e.g. pasta and sauce). A recent paper (Carini et al., 2014) has focused the attention on the sauce phase evaluating the effect of different ingredients commonly used in industrial settings on the status of water in the systems indicating that, for example, sauce thickening induced by flour or gelatin addition corresponded to very different NMR molecular mobilities. Long chain polymers, such as hydrocolloids (polysaccharides and proteins), are extensively used in the food industry, in different food systems as soups, beverages, desserts, ketchups, sauces, salad dressings due to their functional properties (Saha & Bhattacharya, 2010), as thickening (Philips et al., 1986; Philips & Williams, 2000; Gibinski et al., 2006; Sikora et al., 2007; Kok et al. 1999; Wang et al., 2000; Dunstan et al., 2001; Alexander, 1999 a, b; Casas et al., 2000; Kulicke et al., 1996; Murray, 2000; Sahin & Ozdemir, 2004; Koocheki et al., 2009; Saha & Bhattacharya, 2010), gelling (Aguilera 1992; Oakenfull, 1987, Philips and Williams, 2000; Williams, 2006; Saha & Bhattacharya, 2010), emulsifying and stabilizing (Milani & Maleki, 2012) agents. Thus studying the effect of hydrocolloids in tomato sauce might be necessary for understanding how we can modulate the properties of tomato sauce that may be then very important when such sauce is mixed with pasta in an assembled pasta meal.

A deep understanding of the properties of the single phases, their dependence upon formulation, and the changes they undergo during storage is very important to intelligently intervene on products properties to improve its quality at the time of consumer's consumption. Future work should include an investigation of how the interaction between the two phases (pasta and sauce) can be modulated and controlled by controlling the properties of the single phases with the goal of obtaining highly acceptable products also at longer storage times.

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OBJECTIVE

My PhD thesis focused on the characterization of the properties of cooked pasta, shelf-stable RTE pasta and tomato sauce, their dependence upon formulation and storage time with a multi-analytical and multi-dimensional approach. In particular the work can be divided into three sections.

Section A: Characterization of cooked pasta

- Effect of pasta formulation on physico-chemical properties of cooked pasta.

  In particular, pasta formulation was modified by substitution of part of semolina with vital gluten or glycerol. Local market pastas were also used to investigate their quality due to their different formulation, as traditional, enriched and gluten free pastas. Cooked pasta was characterized in terms of water status (macroscopic [moisture content], mesoscopic [frozen water content] and molecular [proton NMR molecular mobility] indicators) and mechanical (texture and viscoelastic properties) attributes.

Section B: Characterization of ready to eat (RTE) pasta.

- Effect of pasta formulation on physico-chemical properties of cooked pasta.

  In particular, pasta formulation was modified by substitution of part of semolina with vital gluten, glycerol or their mixture at different levels and it was cooked to different moisture contents. Cooked pasta was then packed, sterilized and stored at room temperature up to 90 days, and its water status and mechanical properties were studied.

- B1 - Effect of water and gluten on physico-chemical properties and stability of ready to eat shelf-stable pasta. The substitution level was 15% (g gluten / 100 g semolina) and the moisture content 56 and 59% (g water / 100 g product)

- B2 - Effect of glycerol and gluten on physico-chemical properties, water status and stability of ready to eat (RTE) pasta meals during storage. Seven or 14% (g of ingredient(s) / 100 g

semolina) of semolina was substituted by glycerol and /or gluten and the moisture content was 56 and 62% (g water / 100 g product)

- B3 - Effect of water, gluten and glycerol on physico-chemical properties, water status and stability of ready to eat (RTE) pasta during storage. The substitution level was 15% (g ingredients / 100 g semolina) for single ingredient (gluten or glycerol) or its mixture (gluten and glycerol), and moisture content was 59, 62 and 65% (g water / 100 g product).

Section C: Characterization of tomato sauce

- Effect of added hydrocolloids on physico-chemical properties of tamato sauce.

  In particular, tomato sauce was added with some hydrocolloids (Xanthan, Guar, Carboxy Methyl Cellulose, Locust bean gum, soy proteins, potato proteins, milk proteins and potato fiber) at different levels (0, 0.5, 1, and 1.5%) and cooked to obtain a pasta sauce. Pasta sauce was then characterized for water status (moisture content, water activity, and ^{1}H NMR mobility), color and mechanical (Bostwick consistency, viscosity) attributes.

SECTION A

CHARACTERIZATION OF COOKED PASTA

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Effect of Glycerol and Gluten on Mechanical Properties and ^{1}H NMR Mobility of Cooked Pasta

E. Curti, E. Carini, A. Diantom, F. Cassotta & N.E. O. Najm A. D'Alessandro E. Vittadini

Abstract: The effect of gluten and glycerol (5 and 15 % of flour substitution) on physico-chemical properties and ^{1}H Nuclear Magnetic Resonance (NMR) mobility of cooked pasta was evaluated. Pasta was cooked either for the same cooking time (10 min) or to reach 55 g water / 100 g sample. Gluten addition in pasta formulation resulted in a reduced water ab- sorption during cooking, a slower hydration process and harder products (as compared to the control). The presence of glycerol in the formulation, on the contrary, favoured water uptake during cooking and resulted in softer products. At a molecular level, gluten did not significantly alter ^{1}H NMR dynamics, while glycerol increased molecular mobility and proton exchange, suggesting different molecular dynamics and pasta microstructure.

Keywords Pasta · Gluten · Glycerol · Physico-chemical properties · ^{1}H NMR mobility

1- Introduction

Pasta is a relatively simple food system, produced using only two ingredients, wheat semolina and water, that are processed to obtain, in most cases, a dry product (Carini et al., 2013). Dry pasta is cooked in an excess of water prior to consumption. In this process two main events take place: starch gelatinization and denaturation of the gluten network that, if properly formed during the mixing phase, will entrap the gelatinized starch resulting in a high quality product. Pasta is commonly consumed immediately after cooking but products containing precooked pasta are also available in the market. Ready-to-eat (RTE) meals are offered in different storage conditions, such as refrigerated, frozen or shelf-stable. Quality and stability of these products are strictly related to the properties of pasta, which undergoes textural modifications during storage, as reported in previous works on shelf-stable pasta meals with tomato sauce (Carini et al., 2013), refrigerated lasagna meals (Olivera & Salvadori, 2012), frozen lasagne (Redmond et al., 2005), tagliatelle [Olivera & Salvadori, 2009; 2011) and pasta meals (Kindt et al., 2006, 2008; Carini et al., 2014; Diantom et al., 2015). Softening of the product is generally observed in pasta stored in close contact with the sauce due to water migration from the sauce to the pasta, while pasta hardening was reported in shelf-stable pasta stored without sauce.

The reduction of pasta textural modifications during storage is a key factor to improve the quality and stability of shelf-stable RTE meals and it is expected to be strongly affected by product formulation. Physico-chemical changes leading to reduced shelf-stable RTE pasta quality during storage involve its three major components (starch, gluten and water), and resemble the modifications observed in the staling process of bakery products (i.e., increased hardness and retrograded amylopectin content, reduced ^{1}H NMR mobility) (Carini et al., 2014; Diantomet al., 2015). The development of a strong gluten network and the preservation of its plasticity and flexibility during storage, is one way to better preserve the structure that characterizes RTE pasta

(Diantom et al., 20015) and fresh bread (Gray & Bemiller, 2003). Further improvements in the preservation of gluten network plasticity and flexibility can be achieved using a plasticizer, such as glycerol, that has been positively used in bread (Baik & Chinachoti, 2001, 2002) and protein films (Zhang et al., 2005, Gillgren et al., 2009). A previous work (Diantom et al., 20015) investigated the effect of gluten on physico-chemical properties of RTE shelf-stable pasta during 63 days storage, indicating that the presence of gluten (with high moisture level) contributed to control hardness increase and reduced amylopectin retrogradation during storage.

Modification of pasta formulation may have an effect on the pasta cooking process. Higher levels of gluten in pasta formulation, with optimized mixing and drying conditions, were reported to increase cooked pasta hardness (Diantom et al., 20015; Cubadda et al., 2007; Grzybowski & Donnelly, 1979) and to lower the water absorption of pasta during cooking (Sozer & Kaya, 2008; Bemin et al., 2014). To the authors' best knowledge no reports on the addition of glycerol on pasta properties are available in the scientific literature.

The objective of this work was to investigate the effect of the addition of gluten and glycerol into dry pasta formulation on water uptake during cooking and mechanical properties of cooked pasta. Macroscopic (e.g., moisture, mechanical properties) as well as molecular [e.g., ^{1}H Nuclear Magnetic Resonance (NMR) mobility] properties were analyzed in pasta samples as they are key indicators in the characterization and the understanding of cooked pasta quality(Carini et al., 2014; Diantomet al., 2015).

2- Materials and Methods

Pasta Production

Penne shaped dry pasta of different formulation was produced by a local pasta company. Semolina (moisture: 14.5 %, g water / 100 g product; proteins: 12.5 %; ash: 0.85 %, from the same batch)

was partially substituted by either glycerol or vital gluten (5 and 15 %) to obtain dry pasta. Names of pasta containing gluten and glycerol are reported in Table 1. The ingredients were mixed for 20 min and the dough was extruded at 80 bar (40 °C) using a pilot pasta press (Braibanti, Milan, Italy). The pasta was dried to 12 % moisture (g water / 100 g sample) in a pilot drier (Afrem International S.A.S., Dardilly, France) for about 6 h at a maximum temperature of 85 °C, and then allowed to equilibrate at room temperature for 1 week.

Dry pasta of different formulations was then cooked into boiling water (pasta:water ratio 1:10, no salt) for either 10 min (optimal cooking time for the control sample, STD) or for the time required to reach a final moisture content (MC) of 55.0 % in the cooked pasta. Pasta formulations and cooking times are summarized in Table 1. Pasta were cooked to the selected cooking time, drained, stored in sealed multi-layer (polypropylene-PP, polyethylene terephthalate-PET, and polyamide- PA) pouches and kept at 25 °C for 2 h prior to being analysed. Two pasta batches were cooked for each sample.

Moisture Content

Moisture content (MC) (%) of pasta was determined by weight loss by drying in a forced-air oven (ISCO NSV 9035, ISCO, Milan, Italy) at 105 °C to constant weight. At least triplicate samples of cooked pasta were analyzed.

Mechanical Properties

Pasta hardness was measured using a TA.TX2 Texture Analyzer (Stable Micro Systems, Godalming, UK) equipped with a 25 kg load cell. A single 'penna' was cut at a speed of 2 mm/ s with a trigger force of 0.1 N using a flat blade (HDP/BS, 3 mm thickness, Stable Micro Systems, Godalming UK). The maximum height of the cutting peak was taken as sample's hardness (N). At least 15 penne were cut for each sample.

Proton Nuclear Magnetic Resonance (^{1}H NMR) Mobility)

A low resolution (20 MHz) ^{1}H NMR spectrometer (the MiniSpec, Bruker Biospin, Milano, Italy) operating at 25.0± 0.1 °C was used to measure the Free Induction Decay (FID) and the transverse (T_2) relaxation time of pasta. About 4 g of cooked pasta (10 mm high) were placed into a 10 mm NMR tube that was then sealed with Parafilm® to prevent moisture loss during the NMR experiment. Three replicates were measured for each sample.

FIDs were acquired using a single 90° pulse, followed by a dwell time of 7 μs, 32 scans and a recycle delay of 5 s and a 10 ms acquisition window. ^{1}H FIDs were analyzed in the time range 7–100 μs where the homogeneity of magnetic field was assured. The curves were fitted with a two components model (exponential and gaussian, Sigmaplot, v6, Systat Software Inc. USA) (Botlan & Helie-fourel, 1995)

$$f(t) = y_0 + A*exp(-t/T_A) + B*exp[-(t/T_B)^2]$$

where y_0 is the FID decay offset, T_A and T_B the apparent relaxation times, A and B are the are intensities of each relaxation component.

T_2 relaxation times were measured with a CPMG pulse sequence with a recycle delay of 5 s (≥5 ^{1}H T_2), an interpulse spacing of 0.04 ms and 4000 data points. T_2 curves were analyzed as quasi-continuous distributions of relaxation times using a UPENWin software (Alma Mater Studiorum, Bologna, Italy). Default values for all UPEN parameters were used with the exception of one parameter (LoXtrap) that was set to 1 to avoid extrapolation of relaxation times shorter than the first experimental point. ^{1}H T_2 CPMG relaxation decays were also fitted with a discrete exponential model (Sigmaplot, v. 6, Systat Software Inc. USA).

Statistical Analysis

Significant differences ($p \leq 0.05$) among different samples were verified with by one-way-analysis of variance (ANOVA) followed by least significant difference test (LSD) at $p \leq 0.05$

[SPSS statistical software (Version 16.0, SPSS Inc., Chicago, IL, USA)].

3- Results and Discussion

Moisture Content

Moisture content (MC) of cooked pasta samples are shown in Table 1. When samples were cooked for the same time, gluten pasta (GLU-1 and HGLU-1) had a significantly lower moisture content as compared to STD-1, indicating that, when gluten was added in the formulation, less water was absorbed by the pasta matrix during cooking. On the contrary, with addition of glycerol in pasta formulation (GLY-1 and HGLY-1) a larger water uptake was observed and resulted in larger MC of the cooked product (61.1 and 65.1 %, in GLY-1 and HGLY-1, respectively).

Different cooking times were required to obtain pasta with the same MC (55 %, Table 1), depending on pastas' formulation. Gluten lengthened the cooking process (8.5 and 12.0 min in GLU-2 and HGLU-2, respectively) as compared to STD-2 (8.0 min). Glycerol, on the contrary, shortened the cooking time, as compared to STD-2, to 4.5 and 7.0 min in GLY-2 and HGLY-2, respectively. It is important to point out that some of the glycerol (30–40 % of the glycerol content in dry pasta) was lost in the cooking water, due to its high solubility in water. The overall glycerol content in the cooked pasta was, therefore, lower than the theoretical one.

The cooking process implies a competition for water between gluten, that requires water to coagulate, and starch, that needs water to swell and gelatinize (De Noni & Pagani, 2010). Water absorption kinetics and water absorption mechanisms have been previously explained in pasta cooking (Del Nobile & Massera, 2002; Del Nobile et al., 2003; Del Nobile et al., 2005): water is absorbed through holes and cracks on the pasta surface, diffuses towards the inner regions of pasta through the macromolecular matrix, allowing for an increased mobility of the macromolecules, resulting in glass transition of the glassy amorphous matrix and melting of the crystalline starch domains. The cooked product is, therefore, characterized by a rubbery gluten network with

embedded gelatinized starch (Tg<−20 °C, (Cuq et al., 2003; Cuq & Icard-Vernière, 2001). The modification of pasta formulation with molecules (e.g., gluten and glycerol) that are able to interact with water may affect macromolecules plasticization. Our results indicated a hindered water absorption in the presence of gluten while glycerol favored water absorption during cooking. Previous studies reported a lower water absorption in dry spaghetti produced with high protein content semolina (15 % as compared to 13 %, dry basis (Sozer & Kaya, 2008), while no dependence was reported in spaghetti with added gluten (12–44 %, dry basis (Bernin et al., 2014; Del Nobile, 2005). GLU-1 and HGLU-1 had comparable gluten level (18 and 26 % of gluten, dry basis, respectively) to spaghetti (Bernin et al., 2014; Del Nobile, 2005), but exhibited different water absorption. The discrepancy between our results and these works might be related to the different pasta shape (penne vs spaghetti), different drying processes, different cooking conditions (in pot or test tube, without or with salt) that are expected to modify water absorption and kinetics.

The higher MC of glycerol added pasta (GLY-1 and HGLY-1) was attributed to glycerol high water binding capacity that favored moisture uptake and decreased optimal cooking time (Table 1). Glycerol might have also had a plasticizing effect on the gluten network, making it more flexible (Zhang et al., 2005) and facilitating water absorption into the pasta matrix.

Mechanical Properties

Hardness of pasta samples cooked for the same cooking time is shown in Fig. 1a. STD-1 had a hardness of 8.3 N, and it was increased by inclusion of higher level of gluten in the formulation to 10.2 and 18.8 N in GLU-1 and HGLU-1, respectively. On the contrary, glycerol significantly softened pasta texture, with GLY-1 and HGLY-1 having hardness of 8.1 and N, respectively.

To verify if MC was the major contributor to pasta texture, hardness of pasta samples cooked for different times to the same MC (55.0±1.0 %) was measured (Fig. 1b). The presence of gluten in the formulation significantly hardened the cooked product (12.3 and 15.9 N in GLU-2 and HGLU-2,

respectively, versus 10.5 N in STD-2) while glycerol softened the cooked pasta, despite its actual content was lower than the theoretical one (7.9 and 6.6 N in GLY-2 and HGLY-2, respectively, versus 10.5 N in STD-2). These results are in agreement with previous works where stronger and high gluten quality semolina resulted in harder cooked pasta (Cubadda et al., 2007; Grzybowski & Donnelly, 1979; Day et al., 2006). An action of glycerol on product's structure resulting in a macroscopic softening of the matrix was previously hypothesized in bread (Baik & Chinachoti, 2001, 2002).

Statistical differences between pasta samples with the same formulation but different moisture content were also checked (STD-1 vs STD-2, GLU-1 vs GLU-2, HGLU-1 vs HGLU-2). Hardness generally reflected MC differences in gluten enriched pastas, while glycerol enriched samples showed slight differences in hardness (although significant in HGLY, Fig. 1) with larger differences in MC. It might be hypothesized that water, above a certain limit, does not contribute to pasta softness as all the potential binding sites for water of the pasta polymers might have been saturated.

It was concluded that MC was not the sole factor contributing to the texture of pasta samples but gluten and glycerol had an effect on product texture as they might have influenced water–solid interactions, product's microstructure and led to the development of a different consistency at a macroscopic level.

^{1}H NMR Mobility

^{1}H NMR mobility was studied, at 20 MHz, for the fastest relaxing component with a FID experiment, while the slower relaxing protons were characterized in terms of ^{1}H T_2 relaxation time distributions.

^{1}H FIDs of pasta products obtained in this study are reported in Fig. 2. ^{1}H FIDs of GLU-1 and HGLU-1 were faster than STD-1 (Fig. 2a), suggesting a reduced molecular mobility in the gluten samples within this time frame. On the contrary, the FIDs of GLY-1 and HGLY-1 were slower (less

sharp) than STD-1.

The ^{1}H FIDs of pasta with the same moisture content (Fig. 2b) were not significantly modified by the addition of gluten (GLU-2 and HGLU-2) while glycerol (GLY-2 and HGLY-2) resulted in slower decays as compared to STD-2, indicating an increased ^{1}H molecular mobility in this NMR time-frame.

^{1}H FIDs were fitted with a two components model (exponential and Gaussian functions; Fig. 2c and d) to verify which proton fraction contributed more significantly to the different decays. In pastas cooked for the same time (Fig. 2c and d, circles), the most rigid component (A, relaxing at T_A ~0.015–0.017 ms) represented 14 % of the total protons in STD-1, while it was increased to 15 and 20 % in GLU-1 and HGLU-1, suggesting a reduced molecular mobility of the most rigid protons in gluten enriched pasta. On the contrary, this component was less represented in GLY-1 and HGLY-1 (10 and 13 %, respectively) than in STD-1, indicating that glycerol enhanced proton molecular mobility in this NMR time frame. The more mobile component (B) relaxed slightly faster in GLU-1 and HGLU-1 (T_B ~0.611 and ~0.578 ms, respectively) while it relaxed more slowly in HGLY-1 (~0.794 ms) than in STD-1 (~0.685 ms) and GLY-1 (~0.641 ms).

In samples with the same moisture content (Fig. 2c and d, squares), gluten (GLU-2 and HGLU-2) did not alter the mobility of the FID protons in respect to STD-2, while, in the presence of glycerol (GLY-2 and HGLY-2), the most rigid protons were less abundant (~15 %) than in STD-2 (~18 %). Samples with the same formulation but different moisture content were found to have slightly different populations percentages (i.e., lower percentage of population A protons with larger MCs) but comparable relaxation times (T_A and T_B). Previous studies of ^{1}H FID mobility in bread crumb (Farhat et al., 2006; Sereno et al., 2007; Bosmans et al., 2012; Bosmans et al., 2013) reported a decrease in FID mobility during storage as a consequence of moisture loss and amylopectin retrogradation. Two proton FID populations (with relaxation times similar to those found in this

work) were reported in model systems (dough) and bread (Bosmans et al., 2013): the fastest relaxing protons were associated to the solid CH protons of crystalline and amorphous starch not in contact with water and the more mobile protons to amorphous starch and gluten in little contact with water, respectively (Bosmans et al., 2013). The differences observed in the ^{1}H FID populations in our samples, were attributed primarily to MC and, to a lesser extent, to crystalline starch domains.

^{1}H T_2 distributions of relaxation times of pasta with different formulations (STD, GLU, and GLY) are reported in Fig. 3, and they were characterized by the presence of three ^{1}H populations. It was noticed that these populations were more clearly observable in STD and GLU, indicating that protons were more "compartmentalized" in different mobility domains with reduced proton exchange under the experimental timeframe. Overlapped populations were instead observed in GLY, suggesting a more pronounced protons' exchange among the different domains of the pasta matrix. Different solid–water interactions and microstructure are suggested in pasta samples by these data, and similar conclusions were reported in enriched gluten pasta (20 and 40 % substitution level) cooked inside a real time MRI coupled with a microscope (Bernin et al., 2014) where a more heterogeneous water distribution and microstructure was observed at higher gluten contents.

According to the distributions obtained by UPENWin (Fig. 3), a three exponentials model fitting of the ^{1}H T_2 curves was performed. The three components model was considered appropriate also for the GLY samples as the wide and low intensity peak in the 0.1–40 ms range indicates the presence of at least two proton mobility domains. The relative abundances of the proton populations, named C, D and E from the more rigid to the more mobile protons, and their relative relaxation times are reported in Fig. 4. In pastas cooked for the same cooking time (Fig. 4, circles), gluten samples were similar to STD for relaxation times and abundance of the most rigid protons (population C), while the more mobile protons (population E) were less

abundant (HGLU-1). In the glycerol samples, increased relaxation times in the three proton populations (T_{2C}, T_{2D} and T_{2E}) were observed, as well as a much larger presence of the most mobile protons (population E), more markedly in HGLY-1. In the samples with equal moisture content (Fig. 4, squares), gluten enriched pastas were similar to STD for ^{1}H T_2 relaxation times and abundance of the most rigid protons (population C), while the more mobile protons (population E) were slightly less abundant. In the glycerol enriched pasta, slower relaxation times were observed in all proton populations; population D was significantly larger, while population E was less represented at higher levels of glycerol (HGLY-2).

Proton T_2 relaxation analyses suggested a strong influence of pasta formulation on molecular mobility: the relationship of NMR proton mobility indicators was, therefore, analyzed in respect to pasta formulation. Gluten content was found to have a good relationship with the percentage of population D ($R^2 \geq 0.96$, data not shown) and, it might be tentatively hypothesised that protons of population D may represent protein domains. Moisture content of pasta, on the contrary, was found to have a good relation with population E percentage (R^2= 0.8804, data not shown) in the samples cooked for the same time, indicating that changes in MC may be represented in the more mobile protons domains.

4- Conclusions

The effect of gluten and glycerol on physico-chemical properties and ^{1}H NMR mobility was studied in pasta samples cooked for the same cooking time or to the same moisture content (55%). Gluten and glycerol enriched pastas resulted in different water absorption during cooking and in modified mechanical properties. Hardness was greatly influenced by moisture content, but gluten and glycerol also had an impact by hardening or softening, respectively, the products. An effect of these ingredients was also observed at a molecular level. Gluten reduced proton molecular mobility, while glycerol promoted protons exchangeability and enhanced their mobility, possibly contributing to the different macroscopic pasta hardness. The effect of glycerol was particularly important since its actual content in the cooked product was lower than the theoretical one, due to its loss during the cooking process.

This work indicated that modification of pasta formulation may provide significant changes on pasta mechanical properties. Cooked pasta properties have a great importance in the development of ready-to-eat pasta meals where long shelf-life may compromise the quality of the product and may negatively affect consumers' acceptability. Proper and tailored interventions on pasta formulations can be considered to control cooked pasta texture, lengthen storage stability and improve the quality of these products through preservation of mechanical properties during shelf-life.

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Table 1

Gluten and glycerol level substitution, pasta cooking times minutes] and cooked pasta moisture content [MC, g water / 100 g product]minutes] and cooked pasta moisture content [MC, g water / 100 g product]

Experimental condition	Sample	Semolina substitution [g/100 flour]		Cooking time [min]	Moisture content [g water/100 g sample]
		Gluten	Glycerol		
Same cooking time	STD-1	–	–	10.0	59.1±0.2 (c)
	GLY-1	–	5	10.0	61.1±0.8 (b)
	HGLY-1	–	15	10.0	65.1±0.2 (a)
	GLU-1	5	–	10.0	57.6±0.4 (d)
	HGLU-1	15	–	10.0	54.2±0.3 (e)
Same moisture content	STD-2	–	–	8.0	54.5±0.1 (a)
	GLY-2	–	5	7.0	55.7±0.4 (a)
	HGLY-2	–	15	4.5	55.8±0.9 (a)
	GLU-2	5	–	8.5	55.4±0.5 (a)
	HGLU-2	15	–	12.0	55.9±0.1 (a)

Figure 1

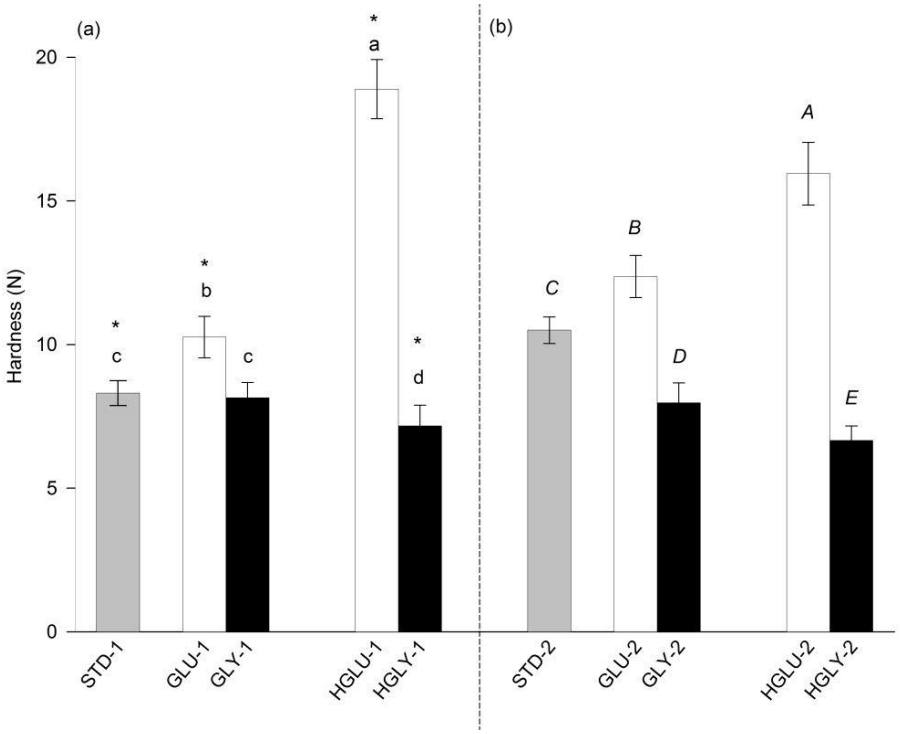


Figure 2

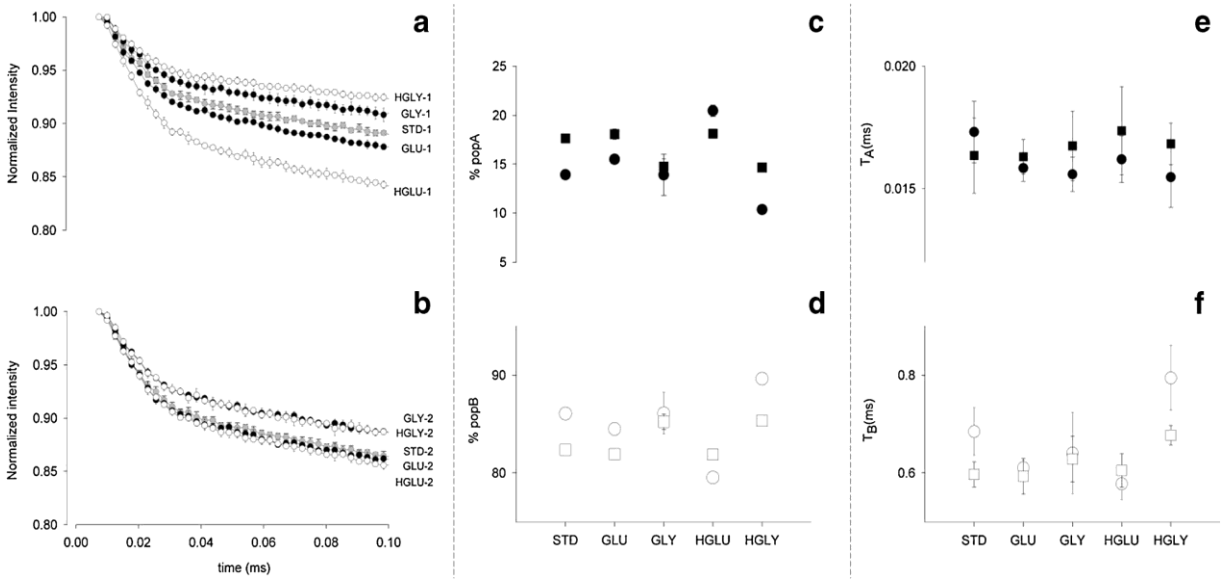


Figure 3

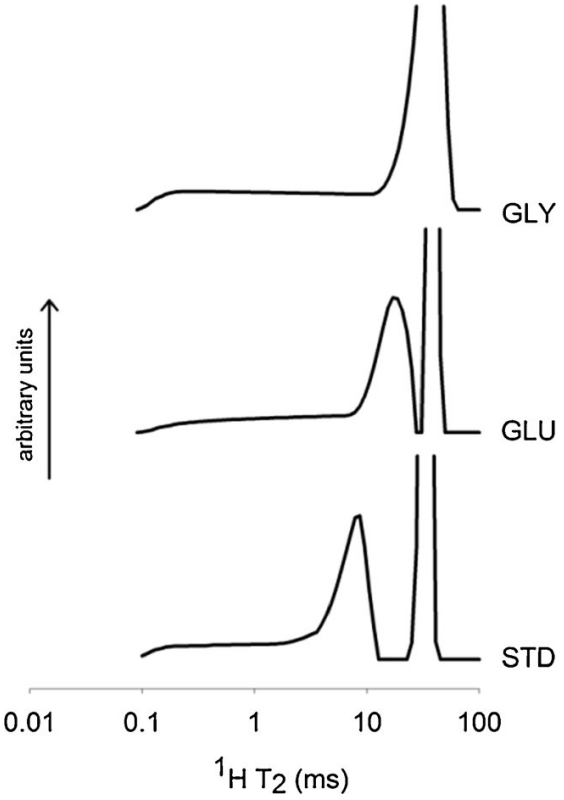
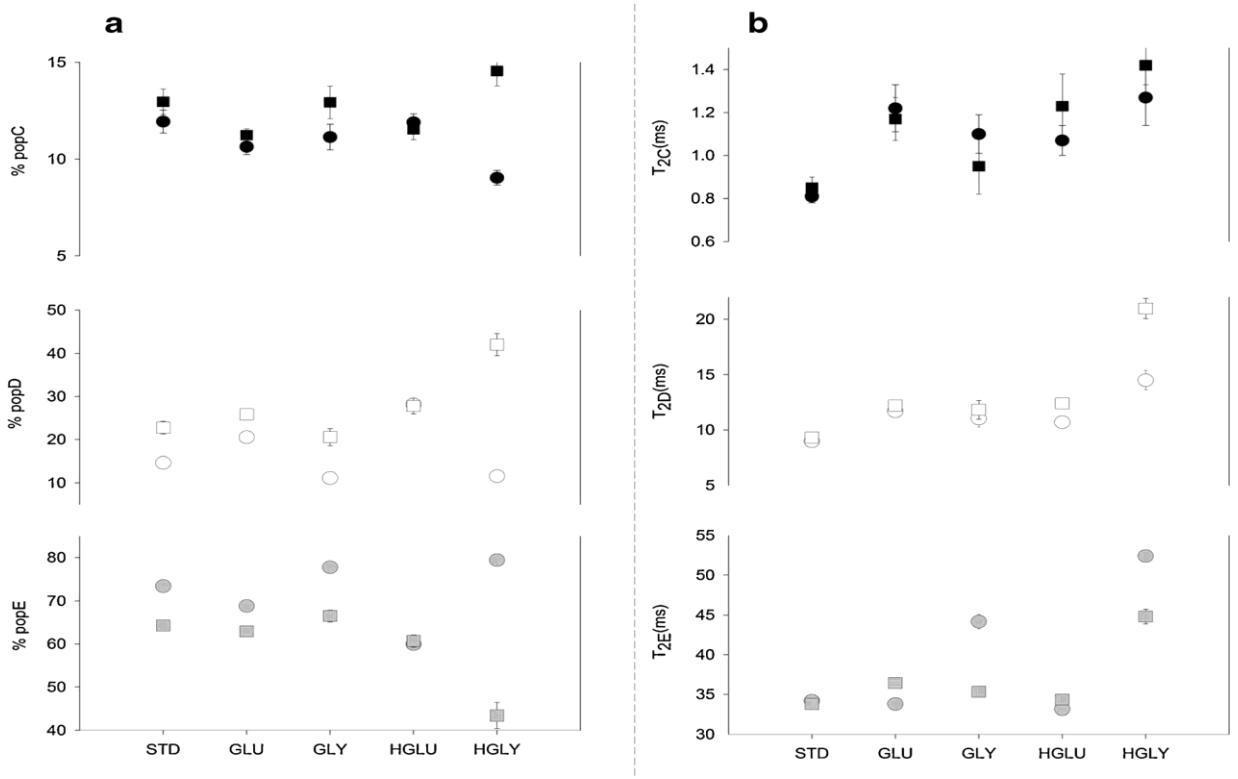


Figure 4


a
% popC
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HGLY
b
T2C(ms)
T2D(ms)
T2E(ms)
STD
GLU
GLY
HGLU
HGLY

Figure caption

Figure 1

Hardness (N) of pasta products with same cooking time (a) and moisture content (b). Different statistical letters indicate significant differences ($p \leq 0.05$) among samples with same cooking time (small letters) and moisture content (capital letters). Stars above bars indicate differences between samples with the same formulation ($p \leq 0.05$)

Figure 2

^{1}H FID curves of samples at the same cooking time (a) and same moisture content (b), ^{1}H relative abundance of population A (%) (c) and population B (%) (d), 1H relaxation times T_A (ms) (e) and T_B (ms) (f), obtained from the discrete fitting for samples with the same cooking time (circles) and samples with the same moisture content (squares)

Figure 3

Representative ^{1}H T_2 distributions of relaxation times of samples with different formulation

Figure 4

Relative abundance (a) and relaxation times (b) of ^{1}H T_2 populations obtained from the discrete fitting for samples with the same cooking time (circles) and samples with the same moisture content (squares)

Mechanical properties and water status of fresh cooked pasta with different formulations

Abstract

Cooked pasta with different formulations was characterized using macroscopic (moisture content, frozen water and water evaporating profile) and molecular (low resolution NMR ^{1}H FID, ^{1}H T_2) water status indicators, as well as macroscopic (texture, hardness) and mesoscopic (DMA storage modulo, loss modulo, tan delta and maximum stress at break) mechanical parameters.

Viscoelastic properties (G', G'') and hardness significantly decreased while moisture content, frozen water and proton molecular mobility significantly increased with increasing cooking time. The water absorbed during cooking was found to be free and tightly bounded water, but it was not correlated to the cooking time and formulation. STD2 seemed to not be significantly different than V and GF2, which exhibited higher texture than STD2. Strong relationship between the hardness and mechanical properties was also observed at all cooking times.

Keywords: Pasta, Physico-chemical properties, Mechanical properties, ^{1}H NMR mobility

1-Introduction

Pasta is a very simple product obtained from durum wheat semolina and water, kneaded to obtain a dough that is than shaped and, most commonly, dried. Pasta is a very common food, largely consumed around the world because of its convenience. Pasta is easy to cook, simple to store, has a long shelf-life. Traditionally, pasta has been produced with durum wheat semolina and water (and sometimes eggs) as only ingredients (Troccoli et al., 1999) in Europe and North America, but nowadays a large variety of pasta products are offered in the market. Pasta is now produced with vegetable, legumes, whole grains, and can also be produced from non-wheat sources (rice, corn, sorghum and pseudo-cereals such as amaranth, quinoa and buckwheat; Marti & Pagani, 2013) to fulfil the requirements for celiac and gluten intolerant individuals.

Pasta eating quality has a strong cultural influence (Carini et al., 2013) and it is strongly dependent on the content and quality of wheat proteins (Matweef, 1966; Matsuo & Ivrine, 1970; Walsh & Gilles, 1971; Matsuo et al., 1972; Grzybowski & Donnelly, 1979), as well as on the length of the cooking process.

During cooking in excess boiling water, pasta absorbs water and undergoes protein coagulation and starch gelatinization that strongly affect eating quality of cooked pasta. Quality of cooked pasta can be evaluated in terms of stickiness, firmness, cooking and overcooking tolerance, water absorption, degree of swelling and loss of solids into the cooking water (Manser, 1981). Water absorption is affected more by the cooking time than the protein content among semolina pasta with different protein content (Edwards et al., 1993). The absorbed water during cooking exhibited different diffusion behaviour among semolina pastas, which is correlated to the protein amount, and decreased with increasing protein content (Del Nobile et al., 2005). Water in cooked pasta exhibited a different behaviour, free and bonded water (Fessas & Schiraldi, 2001), according to its evaporating temperatures (measured by thermogravimetric analysis), lower for free water and

higher for bound water. Moisture content and water status may strongly influence the quality of cooked pasta in terms of texture, viscoelastic properties and proton molecular mobility.

Thus understanding water status in the final product may help in the evaluation of the quality of fresh cooked pasta, in relation to mechanical properties. This study aims to explore the effect of formulation and cooking on physico-chemical, mechanical properties, water status of fresh cooked pasta by means of Thermo-Gravimetric Analysis (to investigate the evaporation profile of water absorbed during cooking) Differential Scanning Calorimiter, Texture Analysis, Nuclear Magnetic Resonance and Dynamic Mechanical Analysis.

2- Materials and Methods

2-1 Materials

Six pastas with a ridged or "rigate" penne" shape with different formulations (Table 1) were used in this study and were obtained from a local supermarket.

Two pasta controls (semolina and water; STD1 and STD2) were studied to represent, respectively, "high" and "medium" quality pasta products. Two gluten free (GF1 and GF2) products were considered and they were made of, respectively, corn flour, rice flour, mono- and di-glycerides of fatty acids (GF1), and white rice, brown rice, corn and quinoa (GF2). Two enriched pasta were studied, a whole grain product [W] made with whole grain semolina, and a veggie [V] product, that included tomato and carrot in the formulation.

Pasta was cooked in boiling water (100 g pasta / 1 l of water ratio) for the optimal cooking time (OCT, "al dente", as indicated on the pasta box), OCT – 3 minutes (undercooked), and OCT + 3 minutes (overcooked) (Table 2). Cooked pasta was cooled to room temperature for 20 minutes prior to analyses.

2-2- Methods

2.2.1- Mechanical properties characterization

Texture

Pasta texture was measured using a TA.TX2 Texture Analyzer equipped with a 25 kg load cell (Stable Micro systems, Goldalming, UK). Single pasta pieces were cut with a flat blade (speed of 2 mm/s; trigger force 0.1 N), and the maximum height of the cutting peak was taken as ‘‘hardness’’. At least 10 pasta pieces for each sample were analyzed.

Dynamic Mechanical Analysis (DMA)

Viscoelastic properties of cooked pasta at 25 °C were measured using a Q800 Dynamic Mechanical Analysis (TA Instruments, New Castle, USA) equipped with a 15mm diameter parallel plate compression clamp (15mm diameter). A multi strain sweep test was preliminarily conducted to identify the linear viscoelastic region for each sample and it was found to be 9-30 µm. A multi-frequency test was then carried out at 15 µm strain over the 1-50 Hz frequency range, to obtain the storage modulus, loss modulus and tan delta as function of frequency. Stress/strain test was carried out in the 0.8 N / min up to 5N, to obtain the maximum stress at break. At least 15 replicates were carried out.

2-2-2 Water status characterization

Thermo-Gravimetric Analysis (TGA)

About 10-15 mg of cooked pasta were placed into aluminium pan and heated from room temperature (25 °C) to 150 °C at 5 °C/min. using a Thermo-Gravimetric Analyzer (TGA Q5000, TA instruments, New Castle, DE, USA). The TGA trace obtained as mass loss (%) vs temperature (°C) was used to calculate moisture content, while its first derivative (Derivative Thermogravimetric [DTG, % / °C]) using Universal Analysis Software, version 3.9A (TA Instruments, New Castle, DE), with subsequent peak deconvolution using PeakfitV4.12 (Systat

Software, Framinghan, Ma, USA) were used to characterize the water evaporation profile. At least three replicates were carried out for each sample.

Differential Scanning Calorimetry (DSC)

Frozen water content was measured using a Differential Scanning Calorimeter (DSC Q100, TA Instruments, New Castle, DE, USA) calibrated with indium (T = 156.6°C; H = 28.71 J/g) and mercury (T = -38.83°C, H = 11.40 J/g). About 10-15 mg of RTE pasta were placed into hermetic stainless steel pans (Perkin Elmer, USA), quench cooled to -50°C and then heated to 100°C at 5 °C/min. DSC thermograms were analyzed using an Universal Analysis Software, version 3.9A (TA Instruments, New Castle, DE).

"Frozen" water content (at the select experimental conditions; FW) was calculated from the endothermic peak around 0 °C (ice melting) using the following equation:

*FW = Enthalpy Ice Fusion * (1/Latent Heat Ice Fusion)* (1/MC)*100*

Where FW is frozen water (%, g frozen water/g water), ice fusion enthalpy (J/g product), latent heat of ice fusion is 334J/g ice, and MC is moisture content (g water/1 g product).

The obtained frozen water was normalized on the moisture content. At least three replicates were carried out.

Proton Nuclear Magnetic Resonance (^{1}H NMR)

A low resolution (20 MHz) ^{1}H NMR spectrometer (the Minispec, Bruker Biospin, Milano, Italy) operating at 25.0 ± 0.1°C was used to study proton molecular mobility by measuring the free induction decay (FID) and transverse relaxation times (T_2). About 2 g of RTE pasta were placed into a 10 mm NMR tube that was then sealed with Parafilm to avoid moisture loss during the NMR experiment. ^{1}H FIDs were acquired using a single 90° pulse, followed by a dwell time of 7 µs, a recycle delay of 3 s and a 10 ms acquisition window. ^{1}H FIDs were analyzed in the time range 7–100 µs where the homogeneity of magnetic field was assured. The curves were fitted with a two

components model (exponential and gaussian; Le Grand, Cambert, & Mariette, 2007; Sigmaplot, v6, Systat Software Inc., USA):

$f(t) = y0 + A*exp(-t/T_A) + B*exp[-(t/T_B)^2]$

where y0 is the FID decay offset, A and B are the intensities of each relaxation component, T_A and T_B are the apparent relaxation times.

T_2 relaxation time was measured with a CPMG pulse sequence with a recycle delay of 3 s (P5 ^1H T_1), an interpulse spacing of 0.04 ms and 4000 data points. T_2 curves were analyzed as quasicontinuous distributions of relaxation times using a UPENWin software (Alma Mater Studiorum, Bologna, Italy). Default values for all UPEN parameters were used with the exception of one parameter (LoXtrap) that was set to 1 to avoid extrapolation of relaxation times shorter than the first experimental point. ^1H T_2 CPMG relaxation decays were also fitted with a discrete exponential model (Sigmaplot, v.6, Systat Software Inc., USA).

2.2.3 Statistical analysis

Means and standard deviations (SD) were calculated with SPSS statistical software (Version 22.0, SPSS Inc., Armonk, New York, USA). Significant differences ($p \leq 0,05$) among different samples were verified with by one-way-analysis of variance (ANOVA) with a Tukey-high and LSD significant difference test.

3-Results and discussion

The different pasta used in this paper were cooked at different cooking time (OCT-3, OCT and OCT+3) considering the cooking time reported on the product packaging as the optimal cooking time. Three group of pasta were used, standards, enriched and gluten free pasta according to their formulation (Table 1). Samples with similar moisture content but different cooking time were used to evaluate the effect of moisture content on mechanical properties and proton molecular mobility.

3.1. Mechanical properties

3.1.1. Hardness

Hardness of cooked pasta was reported in Table 2. Hardness significantly dropped with increasing cooking time in all samples, as expected. STD1 was significantly harder than STD2 at all cooking times. Curti et al., (2015), reported that pasta cooked for 10 minutes exhibited 8.3 N as pasta hardness. In this study STD2 exhibited similar hardness value (8.6 N) when it was cooked for 11 minutes (OCT). Firmness and stickiness are the main quality factor of cooked pasta, which must resist surface disintegration and retain firm structure (Cubadda et al, 2007). Thus higher hardness means higher quality of cooked pasta, therefore STD1 might be considered best quality than STD2. The cooked pasta quality was strictly correlated to the amount and composition of durum proteins, in particular gluten strength (Grant et al, 1993; D'Egidio et al., 1990, Cubadda et al., 2007) and it also depend to the dried temperature (Manser 1980; Manthey & Schorno, 2002; De Stefanis & Sgrulletta, 1993). Considering that STD1 and STD2 have the same amount of proteins, the highest hardness in STD1 might be correlated to the quality of proteins and processing parameters, which conferred to that pasta a good structure. Concerning the enriched pasta, V was generally harder than W, possibly due to the presence of bran in W that might have altered the gluten network, which was associated to the dilution effect of bran on gluten cohesiveness (Kordonowy & Youngs, 1985). Manthey & Schorno, (2002), reported that the presence of bran interfered with the continuity of gluten matrix, reducing hardness of whole wheat spaghetti. Whereas in GF pastas, GF1 was significantly softer than GF2 at all cooking times. Considering the effect of formulation, all samples showed lower texture quality as compared to STD1 at all cooking times, except in the case of GF2 which exhibited higher hardness than STD1 in undercooked pasta. While STD2 resulted to be softer than V and harder than W at all cooking times, but as compared to GF pasta, it resulted to harder than GF1 and softer than GF1 at all cooking times.

Hardness in gluten free pasta is likely to be related to the process of starch gelatinization during cooking, which in GF1 hardness gradually decreased from OCT-3 to OCT+3 while in GF2 it

showed the highest value at OCT-3 and then was halved at OCT, possibly indicating that in this sample more water was required for gelatinization with starch being more resistance to gelatinization. In fact GF2 contains brown rice, where starch behaves as pre-heated starch, that is more resistant to gelatinization, reducing starch swelling (Resmini & Pagani, 1983; Riana et al., 2005) and exhibiting a harder texture, as compared to samples with starch is not untreated.

Higher texture quality in V than STD2 might be due to the added fiber. The presence of fiber, according to its type and amount, induces changes in protein- starch network affecting cooked pasta quality. Tudorica et al., (2002), reported that the presence of pea fiber induced porosity into protein-starch-fiber network, due to higher attitude of gelatinization, while the addition of Inulin exhibited similar structure of protein-starch-fiber network as standard pasta. In fact they reported that the gelatinized starch granules integrated the developed protein matrix to form a compact structure, which determines the hardness in cooked pasta. Thus the presence of fiber in V, might help to control starch gelatinization conferring to V pasta higher hardness.

At the same moisture content, STD1 exhibited the highest hardness as compared to all samples, while STD2 had similar hardness as V, but it was significantly harder than GF2, W and GF1. GF2 was significantly harder than W which had higher hardness than GF2. Considering that hardness was one of the main factor which defines the quality of cooked pasta, it might be possible to classify the different pasta used in this study as following: STD1 > STD and V > GF2 > W > GF1.

3.1.2. Viscoelastic properties

A representative graphs of storage and loss moduli and tan delta is shown in Figure 2. Storage and loss moduli significantly decreased with increasing cooking times. Damping (tanδ) also exhibited changes due to the cooking time, but not in relevant manner as it was observed in storage and loss moduli. All viscoelastic properties significantly increased with increasing frequency in all samples. Similar results were observed in noodles, analysed using a dynamic rheometer (Edwards et al., 1993). Confronting both standards, STD1 exhibited higher ability to store energy (G') than STD2 at

all cooking times and at all frequency. About the enriched pasta, the storage modulus of V was significantly higher than W at all cooking times and at all frequency, except in the case of lower frequency (up to 13 Hz), where both enriched pastas exhibited similar ability to store energy at OCT. Whereas, GF pasta showed similar ability to store energy at all cooking times and at all frequency. Formulation also affected pasta capacity to store energy (Figure 3), with STD1 having a G' significantly higher than enriched and GF pastas, while STD2 exhibited higher storage modulus than both GF pasta, but comparable to enriched pastas.

Maximum stress at break, measured with the stress/strain test, significantly decreased with increasing cooking time in all pastas, as expected. The maximum stress at break was significantly higher in STD1 than STD2 at all cooking times. Regarding the enriched pasta, V showed higher maximum stress at break than W at OCT-3 and OCT+3, but comparable at OCT GF pastas required the maximum stress to brake when overcooked, but GF2 was more difficult to brake when undercooked and al dente. Formulation also played an important role in determining samples capacity to deform under stress. STD1 was always more resistant at breakage than all other samples, (STD1 and enriched and GF pastas) at all cooking conditions. STD2 was comparable to W and higher than V in al dente and overcooked pastas, but it was lower than V and higher than W in undercooked samples. The maximum stress required to brake STD2 was higher than GF1 at all cooking times, but it was similar to GF2 at OCT, while STD2 exhibited higher and lower maximum stress than GF2 in overcooked and undercooked pasta, respectively.

Viscoelastic properties of pasta were, therefore, significantly affected, as expected, by the cooking time but formulation also played an important role. In fact the linear correlation between hardness vs storage modulus and hardness vs maximum stress at break was investigated, excluding GF2 from the fitting, due to its incongruent changes in the structure during cooking, as it was illustrated before in hardness paragraph. A strong correlation ($r^2 > 0.87$) between hardness and maximum stress was observed at all cooking times while the relationship between storage modulus and

hardness values was $r^2 > 0.90$ at all cooking times. A strong correlation was also observed between the stress maximum at break and storage modulus, which resulted more relevant in al dente and undercooked pasta, with $r^2 = 0.99$ while r^2 was = 0.92 in overcooked pasta. It was reported a strong correlation ($r^2 > 0.87$) (Edwards et al., 1993) between the Instron and Rheometer measurements at both optimal cooking time and in overcooked samples. It might be concluded that the small strain rheological determination might allow to investigate changes that are related to macroscopic pasta texture. Thus the ability to store energy was strictly correlated to the product structure, and the evaluation of viscoelastic properties using DMA might be an alternative way to distinguish pasta products quality after cooking.

3.1 Water status

Moisture content of pasta samples considered in this study were reported in Table 2. Moisture content significantly increased with increasing cooking time in all samples, as expected, with the exception of GF1 where OCT and overcooked pastas had comparable moisture content. STD2 and W showed higher moisture content at all cooking time than STD1 and V, respectively, while GF pastas showed similar moisture content at OCT-3 and OCT, but at OCT+3, GF1 moisture content was higher than GF2. The formulation also affected pasta water absorption during cooking. At optimal cooking time, STD1 exhibited lower moisture content than W and V, while the moisture content of STD2 was significantly higher than V but similar as W. Comparing to the GF pastas, STD2 moisture content was higher than GF2 but similar to as GF1, while STD1 exhibited lower moisture content than GF1 but similar as GF2. Therefore to reach the same moisture content samples must be cooked at different cooking time. In fact STD1, STD2, W, V, GF1 and GF2 was cooked at 12, 11, 13, 9, 13 and 11minutes to reach similar moisture content (≈ 48%, g water / 100 g product).

Thermo-gravimentric analysis allowed to investigate the water evaporation profile at increasing temperature for all samples (Figure 4). Water was found to follow a bimodal evaporation pattern,

with about 75% of water molecules evaporating at lower temperature (44.5 ±1.5 to 48.8 ± 2.6 °C range), and the remaining 25% at higher temperatures (102.3 ± 5.0 to 11.8 ± 5.9 °C). A comparable water evaporation profile was found in all samples, indicating that this water status indicator was not affected by the formulation nor by the cooking time. The lower temperature evaporating water population might be correlated to the free water into the pasta matrix, while the higher temperature evaporating water population to the tightly bounded water. Previously Fessas & Schiraldi, (2001) related the higher temperature evaporating water population to water tightly bound to the gluten network, but considering that GF pasta had an evaporating profile comparable to the other samples and that GF pasta did not contain gluten, this hypothesis may not be true in these samples. The more tightly water in GF pasta might be correlated to proteins presented into the sample, which are different than gluten but still bound (and release) water in a similar way and, therefore, confer the product a similar macroscopic structure. Thus the more tightly water might be attributed to the proteins undistinguishably.

Normalized frozen water was found to increase with increasing cooking time and, consequently, moisture content in all samples (Table 2), as expected. STD2 and W exhibited higher frozen water content at all cooking times than STD1 and V, respectively, while GF pastas showed different frozen water content, GF1 lower than GF2, only at OCT-3 and OCT, but similar at OCT+3. For standards and enriched pasta, the changes trend in frozen water content during cooking was similar to that occurred in moisture content, while for GF pasta it was different. Formulation was also influencing frozen water content: STD1 exhibited lower frozen water content than enriched pasta (W and V), while STD2 showed lower frozen water than W, but similar as V. Concerning GF pastas, STD1 exhibited higher frozen water than GF2 but lower than GF1, while frozen water of STD2 was significantly higher than all GF pastas. Similar trend in frozen water was observed in both undercook and overcook, except in the case of both standards, where changes were observed at under and overcook. The changes in frozen water in gluten free pasta, which exhibited similar moisture content, might be correlated to formulation.

At the same moisture content, STD1 and STD2 exhibited similar frozen water, which resulted to be significantly lower than those observed in enriched pasta (W and V), but similar to gluten free pastas.

Proton Nuclear Magnetic Resonance mobility (^{1}H NMR)

Two population of protons and the corresponding relaxation time were obtained with the fitting of FIDs curves. The populations, named population A and B, and the corresponding relaxation times, T_A and T_B, respectively, are showed in Table 3. With increasing cooking time an increase in the molecular mobility was observed in the samples, with a significant decrease of the amount of the more rigid protons (population A) and a consequent increased of population B. This might be correlated to the alteration of the protein-starch network which increased with increasing cooking time conferring to the sample less rigidity. STD1 and GF2 exhibited high percentage of more rigid proton at all cooking times than STD2 and GF1, respectively, while enriched pasta showed difference in more rigid proton only in overcooked pasta, where population A was significantly higher in W than V. Proton mobility was also affected by the formulation of pasta at all cooking times, as compared to the standards (Table 3). In effect, STD1 exhibited higher percentage of the more rigid protons than enriched and GF pastas at all cooking time, except in the case of GF2 which exhibited higher value of more rigid protons than STD1 in overcooked pasta**.** STD2 showed lower values of more rigid protons than that observed in enriched and GF pastas at all cooking time, except in undercooked pasta, where STD1 showed higher value of more rigid protons than enriched pasta, but similar value as GF1 and lower than GF2. At the same moisture content, both standards had similar population A as W and GF2, which resulted to be significantly higher than V and GF1, but GF1 was the lowest.

The more rigid protons was reported to be about 14% in pasta cooked for 10 minutes (Curti et al., 2015), while for STD2, cooked for 11 minutes, the more rigid protons value was about 17%. This difference might be correlated not to the product quality but to the fact that different semolina at

different period of analysis. Thus the highest value, 24%, of more rigid proton in STD1, might be associated to less disintegration into proteins-starch network, which might reduce proton mobility.

Concerning the relaxation time, T_A was about 0.015 ms in all samples and it was not affected neither by the cooking time nor by the formulation. In contrary, T_B significantly increased with increasing cooking time in all samples. STD2 and GF1 exhibited higher T_B at all cooking times than STD1 and GF2, respectively, while the changes in enriched pasta were not clear. T_B was also affected by the formulation at all cooking time. In fact, STD1 exhibited lower T_B than enriched and GF pastas at all cooking times, while STD2 had similar T_B as enriched pastas at all cooking time, but comparing to the GF pasta, T_B of STD2 was lower than GF1 and similar as GF2 in all cooked pasta. At the similar moisture content, T_A was not affected by the formulation, while T_B of both STD was the lower than the rest of samples. GF1 exhibited the highest T_B followed by GF2 and V then by W.

$^1HT_2$ distributions of cooked pasta with different formulation were carried out using UpenWin software. Three unsolved ^1H populations were observed, except in the case of GF2 where the populations were well solved (Figure 6). Proton distribution was strongly affected by the formulation. In effect proton distribution was more evidently defined in STD2 than STD1, where an overlapped population was observed, indicating an eventual proton exchange among the different domains. V and GF1 proton distribution was similar as STD2, but GF2 showed the more distinguished populations, defining its appertaining to a specific domain, and reducing that proton exchange. W and GF1 exhibited similar proton distribution as STD1.

To define the quantity and the relaxation times of proton appertaining to the specific domain, three exponentials model (Curti et al., 2015) was used to fit the ^1H T_2 curves. The proton populations were named population C, D and E, while their corresponding relaxation times were named T_{2C}, T_{2D} and T_{2E}, respectively. The proton intensity of the more abundant and more mobile proton, population E, of all samples was showed in Figure 7A. Cooking time induced changes in the more abundant and more mobile population, which significantly increased with increasing cooking time

in all samples, except in the case of V where changes in population E were observed in overcooked pasta.

Considering different groups of pasta, STD2 showed the more represented population E than STD1 at all cooking times, confirming the less disintegration of gluten structure during cooking. GF pasta exhibited similar value of population E in all cooked pasta, suggesting that disintegration in these pastas structure, meanly starch network, occurred in the same manner in both pasta. Whereas in reached pasta, population E value was higher in V than W in undercooked pasta, but W showed higher value at OCT and OCT+3, suggesting a strong disintegration of gluten network in W.

The more abundant and more mobile population was affected by the formulation. In effect the more mobile and more abundant population of STD1 resulted to be significantly lower than enriched and GF pastas at all cooking times. STD2 exhibited lower and higher population E than W and V, respectively, at OCT and OCT+3, but it was lower than V and similar as W in undercooked pasta; while it resulted to be lower than both Gf pastas in undercooked pasta and similar as both GF pasta in OCT and overcooked pasta. The other populations (data not shown) exhibited changes due to pasta formulation and cooking times. At the same moisture content, both standards and GF2 had the same percentage of the more abundant and more mobile proton, which was higher than V but lower than GF1 and W.

The corresponding relaxation times were affected by the formulation and cooking time. The relaxation time of population E, T_{2E}, increased with increasing cooking time in all samples (Figure 7B). STD1 and GF2 exhibited lower relaxation time than STD2 and GF1, respectively, while T2E of W was higher and lower than V, at OCT-3 and OCT+3, respectively, but similar to V at optimal cooking time. Concerning the formulation effect, STD1 exhibited lower relaxation time than enriched pastas at OCT and OCT+3, but similar to W and higher then V in undercooked pasta while it resulted to be higher than GF pastas relaxation time at all cooking times. In other hands, STD2 relaxation time was significantly higher than enriched and GF pastas at all cooking times.

The changes in proton mobility induced by cooking might correlated to the changes into gluten network, which slowly loses it rigidity becoming more elastic, conferring to the proton high mobility. The difference observed into T_{2E} of standards pasta might be in part correlated to the different moisture content, which resulted to be lower in STD1 conferring it the more rigid structure, and this might affect the proton mobility. In effect changes population E were strongly correlated ($R^2 = 0.94$) to the sample moisture content only at optimal cooking time, suggesting that a perfect starch gelatinization and proteins coagulation was reached. However changes into pasta structure was not related only to the absorbed water during cooking but also to the quality of gluten network, which disintegration rate affects the proton mobility. The more changes in enriched pasta compared to STDs might be correlated to the presence of fiber and bran, in V and W, respectively which alter the pasta structure. The strong difference W compared to V, might be correlated to the presence of bran which boosted the disintegration of the protein network. Tudorica et al., (2002), observed Scanning Electron Microscopy micrograph of cooked pasta and they reported that the addition of pea fiber into pasta formulation disrupted the starch-protein-fiber network altering pasta structure. The strong changes in proton mobility of GF pasta compared to STDs might be correlated to the fact that only starch network was formed in these pastas. Thus the swelling of starch network, due to the gelatinization, significantly increases the rigidity losing in these samples.

4-Conclusions

The effect of formulation and three different cooking time on cooked pasta was explored using sophisticated methods. The increasing of cooking time affected all parameters: moisture content, frozen water content and protons molecular mobility indicators increased with increasing cooking time, while hardness sand mechanical properties significantly decreased with increasing cooking time. Only the evaporated water profile, amount and temperature, was not affected by cooking time nor by formulation. Pasta formulation significantly affected the investigated parameters. STD1 exhibited the highest quality compared to STD2 and all the samples. STD2 exhibited lower texture quality at all cooking time compared to V and GF2. However changes in GF2 were stronger than STD2. GF1 and W resulted to be the worst pasta. In this study a strong correlation between the Texture Analyzer and Dynamic Mechanical Analyzer measurements was also observed, suggesting that small deformation analysis might be used to explore the changes into the cooked pasta quality.

These results confirmed that the quality of cooked pasta strongly depends to the quality of dry pasta and cooking time. Suggesting that to obtain the best quality cooked pasta, as fresh and RTE pasta, it might be necessary to investigate the effect of formulation on the quality of that pasta, as changes in physico-chemical, mechanical properties and water status. They also suggested that sophisticated methods might be used to explore the quality of cooked pasta.

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Table 1

Chemical composition of different pasta used in this study. The composition is based on 56 g of pasta as reported on product packaging.

	Fat (g)	Carbohydrate (g)	Protein (g)
STD1	1	41 (2)	7
STD2	1	42 (2)	7
V	1	41 (2)	8
W	1.5	39 (6)	8
GF1	1	44 (1)	4
GF2	1.5	44 (2)	4

Table 2

Physico-chemical properties of cooked pasta with different formulations at different cooking time: MC (Moisture Content), FW (Frozen Water), Hardness, and stress maximum at break. Standard deviations are given in parenthesis following the means values; small letters indicate the differences in the same samples at different cooking time; capital letters indicate differences among the samples at equal cooking time; "a" and “A” letters were assigned to the highest mean.

		Cooking times (minute)	Moisture content (%, g H_20/100 g sample)	Frozen water (%, g H_2O / g H_2O)	Hardness (N)	Maximum stress at break (MPa)
STD1	OCT-3 OCT OCT+3	6 9 12	39.35 (0.64) c/B 42.72(0.10) b/D 48.63(0.89) a/DE	33.79 (0.44) c/C 37.37 (0.08) b/D 43.24 (0.59) a/C	18.99 (0.59) a/B 13.89 (0.44) b/A 11.83(0.44) c/A	0.27(0.01) a/A 0.25(0.01) b/A 0.22(0.01) c/A
STD2	OCT-3 OCT OCT+3	8 11 14	42.97(0.53) c/A 48.97(0.90) b/AB 52.31(0.80) a/B	35.18 (0.04) c/B 42.19 (0.36) b/B 48.32(0.25) a/B	10.13(0.22) a/D 8.66(0.33) b/D 7.62(0.30) c/C	0.15(0.01) a/D 0.12(0.01) b/B 0.11(0.01) c/B
V	OCT-3 OCT OCT+3	7 10 13	39.38(0.53) c/B 46.29(0.66) b/C 50.09(0.64) a/CD	33.92 (0.13) c/C 41.48 (0.66) b/B 47.04 (0.33) a/B	12.56(0.57)a/C 9.27(0.24) b/C 8.56(0.29) c/B	0.16(0.01)a/C 0.12(0.01) b/C 0.10(0.01) c/C
W	OCT-3 OCT OCT+3	6 9 12	42.41 (0.98) c/A 50.14 (0.59) b/A 56.71 (0.94) a/A	39.36 (0.15) c/A 47.89 (0.44) b/A 51.51 (0.11) a/A	7.57(0.30) a/E 6.30(0.29) b/E 5.54(0.26) c/D	0.12(0.01) a/E 0.12(0.01) b/BC 0.09(0.01) c/D
GF1	OCT-3 OCT OCT+3	7 10 13	37.12(0.49) b/C 46.90(0.91) a/BC 47.18(0.89) a/E	25.68 (0.57) c/E 35.87 (0.21) b/E 43.47 (0.68) a/C	9.30(0.47) a/F 5.38(0.18) b/F 5.02(0.27) c/E	0.12(0.01) a/E 0.07(0.01) b/D 0.06(0.01) c/E
GF2	OCT-3 OCT OCT+3	5 8 11	36.58(1.03) c/C 44.66(0.52) b/CD 50.81(0.27) a/BC	28.77 (0.34) c/D 39.66 (0.53) b/C 44.57 (0.40) a/C	20.51(0.74) a/A 10.99(0.36) b/B 7.41(0.38) c/C	0.24(0.01) a/B 0.12(0.01) b/B 0.06(0.01) c/E

Table 3

^{1}H FID populations and corresponding relaxation times. Standard deviations are given in parenthesis following the means values; different letters close to numbers indicate significant difference among samples ($p \leq 0.05$), small letters indicate the differences in the same samples at different cooking time; capital letters indicate differences among the samples at equal cooking time; "a" and “A” letters were assigned to the highest mean

		Population A (%)	T A (ms)	Population B (%)	T B (ms)
STD1	OCT-3 OCT OCT+3	29.59 (0.14) a/A 24.03(0.20) b/A 17.54(0.29) c/B	0.015 (0.001) a/A 0.015 (0.001) a/A 0.015 (0.001) a/A	70.41(0.14) c/D 75.97(0.20) b/C 82.46(0.29) a/C	0.47(0.01) c/C 0.49(0.01) b/D 0.54(0.01) a/D
STD2	OCT-3 OCT OCT+3	23.85 (1.03) a/C 17.42(0.54) b/E 13.65(0.38) c/E	0.015 (0.001) a/A 0.015 (0.001) a/AB 0.015 (0.001) a/A	76.15(1.03) c/B 82.58(0.54) b/B 86.35(0.38) a/A	0.52(0.02) c/B 0.56(0.02) b/C 0.67(0.02) a/B
V	OCT-3 OCT OCT+3	20.48(1.17) a/D 16.79(0.57) b/D 13.72(0.24) c/D	0.015 (0.001) a/A 0.015 (0.001) a/AB 0.014 (0.001) a/AB	79.52(1.17)c/A 83.21(0.57) b/A 86.28(0.24) a/A	0.52(0.01)c/B 0.57(0.01) b/C 0.66(0.01) a/B
W	OCT-3 OCT OCT+3	21.36 (0.19) a/D 17.13(0.33) b/D 15.42 (0.16) c/C	0.014 (0.001) a/A 0.014 (0.001) a/B 0.013 (0.001) a/B	78.64(0.19) c/A 82.87(0.33) b/AB 84.58(0.16) a/C	0.51(0.01) c/B 0.60(0.02) b/B 0.63(0.02) a/C
GF1	OCT-3 OCT OCT+3	24.36 (0.15) a/C 19.38(0.18) b/C 15.64(0.58) c/C	0.015 (0.001) a/A 0.015 (0.001) b/B 0.015 (0.001) a/C	75.64(0.15) c/B 80.62(0.18) b/D 84.36(0.58) a/D	0.57(0.02) c/A 0.62(0.02) b/A 0.71(0.03) a/A
GF2	OCT-3 OCT OCT+3	27.18(0.37) a/B 21.82(0.15) b/B 18.09(0.36) c/A	0.015 (0.001) a/A 0.014 (0.001) ab/BC 0.014 (0.001) b/C	72.82(0.37) c/C 78.18(0.15) b/D 81.91(0.36) a/C	0.46(0.01) c/C 0.55(0.01) b/C 0.64(0.03) a/BC

Figure 1

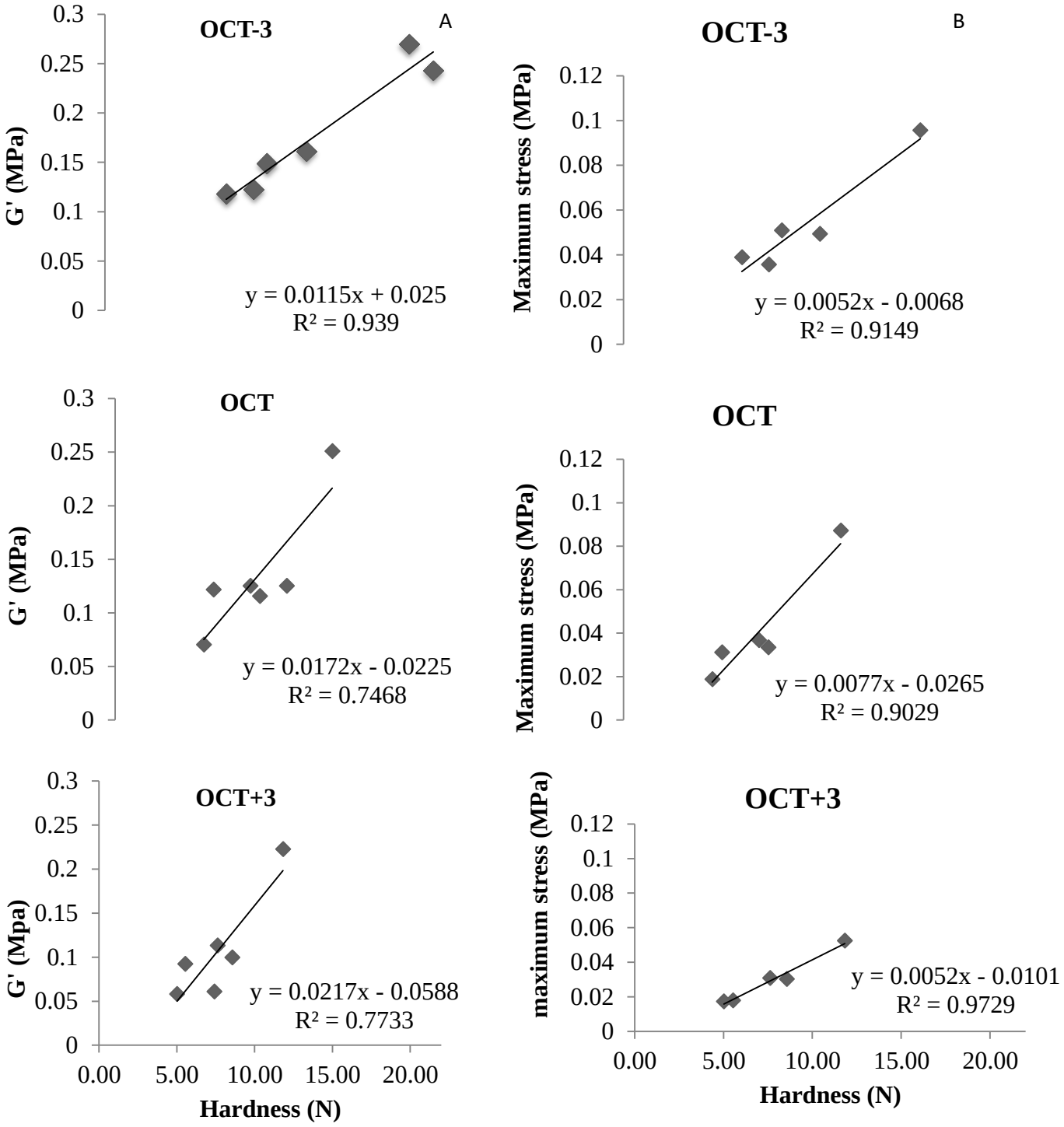
A
OCT-3
G' (MPa)
0.3
0.25
0.2
0.15
0.1
0.05
0
y = 0.0115x + 0.025
R² = 0.939
B
OCT-3
Maximum stress (MPa)
0.12
0.1
0.08
0.06
0.04
0.02
0
y = 0.0052x - 0.0068
R² = 0.9149
OCT
G' (MPa)
y = 0.0172x - 0.0225
R² = 0.7468
OCT
Maximum stress (MPa)
y = 0.0077x - 0.0265
R² = 0.9029
OCT+3
G' (Mpa)
y = 0.0217x - 0.0588
R² = 0.7733
0.00
5.00
10.00
15.00
20.00
Hardness (N)
OCT+3
maximum stress (MPa)
y = 0.0052x - 0.0101
R² = 0.9729
Hardness (N)

Figure 2

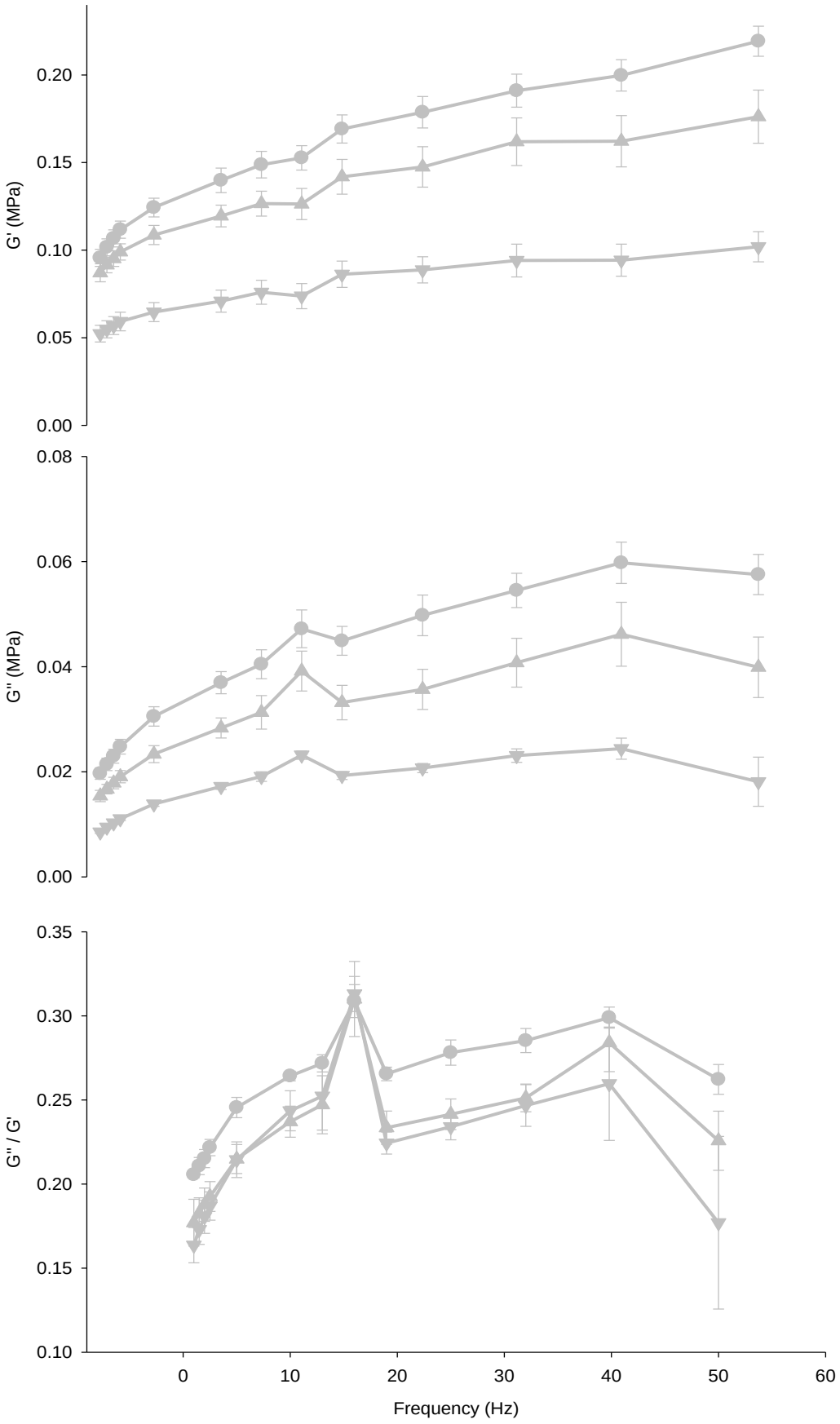
G' (MPa)
0.00
0.05
0.10
0.15
0.20
G'' (MPa)
0.00
0.02
0.04
0.06
0.08
G'' / G'
0.10
0.15
0.20
0.25
0.30
0.35
0
10
20
30
40
50
60
Frequency (Hz)

Figure 3

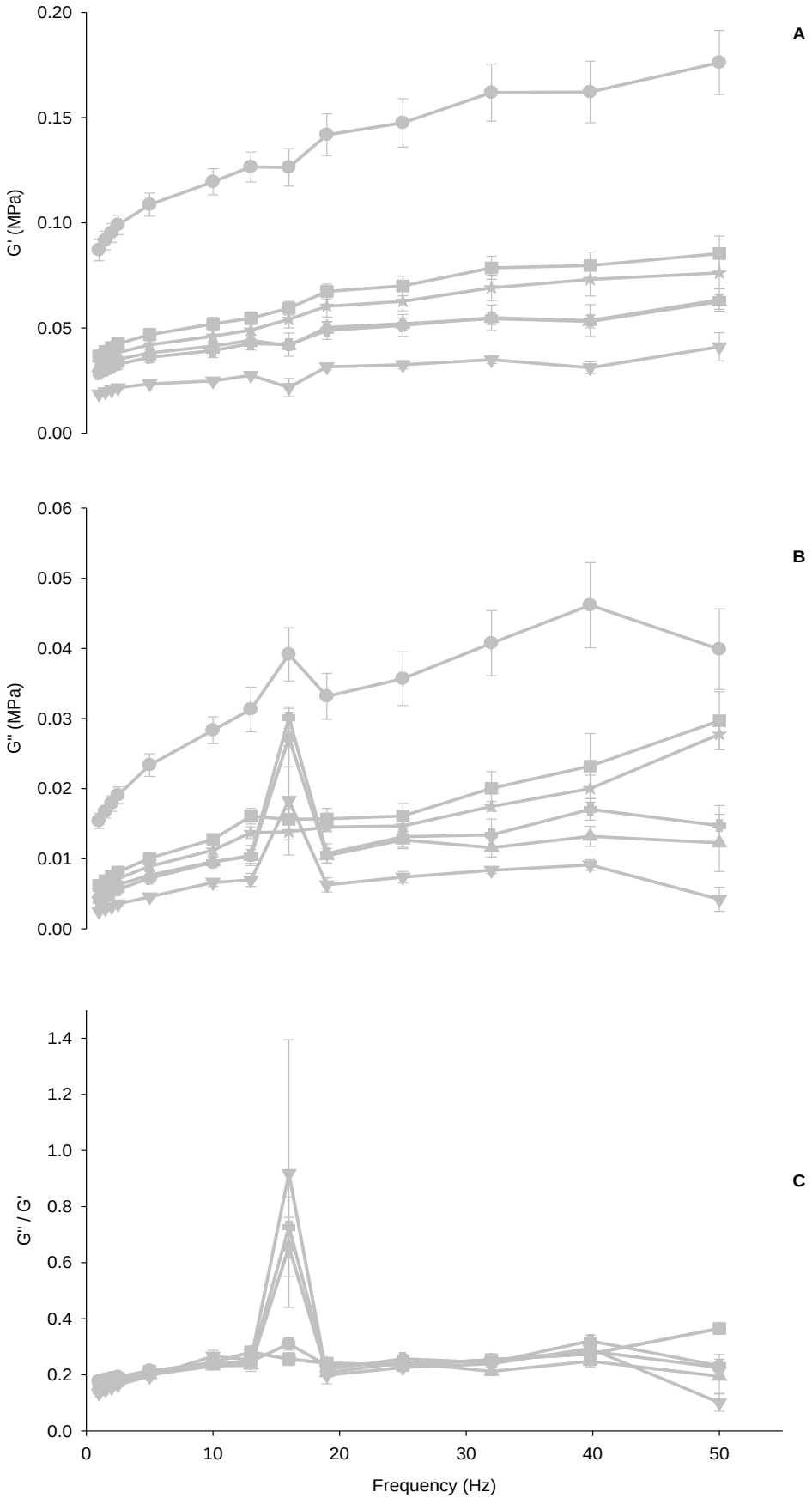
A
B
C
G' (MPa)
G'' (MPa)
G'' / G'
Frequency (Hz)
0.20
0.15
0.10
0.05
0.00
0.06
0.05
0.04
0.03
0.02
0.01
0.00
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50

Figure 4

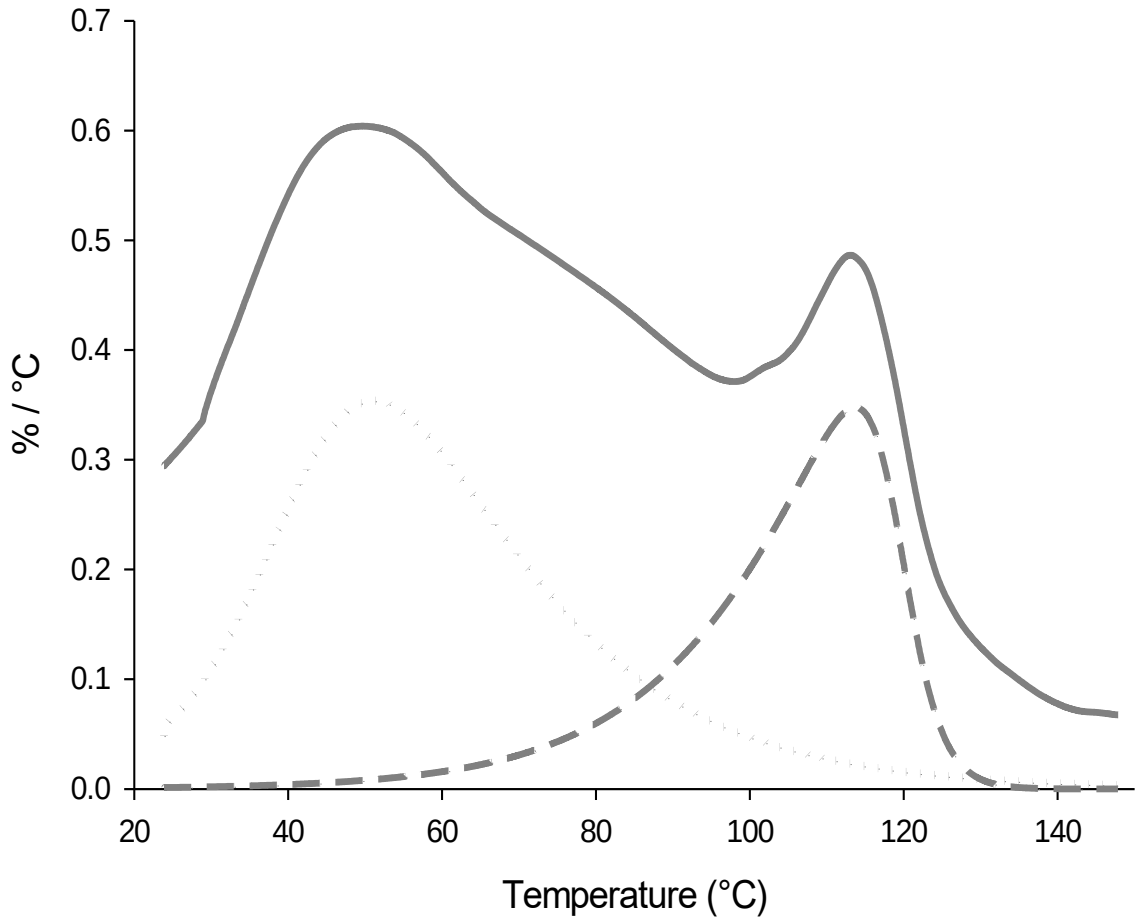

Figure 5

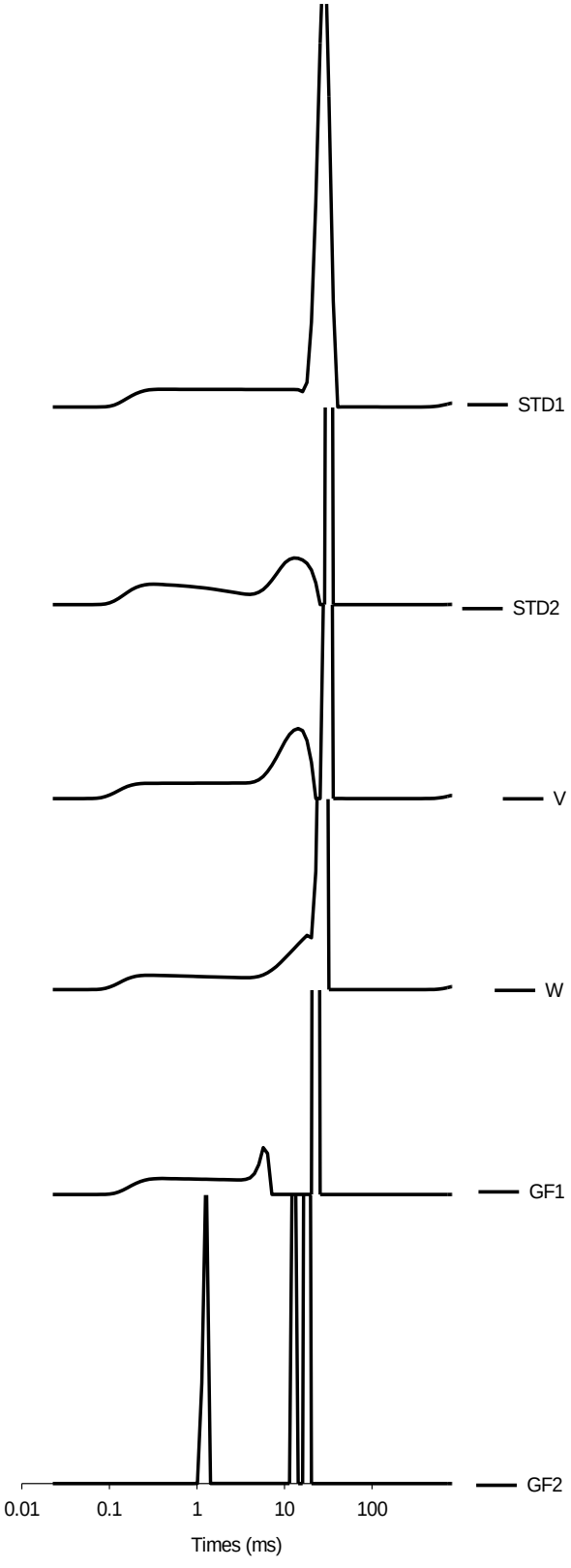

Figure 6

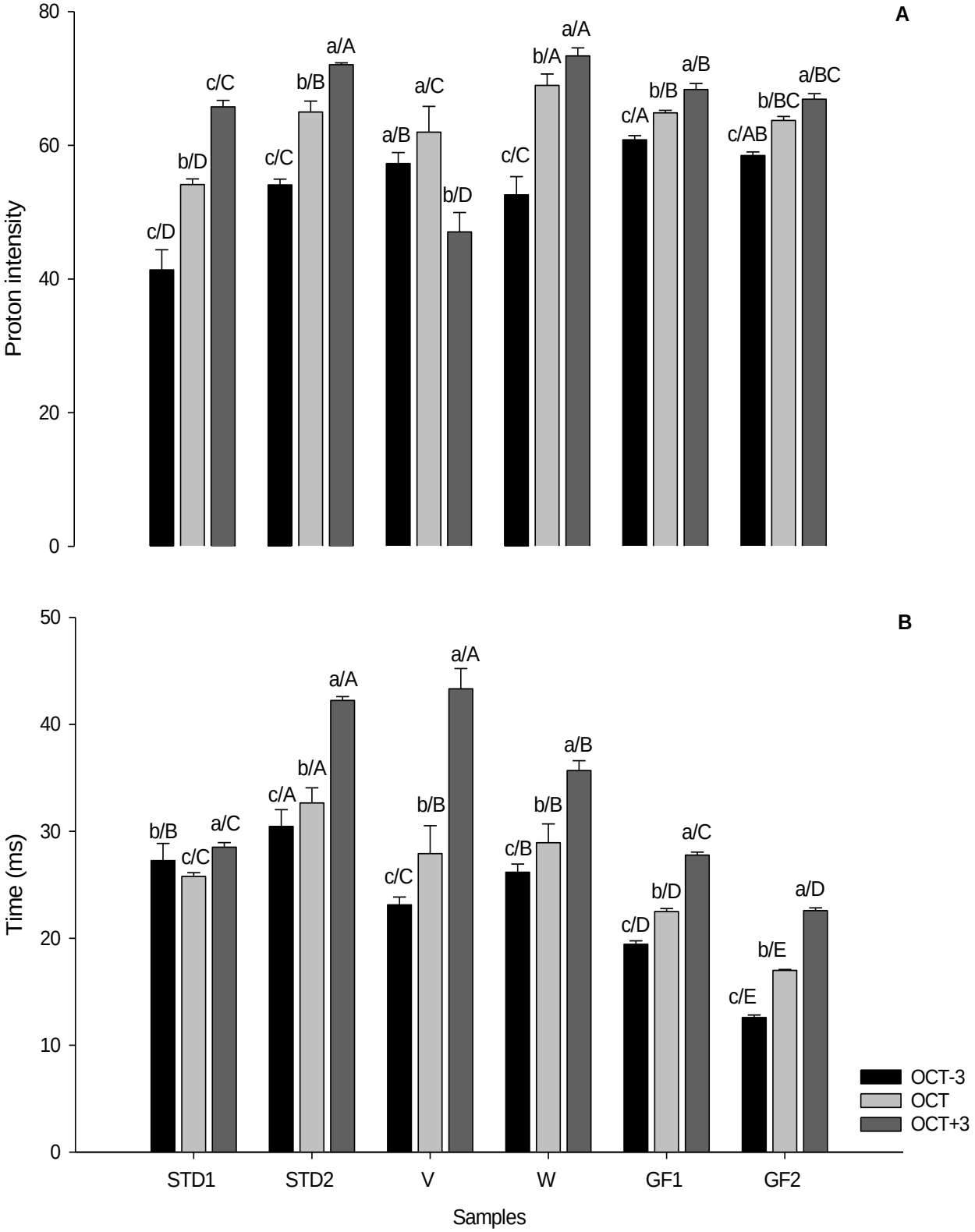
A
Proton intensity
80
60
40
20
0
c/D
b/D
c/C
c/C
b/B
a/A
a/B
a/C
b/D
c/C
b/A
a/A
c/A
b/B
a/B
c/AB
b/BC
a/BC
B
Time (ms)
50
40
30
20
10
0
b/B
c/C
a/C
c/A
b/A
a/A
c/C
b/B
a/A
c/B
b/B
a/B
c/D
b/D
a/C
c/E
b/E
a/D
STD1
STD2
V
W
GF1
GF2
Samples
OCT-3
OCT
OCT+3

Figure captions

Figure 1

Relationship between hardness vs storage modulus (A) and hardness vs stress maximum at break (B) of cooked pasta at different cooking time

Figure 2

Storage modulus (A), loss modulus (B) and tan delta (C) of cooked pasta (STD1) at different cooking time, OCT-3 (circle), OCT (triangle up) and OCT+3 (triangle up).

Figure 3

Storage modulus (A), loss modulus (B) and tan delta (C) of cooked pasta with different formulation at optimal cooking time: STD1 (circle), STD2 (square), V (star), W (triangle up), GF1 (triangle down) and GF2 (cross).

Figure 4

Characteristic ^{1}H T_2 relaxation time distribution for cooked pasta

Figure 5

Characteristic ^{1}H T_2 relaxation time distribution for cooked pasta with different formulation at optimal cooking time

Figure 6

^{1}H T_2 of the abundant and the more mobile population (A) and corresponding relaxation (B) of cooked pasta with different formulation at different cooking time; different letters in the figure indicate significant difference among samples ($p \leq 0.05$), where the small letter referred the difference due to the cooking time and the big one to the formulation; the "a" and “A” letters were assigned to the highest value

SECTION B

CHARACTERIZATION OF READY TO EAT (RTE) PASTA

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Effect of water and gluten on physico-chemical properties and stability of ready to eat shelf-stable pasta

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Abstract

A multi-analytical and multi-dimensional approach was used to investigate the effect of moisture and gluten on physico-chemical properties of shelf-stable ready to eat (RTE) pasta.

Moisture and frozen water contents were not affected by formulation nor storage time. Hardness and retrograded amylopectin significantly increased during storage in all samples, more markedly in pasta with the lowest moisture content. Higher amounts of water and gluten reduced pasta hardening and contributed to control RTE pasta quality. ^{1}H FID became steeper in samples during storage, more evidently in the sample with the lowest moisture content. Three proton T_2 populations were observed (population C, population D and population E). Population C and D were not resolved during all storage. ^{1}H T_2 relaxation time of the most abundant population (population E) shifted to shorter times and the amount of protons increased during storage, more importantly in the samples with lower moisture and gluten content.

Keywords: shelf-stable pasta; gluten; physico-chemical properties; ^{1}H NMR mobility

1- Introduction

Ready to eat (RTE) meals are becoming popular among consumers due to their convenience and the changes of eating habits. This trend is reflected by their significant market share (~10%) in recent years. Among RTE meals, pasta meals are indeed the main category as they represent a very large market segment, especially in Asia, Latin America, Middle and North Africa.

Pasta industry is introducing RTE pasta meals in the retail market in different storage conditions, primarily frozen, refrigerated or shelf-stable products. RTE pasta meals are generally constituted by a pasta phase and a sauce phase that may be in contact during storage or mixed only at time of consumption. The physical contact (or lack of it) between pasta and sauce are expected to have a key role on the dynamic interaction among meal components and the evolution of product's quality during storage.

To the authors' best knowledge, only few scientific reports have focused on the characterization of RTE pasta meals. Some works focused the attention on the changes occurring in the pasta phase during storage. Olivera and Salvadori (2009, 2011) studied quality parameters of cooked tagliatelle during frozen storage (up to 12 months) and the effect of freezing on tagliatelle properties. They reported a decrease of moisture content during the first 4 months and of hardness (first 2 months), that then remained almost constant during the rest of storage, and concluded that freezing had a negative effect on pasta structure. Carini, Curti, Cassotta, Najm and Vittadini (2014) focused their work on shelf-stable RTE pasta during 2 months of storage and reported an increase in pasta hardness and retrograded amylopectin as well as an increased molecular rigidity as measured by ^{1}H NMR (increased ^{1}H FID steepness and decreased ^{1}H T_2 relaxation times).

Other works investigated physico-chemical changes on RTE pasta meals in presence of sauce. Olivera and Salvadori (2012) observed decreased pasta hardness and increased moisture content in refrigerated lasagna (pasta with sauce) over 8 days storage. Carini, Curti, Littardi, Luzzini and Vittadini (2013) investigated water status in shelf-stable pasta meals with a tomato based sauce during 34 days of storage, and reported pasta softening and water migration between pasta and

sauce phases detected only at a molecular level (^{1}H T_1 and T_2), while moisture content and water activity did not reveal a macroscopic water migration between the pasta and sauce phases. The properties of the sauce should, also be carefully considered when used in a multiphasic meal (e.g. pasta and sauce).

A recent paper (Carini, Mora, Curti, Luzzini, & Vittadini, in press) has focused the attention on the sauce phase evaluating the effect of different ingredients commonly used in industrial settings on the status of water in the systems indicating that, for example, sauce thickening induced by flour or gelatin addition corresponded to very different NMR molecular mobilities.

Water and water dynamics are indeed a key factor in defining RTE meals quality and stability and a good understanding of the relation of water status indicators with product's composition, quality and stability is still a big challenge for food technologists.

In this work the effect of moisture content and gluten addition into RTE shelf-stable pasta was evaluated, during storage, in terms of physico-chemical properties and water status.

2- Materials and methods

2.1. Ready to eat shelf stable pasta production

Dry pasta (penne shaped) was produced by a local pasta maker using a standard formulation (semolina and water, STD) and was cooked into boiling water (pasta/water ratio 1:10) to reach 56% moisture (g H_2O / 100 g product, control sample, STD-56). Pilot plant trials were carried out to optimize the moisture content and gluten level to have a high quality product and the optimal conditions were defined as 59% moisture and 15%, semolina substitution, dry mass. Dry gluten enriched (15%) pasta was produced and cooked into boiling water to 59% moisture (g H_2O / 100 g product, GLU-59). A standard formulation pasta sample was also cooked to 59% moisture (STD-59). About 60 g of cooked pastas were packed into multi-layer (polypropylene-PP, polyethylene terephthalate-PET, and polyamide-PA) pouches and sterilized in autoclave ($F_0 \geq 7$) to obtain ready to eat shelf-stable pasta (RTE pasta). RTE pasta pouches were then kept at 22.5°C for 63 days, and

analyzed within 24 hours from production (day 0) and after 3, 7, 10, 21, 28, 35, 42, 49 and 63 days of storage.

STD-56 sample was beforehand characterized and the results are included in previous study (Carini, Curti, Cassotta, Najm, & Vittadini, 2014) but they were also presented in this paper to better study the effect of water and gluten contents on physico-chemical properties of RTE pasta during storage.

2.2. Moisture content

Moisture content (MC, % g water /100 g product) of RTE pasta was determined by weight loss by drying in a forced-air oven (ISCO NSV 9035, ISCO, Milan, Italy) at 105 °C to constant weight. At least ten pasta pieces of each RTE pasta sample at each storage time were analyzed.

2.3. Texture

Pasta texture was measured using a TA.TX2 Texture Analyzer equipped with a 25 kg load cell (Stable Micro systems, Goldalming, UK). Single pasta pieces were cut with a flat blade (speed of 2 mm/s; trigger force 0.1 N). The maximum height of the cutting peak was taken as “hardness”. A small peak before the major peak was also observed in some samples, indicating a significant breakage of the pasta piece along its main axes when touched by the cutting blade. This peak was taken as evidence of pasta “fracturability ”. 15 pasta pieces for each sample at each storage time were analyzed.

2.4. Thermal properties

Frozen water content and amylopectin melting were measured using a Differential Scanning Calorimeter (DSC Q100, TA Instruments, New Castle, DE, USA) calibrated with indium (T = 156.6°C; H = 28.71 J/g) and mercury (T = -38.83°C, H = 11.40 J/g). About 5-10 mg of RTE pasta were placed into hermetic stainless steel pans (Perkin Elmer, USA). Samples were heated from -80°C to 100°C at 5 °C/min. DSC thermograms were analyzed using an Universal Analysis Software, version 3.9A (TA Instruments, New Castle, DE).

“Frozen” water content (at the select experimental conditions; FW) was calculated from the endothermic peak around 0 °C (ice melting) using the following equation:

$$FW = EnthalpyIceFusion * (\frac{1}{LatentHeatIceFusion}) * (\frac{1}{MC}) * 100$$

Where FW is frozen water (%, g frozen water/g water), ice fusion enthalpy (J/g product), latent heat of ice fusion is 334J/g ice, and MC is moisture content (g water/1 g product).

The occurrence of an endothermic peak in the 50-80 °C range was taken as recrystallized amylopectin melting. Enthalpy of this peak was measured (J/g product) and normalized to the grams of starch of the sample (J/g starch). At least triplicated samples of each product were analyzed at each storage time.

2.5. Proton Nuclear Magnetic Resonance mobility (^{1}H NMR)

A low resolution (20MHz) ^{1}H NMR spectrometer (the Minispec, Bruker Biospin, Milano, Italy) operating at 25.0 ± 0.1°C was used to study proton molecular mobility by measuring the free induction decay (FID) and transverse relaxation times (T_2). About 2 g of RTE pasta were placed into a 10 mm NMR tube that was then sealed with Parafilm® to avoid moisture loss during the NMR experiment. ^{1}H FIDs were acquired using a single 90° pulse, followed by a dwell time of 7 µs, a recycle delay of 3 s and a 10 ms acquisition window. ^{1}H FIDs were analyzed in the time range 7 µs -100 µs where the homogeneity of magnetic field was assured. The curves were fitted with a two components model (exponential and gaussian; Le Grand, Cambert, and Mariette**,** 2007; Sigmaplot, v6, Systat Software Inc. USA):

$$f(t) = y0 + A * \exp(-t / T_A) + B * \exp[-(t / T_B)^2]$$

where y0 is the FID decay offset, A and B are the intensities of each relaxation component, T_A and T_B are the apparent relaxation times.

T_2 relaxation time was measured with a CPMG pulse sequence with a recycle delay of 3 s (≥ 5 ^{1}H T_1), an interpulse spacing of 0.04 ms and 4000 data points. T_2 curves were analyzed as quasi-continuous distributions of relaxation times using a UPENWin software (Alma Mater Studiorum, Bologna, Italy). Default values for all UPEN parameters were used with the exception of one parameter (LoXtrap) that was set to 1 to avoid extrapolation of relaxation times shorter than the first

experimental point. ^{1}H T_2 CPMG relaxation decays were also fitted with a discrete exponential model (Sigmaplot, v.6, Systat Software Inc. USA).

2.5. Statistical analysis

Means and standard deviations (SD) were calculated with SPSS statistical software (Version 20, IBM SPSS Inc., Chicago, IL, USA). Significant differences ($p \leq 0.05$) among different samples were verified with by one-way-analysis of variance (ANOVA) followed by least significant difference test (LSD) at $p \leq 0.05$.

3- Results and discussion

3.1 Moisture content and frozen water content

Moisture content (MC) of RTE pasta samples was found to be 56.5 ± 0.7% (g H_2O / 100 g product) in STD-56, 58.6 ± 0.9% in STD-59, and 59.0 ± 0.9% in GLU-59, confirming the achievement of the desired moisture contents. About 77% (g frozen water/100 g water) of the total water present in RTE pasta samples was measured to be frozen (FW) under the selected experimental conditions and no significant changes were ascribable to the different composition of the products. Both MC and FW did not significantly change during storage in all samples.

3.2. Texture

Hardness of RTE pasta during storage is shown in Figure 1a. Statistical significances are reported in Table 1.

At day 0, STD-59 was softer than STD-56 and GLU-59 was harder than STD-59. All samples became significantly harder with increasing storage time, as expected (Carini et al., 2014). Nevertheless, the hardness increase during storage was formulation dependent. STD-56 hardness increased very fast (from 9.9 to 17.3 N) up to 21 days of storage and then more gradually to the end of storage (17.7 N at 63 days; Carini et al., 2014). On the contrary, hardness increase in STD-59 was steady throughout the entire storage time (from 9.1 N at day 0, to 12.8 N at days 63), and less important than in STD-56. GLU-59 exhibited the lowest hardness increase (from 11.7 N, day 0, to

13.8 N, days 63) among all samples and it could, therefore, be considered the sample with the best texture retention during storage.

STD-56 was generally harder than STD-59 over storage, indicating that the higher moisture content contributed to material plasticization/softening, and might have helped to control hardness increase during storage. GLU-59 was significantly harder than STD-59 up to days 28, then no significant differences were observed for the remaining of the storage. Large amounts of gluten in pasta formulation (high gluten semolina) are known to harden cooked pasta texture (Cubadda, Carcea, Marconi, & Trivisonno, 2007; Day, Augustin, Batey, & Wrigley, 2006). GLU-59 generally exhibited hardness intermediate between STD-56 and STD-59 during storage, indicating that the increased moisture content may have allowed for a better plasticization of the gluten network and partially counteracted the hardening contribution of gluten to pasta texture. The higher softness of GLU-59 than STD-56 suggested that the softening effect of moisture content was probably more important than the hardening effect of gluten in determining hardness.

STD-56 exhibited fracturability (presence of a small peak in cut test trace) after 28 days of storage, that was not observable in STD-59 and GLU-59 until the end of storage. These samples, therefore, remained flexible and plastic during storage, probably due to their larger moisture (and gluten) content that might have contributed to the maintenance of pasta texture over storage.

3.3. Retrograded amylopectin

At days 0, no amylopectin retrogradation (J/g sample) was found in all pasta samples, as expected, due to the cooking and sterilization processes that allowed for a complete starch gelatinization in the products. Retrograded amylopectin was detected in STD-56 and STD-59 after 3 days of storage and, in GLU-59 only at 7 days of storage (Figure 1b). Retrograded amylopectin increased in all samples during storage, more significantly in STD-56 than in the 59% moisture content samples. STD-56 had significantly more retrograded amylopectin than STD-59 after 10 days, and also at day 63, probably due to its lower MC (Piazza & Masi, 1995). GLU-59 was, generally, less retrograded than STD-59 (with the exception of day 28 and 42). GLU-59 also had significantly lower

retrograded amylopectin than STD-56 after 10 days up to the end of storage. Chemical interactions of gluten with starch may have prevented formations of crystals as previously reported (Kim & D'Appolonia,1977; Martin, Zeleznak, & Hoseney, 1991). However the effect of gluten on amylopectin retrogradation has not yet been clearly elucidated, as some studies, in model systems and bread, reported that gluten enrichment have promoted (Curti et al., 2014) and had no effect (Wang, 2004; Ottenhof and Farhat, 2004).

Higher moisture, more importantly, and gluten levels had, therefore, a reducing effect on amylopectin retrogradation in RTE pasta during storage.

Amylopectin retrogradation enthalpy was plotted against hardness during storage, but only a weak relationship was found (R^2 of linear regression = 0.71 for STD-56, 0.91 for STD-59 and 0.70 for GLU-59, data not showed) indicating that other factors in addition to amylopectin retrogradation concurred to hardness increase.

3.4. Protons Nuclear Magnetic Resonance mobility (^{1}H NMR)

Proton molecular mobility was studied using a low resolution ^{1}H NMR spectrometer to investigate the effect of formulation on pasta products at a molecular level. ^{1}H FIDs (data not shown) had comparable decay at day 0 and became progressively steeper during storage, indicating rigidity increase in pasta samples. ^{1}H FIDs were fit with a two components model (exponential and Gaussian functions) to quantify the mobility of protons detected in this time-frame window. The two proton populations were named population A and population B. In STD-56, the most rigid population A relaxed at about 0.017 ms while population B at about 0.670 ms. In STD-59 and GLU-59, relaxation times of population A and B were comparable to those found in STD-56 and they were, respectively, 0.016 and 0.640 ms. Relaxation times did not significantly change during storage in all samples. At day 0, population A was found to be ~16-17% of all detectable protons in all samples. Population A increased in all samples during storage, up to 20-22%, with a consequent decrease of population B, indicating an increase of system rigidity, mainly due to amylopectin retrogradation (Fahrat, Ottenhof, Marie, & De Bezenac, 2003; Sereno, Hill, Mitchell, Scharf, &

Farhat, 2007). The presence of higher moisture and gluten content in pasta product did not change the mobility of the protons observed in this NMR time-frame window.

A representative ^{1}H T_2 distribution of pasta during storage, obtained with UpenWin, is showed in Figure 2a for a fresh sample (STD-56) and indicated the presence of three proton populations. The observed proton populations were not always resolved in all samples during storage, indicating an important molecular exchange among the protons with relaxation times in the 0.1 to 10 ms range (Figure 2b). A three exponential model was used to quantify proton populations' mobilities. The three ^{1}H populations were named population C, population D and population E, from the more rigid (shorter relaxation times) to the more mobile (longer relaxation times) protons, respectively (Table 2). Population C and population D relaxed at shorter times and represented the most rigid protons detected in this NMR time-frame.

In fresh samples, population C, represented about 10-11% (comparable among samples) of the total detectable protons and relaxed at 1.5 ms (T_{2C}) in STD-56, and 0.7-0.8 ms in STD-59 and GLU-59. Population D, represented about 11-12% (comparable among samples) and relaxed (T_{2D}) at 13.5 ms in STD-56 and 6-8 ms in STD-59 and GLU-59. Population E was the most abundant population in all samples, and represented, at day 0, about 74, 76 and 78% of total protons for STD-56, STD-59 and GLU-59, respectively. ^{1}H T_{2E} relaxation times were ~39.6 ms, ~33.2 ms, and ~34.8 ms in STD-56, STD-59, GLU-59, respectively. Generally, the three protons populations relaxed at slightly higher relaxation times in STD-56 than in the 59% moisture samples. Population D (intermediate mobility protons), was slightly more abundant, and population E (more mobile protons) slightly less abundant in STD-56 than in the 59% moisture samples. No remarkable different molecular mobilities were found among fresh RTE pasta samples that were, therefore, considered comparable.

^{1}H T_2 mobilities, on the contrary, evolved differently in the samples with different formulation during storage. Population percentages and relaxation times of protons belonging to population C and population D decreased during storage, more importantly in STD-56 than in STD-59 and GLU-59 (Table 2). STD-56 showed a larger decrease than the 59% samples for protons abundance of

population C (4% in STD-56 vs 2% in STD-59 and GLU-59) and D (8% in STD-56 vs 4% in STD-59 and GLU-59). Similarly, T_{2D} of STD-56 decreased of 7 ms vs 2-3 ms in STD-59 and GLU-59 during the 63 days of storage. Pop E, on the contrary, increased in the percentage of protons and decreased in relaxation time in all samples during storage. STD-56 showed a larger increase in population E (12% vs 5 and 8% in STD-59 and GLU-59, respectively) and a larger decrease of T_{2E} (10 ms vs 3 and 6 ms in STD-59 and GLU-59, respectively) among the samples considered. The shortest relaxing ^{1}H population (C) was tentatively related to rigid CH protons of amorphous starch and gluten; the population D to some CH protons of gluten and exchanging protons to confined water, starch, and gluten. Lastly, the predominant ^{1}H T_2 population (E) was tentatively related to mobile exchanging protons of water, starch, and gluten present in the formed gel network (Bosmans, Lagrain, Ooms, Fierens, & Delcour, 2013; Carini et al., 2014).

The decrease of T_{2E} and the corresponding increase of protons abundance (population E) during storage indicated changes in mobility within the amorphous gel network during storage. However, a good linear relationship did not subsist between ^{1}H T_{2E} (and % population E) and hardness (data not shown). On the contrary, Bosmans and colleagues (2013) correlated T_{2E} with hardness in bread, but the relationship was established with a limited number of data points (4) due to the short storage time considered (7 days).

Overall the results of this study indicated that the ^{1}H T_2 molecular changes during storage were more pronounced in STD-56 than in the 59% moisture samples. STD-56 retrograded and hardened to a greater extent during storage. Both amylopectin retrogradation and water molecular redistribution concurred to the hardening of RTE pasta during storage but the complexity of the system did not allow for a complete comprehension of the mechanisms. Proper hydration/plasticity of gluten could also be a key factor in preserving product's structure. The presence of higher moisture and/or gluten levels may have had a role in the softening of the macroscopic structure and have mitigated the protons/water redistribution among starch and gluten domains at a molecular level.

4. Conclusions

The effect of moisture content and gluten addition into RTE shelf-stable pasta was studied during 63 days storage.

A higher moisture content of pasta resulted in softer samples and reduced amylopectin retrogradation. Conversely, the presence of gluten in the sample with higher moisture level, resulted in increased hardness and decreased amylopectin retrogradation. Molecular mobility measurements showed that the most rigid protons (observed with ^{1}H FID) were not affected neither by moisture content nor gluten. Changes in molecular mobility were more clearly observed in the more mobile domains (^{1}H T_2), where a more pronounced increase in rigidity was observed in the sample with lower moisture and gluten content during storage.

It was concluded that, under the selected experimental conditions, properties of RTE pasta may be positively modulated by changes in formulation. An increase of moisture level improved quality and shelf-stability of RTE pasta and has to be taken in consideration when formulation of these products are conceived. Moreover, high levels of gluten also showed a positive effect on the physico-chemical properties evaluated and, for this reason, it should be considered, a key ingredient in the formulation of these products.

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Table 1

Table 1: Statistical significance (LSD Test, t-test; $p<0.05$), for hardness and retrograded amylopectin during storage[1]

	Hardness				**Retrograded amylopectin**			
	LSD Test			T-test	LSD Test			T-test
days	STD-56	STD-59	GLU-59		STD-56	STD-59	GLU-59	
0	g	f	de	(*) (*) (*)	n.d.	n.d.	n.d.	n.d.
3	f	def	e	(*) (*) (*)	d	de	n.d.	(*)
7	e	ef	bcd	(*) (*) (*)	cd	e	de	(-) (-) (*)
10	d	de	de	(*) (*) (*)	cd	cd	c	(-) (-) (-)
14	d	de	cde	(*) (*) (*)	ab	cde	de	(*) (*) (*)
21	c	cd	bc	(*) (*) (*)	bc	c	d	(*) (*) (*)
28	c	cd	bc	(*) (*) (*)	ab	c	bc	(*) (-) (*)
35	c	abc	b	(*) (-) (*)	ab	b	bc	(*) (*) (*)
42	b	abc	b	(*) (-) (*)	ab	b	ab	(-) (-) (*)
49	a	ab	b	(*) (-) (*)	abc	b	bc	(-) (*) (*)
63	bc	a	a	(*) (-) (*)	a	a	a	(*) (*) (*)

[a]Letters indicate significant differences among samples with the same formulation at different storage times; T-test was carried out between samples with different MC (STD-56 and STD-59) (indicated by the first symbol in brackets), gluten content (STD-59 and GLU-59) (indicated by the second symbol in brackets), different MC and gluten content (STD-56 and GLU-59) (indicated by the third symbol in brackets); stars indicate statistical difference; dashes indicate no statistical difference.

Table 2: Protons abundance (%) and relaxation times (ms) of T_2 experiments at selected storage times[2]

	STD-56			STD-59			GLU-59		
	T_2			T_2			T_2		
days	pop C	pop D	pop E	pop C	pop D	pop E	pop C	pop D	pop E
0	10.6 (0.5)	15.3 (1.2)	74.1 (0.8)	11.8 (0.8)	12.1 (0.5)	76.1 (0.7)	11.2 (0.8)	11.0 (0.3)	77.7 (0.7)
7	8.5 (0.8)	9.4 (0.5)	82.1 (0.5)	9.3 (0.7)	9.1 (0.3)	81.6 (0.7)	9.9 (0.6)	9.2 (0.6)	80.9 (0.3)
14	7.9 (0.5)	7.7 (0.3)	84.4 (0.5)	10.4 (0.9)	8.9 (0.4)	80.7 (1.2)	10.4 (0.8)	9.1 (0.2)	80.5 (0.8)
21	7.6 (0.7)	7.4 (0.6)	84.9 (0.5)	9.4 (0.5)	8.3 (0.3)	82.3 (0.5)	9.4 (0.4)	9.1 (0.2)	81.5 (0.4)
35	7.5 (0.8)	6.9 (0.5)	85.6 (0.6)	9.8 (0.6)	7.9 (0.5)	82.2 (0.9)	9.7 (0.6)	8.4 (0.2)	82.0 (0.5)
63	7.1 (0.4)	7.0 (0.5)	85.9 (0.5)	8.7 (0.5)	7.3 (0.5)	84.0 (0.9)	9.4 (0.6)	7.7 (0.4)	82.8 (0.8)
days	T_{2C}	T_{2D}	T_{2E}	T_{2C}	T_{2D}	T_{2E}	T_{2C}	T_{2D}	T_{2E}
0	1.50 (0.15)	13.54 (0.15)	39.59 (1.39)	0.81 (0.07)	7.79 (0.49)	33.22 (0.17)	0.67 (0.07)	6.48 (0.58)	34.79 (0.35)
7	0.93 (0.12)	7.65 (0.15)	30.63 (0.45)	0.48 (0.08)	5.04 (0.38)	35.56 (0.13)	0.56 (0.10)	5.30 (0.08)	33.29 (0.59)
14	0.76 (0.09)	6.14 (0.15)	28.63 (0.48)	0.41 (0.07)	4.08 (0.32)	29.02 (0.29)	0.51 (0.09)	5.10 (0.44)	31.73 (0.11)
21	0.38 (0.10)	4.15 (0.15)	27.84 (0.54)	0.42 (0.05)	4.34 (0.35)	31.02 (0.32)	0.52 (0.08)	5.30 (0.41)	32.31 (0.15)
35	0.34 (0.13)	3.60 (0.57)	28.50 (1.00)	0.43 (0.07)	3.96 (0.32)	27.86 (0.41)	0.54 (0.06)	5.78 (0.23)	32.62 (0.27)
63	0.70 (0.13)	7.05 (2.01)	29.87 (1.21)	0.48 (0.10)	4.37 (0.34)	30.10 (0.55)	0.43 (0.07)	4.27 (0.29)	29.12 (0.37)

Figure 1

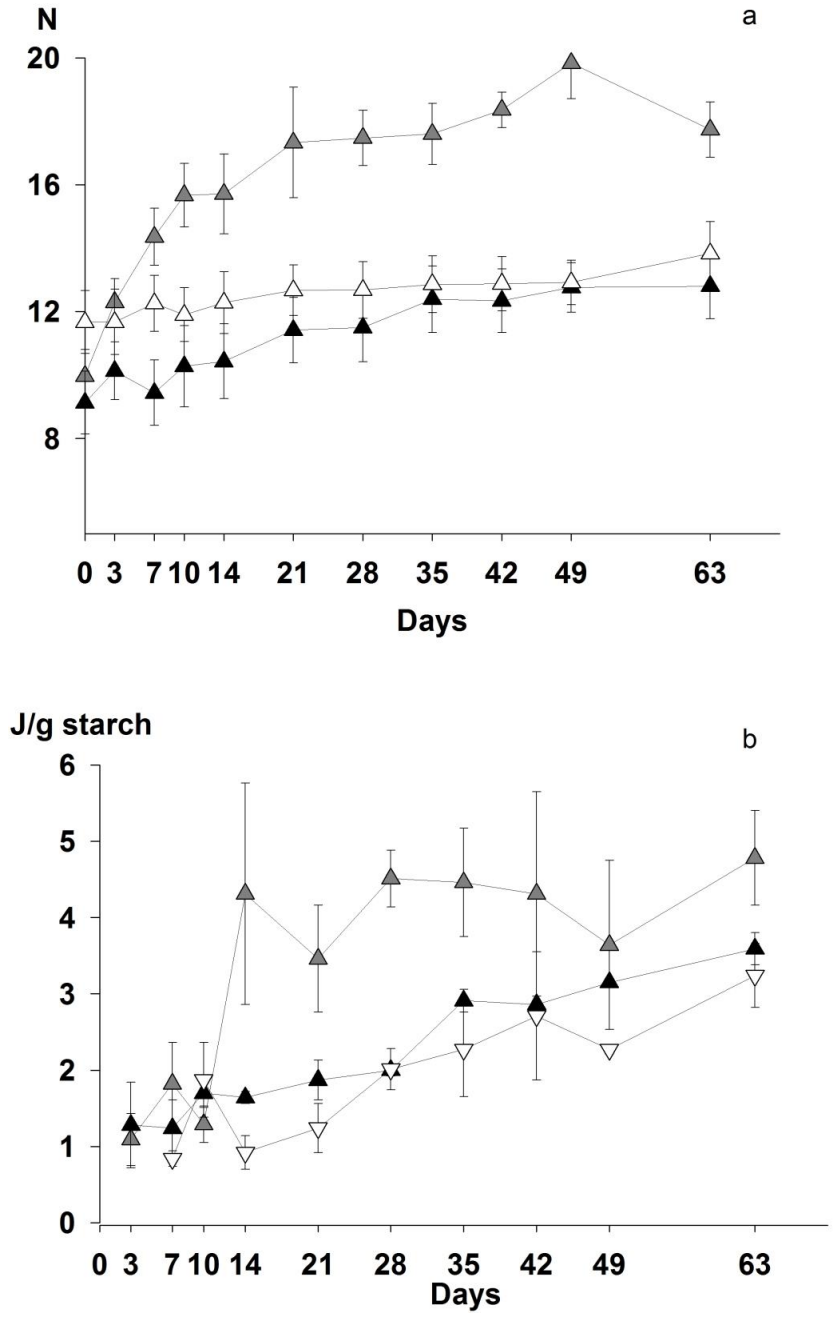
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Figure 2

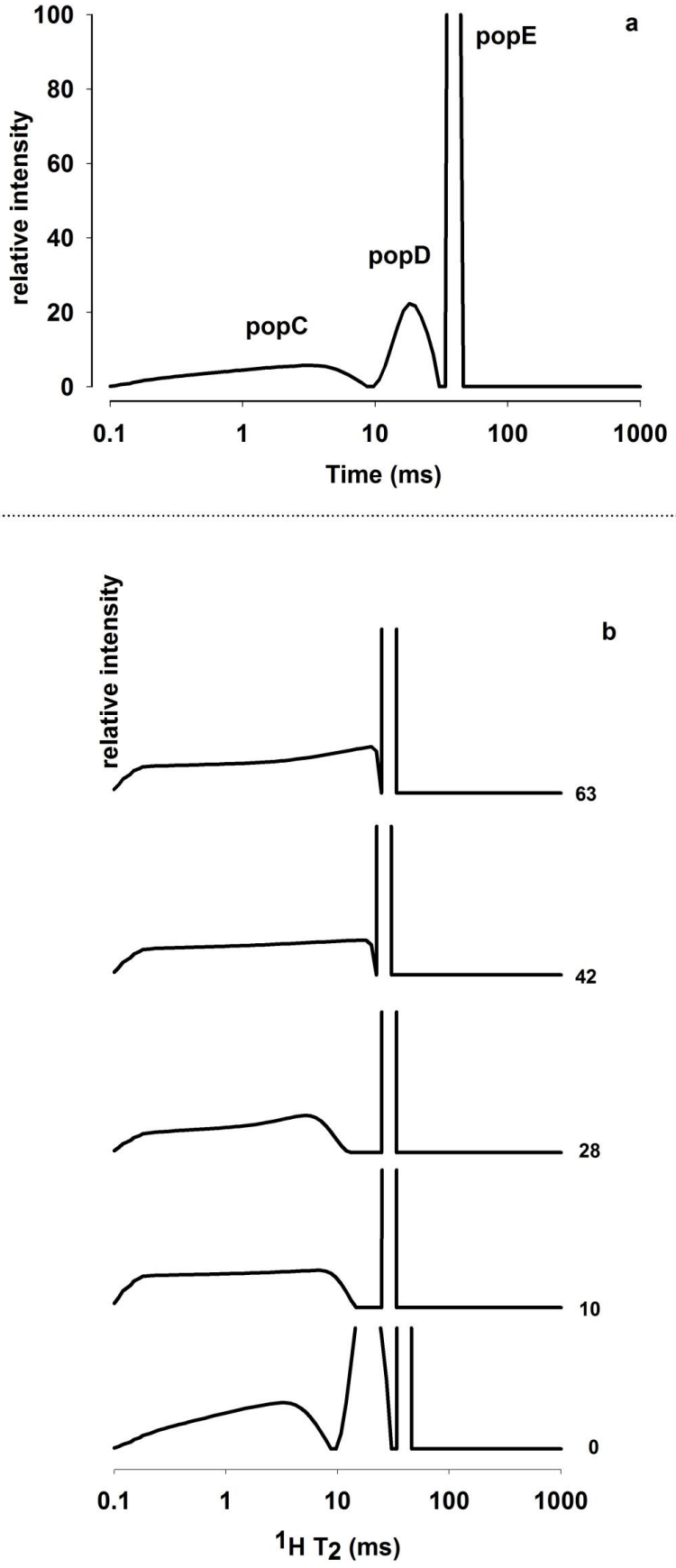
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Figure captions

Figure 1: (a) Hardness of STD-56 (gray triangle), STD-59 (black triangle) and GLU-59 (white triangle) during storage; (b) Retrograded amylopectin of RTE pasta samples during storage.

Figure 2: (a) Characteristic ^{1}H T_2 relaxation time distribution for ready-to-eat shelf-stable pasta; (b) Characteristic ^{1}H T_2 relaxation time distributions during storage.

Effect of glycerol and gluten on physico-chemical properties of ready to eat pasta during storage

Abstract

This work aims to investigate the effect of glycerol and gluten on physico-chemical properties of ready to eat pasta at two levels of water (56% and 62%, g water /100 g product). Macroscopic (moisture content, frozen water) and molecular (^{1}H FID, ^{1}H T_2) water status indicators, rheological properties and retrograded amylopectin content were measured to characterize the product with a multi-analytical and multi-dimensional approach.

Neither glycerol nor gluten affected macroscopic waters status. Glycerol did not affect rheological properties at both moisture contents, but it induced important modifications at a molecular level (increased proton mobility at lower moisture content and, at higher moisture content, reduced amount of the more abundant proton population that relaxed faster). Gluten hardened samples but it did not affect the proton molecular mobility. The combination of both ingredients at a lower moisture content resulted in harder samples ("gluten-effect") with a higher proton molecular mobility ("glycerol-effect"). At higher moisture content the combination of both ingredients delayed amylopectin retrogradation, which was almost constant over the storage.

Keywords Pasta . Gluten . Glycerol . Physico-chemical properties . ^{1}H NMR mobility

1- Introduction

Traub and Odland (1979), defined convenience foods as “any fully or partially prepared food in which significant preparation time, culinary skills, or energy inputs have been transferred from the home kitchen to the food industry or distributor. The increasing demand for convenience foods has been correlated to the different human socio-economic habits, such as female participation in the workforce, increased time pressure, growing number of single-person households, and a lack of abilities and experience with preparing meals at home (Costa et al., 2007; Geeroms et al., 2008). In the recent years, pasta industry has introduced different typologies of ready to eat (RTE) pasta that can be included in the category of convenience food meals. RTE pasta meals are found in the market in different typologies, based on their storage conditions: frozen, refrigerated and shelf-stable products. Olsen et al. (2012) reported that the consumers’ probability to buy convenience food is strongly correlated to the product properties, such as flavor, appearance, texture and odor. RTE pasta meals are subjected to enormous changes during storage, with important effects on consumers acceptability: water migration from surface to center of lasagna piece was observed during storage/cooling time (McCarthy et al., 2002) while increase of moisture content at the beginning of storage, firmness increase and surface color changes were observed in refrigerated and frozen lasagna (Olivera & Salvadori, 2011, 2012). Changes in ready to eat shelf-stable pasta meals were associated to an overall quality loss, resulting from physico-chemical changes: Carini et al. (2013) reported an increase of hardness and recrystallized amylopectin in cooked and sterilized pasta products, and changes in the water status molecular mobility indicators. Another study (Carini et al., 2014) investigated changes in RTE pasta meal, where pasta and a tomato sauce phase were mixed together. They highlighted pasta softening during storage, possibly related to a molecular water migration from the sauce to pasta. The investigation of the water role might, therefore, help in understanding these phenomena. Changes occurring in pasta during storage are similar to those observed in bread staling, which were deeply investigated (Hallberg & Chinachoti, 2002; Lin & Lineback, 1990; Imberty & Perez, 1988; Slade & Levine, 1991; Vittadini & Vodovotz, 2003,

Sereno et al., 2007). The main goal to improve RTE pasta quality is to prevent physico-chemical changes during storage and, for example, a proper pasta formulation might contribute to modulate these changes. Glycerol was used to extend the shelf-life of bread (Berkowitz & Oleksyk, 1991) and it was used in bread where they observed that the presence of glycerol decreased the retrograded amylopectin (Baik & Chinachoti, 2002). Curti et al. (2015) investigated the effect of gluten and glycerol on fresh cooked pasta quality. They observed limited water absorption during cooking and an increased pasta hardness in the presence of a high level of gluten, while the addition of glycerol enhanced water absorption and softened pasta. Diantom et al. (2015) investigated the influence of moisture content and gluten on physico-chemical properties of 'fresh' cooked pasta. They reported a softening action at higher moisture content (59% g water / 100 g product as compared to 56%, also in combination with higher level of gluten (15%, g gluten / 100 g product), associated to a water plasticizing effect on pasta structure.

This paper aims, therefore, to explore the effect of glycerol and gluten, also mixed together, on physico-chemical properties (water status, texture) and proton molecular mobility of RTE pasta meals during storage.

2- Materials and methods

2.1- Ready to eat shelf stable pasta production

Dry pasta (penne shaped) was produced by a local pasta maker using a standard formulation (semolina and water, STD) and was cooked into boiling water (pasta/water ratio 1:10) to reach 56% and 62% moisture (g H_2O /100 g product, control sample, STD-56 and STD-62, respectively). Dry pastas with added glycerol, gluten and their mixture in equal amounts were also produced. Dry pasta with 7% (g added ingredient /100 product) of gluten and glycerol, and pasta with both glycerol (7%) and gluten (7%) and cooked into boiling water to reach 56% (GLU7-56, GLY7-56 and GLU7GLY7-56, respectively) and 62% (GLU7-62, GLY7-62 and GLU7GLY7-62, respectively) moisture. About 60 g of cooked pastas were packed into multi-layer (polypropylene-PP, polyethylene terephthalate-PET, and polyamide-PA) pouches and sterilized in autoclave ($F_0 \geq$

7) to obtain ready to eat shelf-stable pasta (RTE pasta). RTE pasta pouches were then kept at 22.5 °C for 70 days, and analyzed within 24 h from production (day 0) and after 7, 14, 21, 28, 42, and 70 days of storage.

2.2- Methods

2.2.1- Water status

Moisture content (MC, % g water/100 g product) of RTE pasta was determined by weight loss by drying in a forced-air oven (ISCO NSV 9035, ISCO, Milan, Italy) at 105 °C to constant weight. At least ten pasta pieces of each RTE pasta sample at each storage time were analyzed.

2.2.2- Texture

Pasta texture was measured using a TA.TX2 Texture Analyzer equipped with a 25 kg load cell (Stable Micro systems, Goldalming, UK). Single pasta pieces were cut with a flat blade (speed of 2 mm/s; trigger force 0.1 N). The maximum height of the cutting peak was taken as ‘‘Hardness’’. 15 pasta pieces for each sample at each storage time were analyzed.

2.2.3- Thermal properties

Frozen water content and amylopectin melting were measured using a Differential Scanning Calorimeter (DSC Q100, TA Instruments, New Castle, DE, USA) calibrated with indium (T = 156.6 °C; H = 28.71 J/g) and mercury (T = -38.83 °C, H = 11.40 J/g). About 5–10 mg of RTE pasta were placed into hermetic stainless steel pans (Perkin Elmer, USA). Samples were heated from -80 °C to 100 °C at 5 -C/min. DSC thermograms were analyzed using an Universal Analysis Software, version 3.9A (TA Instruments, New Castle, DE). Frozen water content (at the select experimental conditions; FW) was calculated from the endothermic peak around 0 °C (ice melting) using the following equation:

*FW = Enthalpy Ice Fusion * (1/Latent Heat Ice Fusion)* (1/MC)*100*

where FW is frozen water (%, g frozen water/g water), ice fusion enthalpy (J/g product), latent heat of ice fusion is 334 J/g ice, and MC is moisture content (g water/1 g product).

The occurrence of an endothermic peak in the 50–80 °C range was taken as recrystallized amylopectin melting. Enthalpy of this peak was measured (J/g product) and normalized to the grams of starch in the sample (J/g starch). At least triplicated samples of each product were analyzed at each storage time.

2.2.4- Proton molecular mobility

A low resolution (20 MHz) ^{1}H NMR spectrometer (the Minispec, Bruker Biospin, Milano, Italy) operating at 25.0 ± 0.1°C was used to study proton molecular mobility by measuring the free induction decay (FID) and transverse relaxation times (T_2). About 2 g of RTE pasta were placed into a 10 mm NMR tube that was then sealed with Parafilm to avoid moisture loss during the NMR experiment. ^{1}H FIDs were acquired using a single 90° pulse, followed by a dwell time of 7 µs, a recycle delay of 3 s and a 10 ms acquisition window. ^{1}H FIDs were analyzed in the time range 7–100 µs where the homogeneity of magnetic field was assured. The curves were fitted with a two components model (exponential and gaussian; Le Grand, Cambert, & Mariette, 2007; Sigmaplot, v6, Systat Software Inc., USA):

$f\ (t) = y0 + A*\exp(-t/T_A) + B*\exp[-(t/T_B)^2]$

where y0 is the FID decay offset, A and B are the intensities of each relaxation component, T_A and T_B are the apparent relaxation times.

T_2 relaxation curves were acquired with a CPMG pulse sequence with a recycle delay of 3 s (≥ 5 ^{1}H T_1), an interpulse spacing of 0.04 ms and 4000 data points. T_2 curves were analyzed as quasi-continuous distributions of relaxation times using a UPENWin software (Alma Mater Studiorum, Bologna, Italy). Default values for all UPEN parameters were used with the exception of one parameter (LoXtrap) that was set to 1 to avoid extrapolation of relaxation times shorter than the first experimental point. ^{1}H T_2 CPMG relaxation decays were also fitted with a discrete exponential model (Sigmaplot, v.6, Systat Software Inc., USA).

2.2.5- Statistical analysis

Means and standard deviations (SD) were calculated with SPSS statistical software (Version 22.0, SPSS Inc., Armonk, New York, USA). Significant differences ($p \leq 0,05$) among different samples at equal moisture content were verified with by one-way-analysis of variance (ANOVA) with a Tukey-high and LSD significant difference test

3- Results and Discussions

Moisture content of STD-56 and STD-62 was found to be 55.7 ± 0.5 and 61.6 ± 0.4% (g H_2O / 100 g product), respectively, confirming that the desired moisture content was reached during cooking. Also samples with glycerol and gluten also exhibited the desired moisture content (data not shown) and were comparable to the controls (STD-56 and STD-62). Moisture content of all samples did not significantly change during storage, as previously reported (Diantom et al., 2015; Carini et al., 2014).

Frozen water content of STD-56 and STD-62 was 68.0 ± 3.2 and 71.7 ± 6.0% (g frozen water / 100 g water), respectively, showing that frozen water was not related to the sample's moisture content. Glycerol and gluten added samples exhibited similar frozen water content (data not shown), indicating that frozen water content was not dependent on formulation, in these samples. Moreover, frozen water content did not change in all samples during storage, suggesting that this water status indicator was not able to discriminate among samples.

Hardness

Hardness of RTE pasta with different formulation during storage is showed in Table 1. At day 0, GLU7-56 and GLU7GLY7-56 showed similar hardness but resulted to be significantly harder than STD-56 as previously reported (Curti et al, 2015), while GLY7-56 exhibited similar harness as STD-56. At 62% moisture content, GLU7-62 showed similar hardness to STD-62 while GLY7-62 was softer than STD-62, but GLU7GLY7-62 had similar hardness to STD-62. Hardness significantly increased during storage (70 days) in all samples, as expected (Carini et al., 2014; Diantom et al., 2015) but it reached a plateau after days 28 of storage at both moisture contents and then it remained almost constant over storage in all samples, except for GLU7-62. The presence of

gluten hardened pasta during storage, as previously reported (Diantom et al., 2015). This might be associated to a strengthening of the gluten network, since it is known that higher quantity and better quality of gluten in semolina has an effect on pasta hardness of final product (Cubadda et al., 2007). On the contrary, at 62% moisture, gluten did not significantly affect pasta hardness, and this might be associated to the predominant plasticizing effect of water, which softened pasta structure.

The presence of glycerol significantly softened pasta, at 56% and 62% moisture, as previously reported (Curti et al, 2015), and the softening effect was more evident only at day 28. Hallberg & Chinachoti, (1992) and Taub et al., (1994) reported that added glycerol to white bread softened.

The simultaneous presence of two ingredients (GLU7GLY7-56 and 62), that were expected to have very different effect on pasta texture (hardening and softening), resulted to be similar to glu7-56 and 62. This result suggested a predominant effect of gluten on glycerol at a macroscopic level.

Retrograded amylopectin

The retrograded amylopectin content of RTE pasta with different formulation during storage was showed in Table 2. At day 0, there was no amylopectin melting peak in all samples, as expected, indicating a complete starch gelatinization after cooking and sterilization. Amylopectin retrogradation was observed at day 7 in all samples at 56% moisture, but no significant difference was found among samples.

At 62% moisture, only GLY7-62 exhibited amylopectin retrogradation at day 7, but in STD-62 and GLU7-62 the retrograded amylopectin was observed at day 14, while it was detected after 21 days of storage in GLU7GLY7-62. Retrograded amylopectin content increased significantly during storage in all samples, except in the case of GLU7GLY7-62, where no differences were observed over storage. The presence of gluten did not significantly affect amylopectin retrogradation, however Diantom et al. (2015) reported that the addition of higher level of gluten and moisture contents reduced amylopectin retrogradation over storage. In this case the no effect of added gluten neither at lower nor at higher moisture content, might be associated to its lower quantity (7% g gluten / 100 g semolina). Thus, the changes in samples with higher gluten content might be also

correlated to the reduced starch content, and it could be useful to investigate if changes in amylopectin retrogradation in samples with similar value of starch content are related to starch content or added gluten. On the contrary, the presence of glycerol in combination with higher moisture content seemed to accelerate amylopectin retrogradation. Although it was previously reported (Baik & Chinachoti, 2001) added glycerol in bread delays amylopectin retrogradation, due to its penetration into the amorphous regions of amylopectin which may lower the local mobility of the amylopectin chain, and preventing the reorienting into crystalline structure. Finally, it could be observed that the combination of gluten, glycerol and higher moisture content could delay amylopectin retrogradation and seemed to be important key to control amylopectin retrogradation over storage. In fact GLU7GLY7-62 showed the lowest retrograded amylopectin content after 21 days with no further changes over storage.

Proton molecular mobility

^{1}H NMR investigation was used to explore the effect of formulation on proton molecular mobility. In particular, ^{1}H FID was used to evaluate the effect of formulation on the more rigid protons while ^{1}H T_2 on the more mobile protons. ^{1}H FIDs were fit with a two components model (data not shown). Populations were named population A and B, and the corresponding relaxation times, T_A and T_B. At day 0 and 56% moisture content, population A of all samples was similar to STD-56, while at higher moisture content, GLU7-62 had a larger population A (more rigid protons) than STD-62, and GLY7-62 and GLU7GLY7-62 comparable to STD-62. The more rigid population significantly increased during storage in all samples at 56% and 62%, except for GLU7-62, where population A remained approximately constant (about 15.4%) up to days 42 then it significantly increased to 17.2% at days 70. The increase of more rigid protons (population A) indicates that changes occurred in the product structure over storage, which conferred to the sample higher rigidity.

The corresponding relaxation times, T_A and T_B, were found to be about 0.016 ms and 0.620 ms, respectively, in all samples. Both relaxation times did not depend on storage time and were not significantly affected by the presence of glycerol and gluten over storage.

Representative ^1H T_2 distributions of pasta with different formulation at 56% and 62% obtained with UpenWin indicated the presence of four protons populations (Figure 1). According to these distributions, ^1H T_2 curves were fitted with a four exponential model and the population were named population C, D, E for all samples (Figure 1A), except in the case of GLY15-56 and GLU7GLY7-56, where population C and D were merged in one broad population, population E was no more observable and a resolved population G was observed at higher relaxation times (Figure 1B).

At day 0 and 56% moisture content, GLU7-56 had a lower percentage of population F than STD-56, while GLY7-56 and GLU7GLY7-56 were comparable to STD-56. At 62% moisture, the presence of added gluten and/or glycerol significantly decreased the population F as compared to STD-62, with GLU7-62 and GLU7GLY7-62 comparable between each other and significantly higher than GLY7-62.

At lower moisture content (56%), population F significantly increased in GLY-56 and GLU7GLY7-56 during the first 14 days and then it remained constant up to the end of storage, while it seemed to decrease during storage in GLU7-56 and remained almost constant in STD-56. At higher moisture content, population F seemed to increase in STD-62 and GLU7-62 while it seemed to decrease in GLY7-62 and GLU7GLY7-62.

The presence of glycerol significantly increased the more abundant population at lower moisture content while it significantly reduced population F at higher moisture content during storage. The presence of gluten did not affect the proton mobility neither at lower nor at higher moisture content. Changes in samples with the mixture of gluten and glycerol were similar to those observed in sample with glycerol, suggesting that glycerol effect was more relevant than that of gluten, even if these samples exhibited similar hardness to gluten samples. Thus, samples with the mixture of

gluten and glycerol behaved as gluten samples at macroscopic level, while glycerol promoted the proton mobility at a molecular level.

The other proton populations (data not shown) indicated major changes in the presence of glycerol. In fact populations C, D and E were differently distributed at lower moisture content, where population E was not observed and the additional population G with a higher mobility was present (≈10% at day 0 and then it significantly decreased during storage). At higher moisture content population E was observed, being significantly higher (≈ 21%) in GLY7-62 and GLU-GLY62 than in STD-62 and GLU7-62, where was about 12% and 14%, respectively.

The relaxation time of the more abundant proton population (T_{2F}) is reported in Table 1. At day 0, T_{2F} of GLY7-56 was higher than STD-56, while GLU7-56 and GLU7GLY7-56 exhibited lower relaxation time than STD-56, with GLU7GLY7-56 having the lowest relaxation time. At 62% moisture, GLU7-62 had similar relaxation time to STD-62, while the T_{2F} of GLY7-62 and GLU7GLY7-62 was significantly higher than STD-62 but GLU7GLY7-62 exhibited the highest relaxation time. The relaxation time of the more abundant proton shifted towards shorter times during storage in all samples, except in STD-56 were it remained almost constant.

The presence of glycerol significantly decreased T_{2F} at lower moisture content, as previously reported (Curti el al., 2015). An increased in proton mobility was however observed and possibly associated to a plasticizing effect, as indicated by the additional more mobile proton population observed at higher relaxation time (population G). At a higher moisture content, the presence of glycerol increased the relaxation time of the more abundant and more mobile proton population during storage. These changes might suggest that the presence of glycerol at higher moisture content seemed to increase sample rigidity, as indicated by the increase of pop C. This might be explained by a possible anti-plasticizing effect of glycerol at higher moisture content. Lourdin et al. (1997) reported that in water-glycerol-starch systems, the roles the two competing plasticizers are related to their respective concentration. Thus at higher moisture content, water plays in this case

the most relevant plasticizing effect, increasing the proton mobility, while glycerol induced an increase of rigidity.

On the contrary, the presence of added gluten increased and decreased T_{2F} at lower and higher moisture content, respectively. However the presence of gluten did not significantly affect the proton redistribution neither at lower and higher moisture content.

GLU7GLY7-56 exhibited similar T_{2F} as GLY7-56, while GLU7GLY7-62 showed the highest T_{2F} at higher moisture content, suggesting an eventual interaction between the glycerol and gluten at higher moisture content.

4- Conclusions

The effect of added glycerol and gluten on physico-chemical properties of ready to eat pasta with two levels of water was explored during storage. Both moisture content and frozen water content were not affected neither by storage time nor by formulation. The presence of gluten hardened samples, more markedly at lower moisture content, but it did not affect the proton molecular mobility and amylopectin retrogradation. In fact the effect of gluten was more highlighted at macroscopic level than at molecular level. In contrast, the presence of glycerol softened pasta and induced strong changes at molecular level, affecting the proton molecular mobility. Glycerol effect seemed to be related to the moisture content into the samples, exhibiting the higher plasticizing effect at lower moisture content while at higher moisture content it seemed to play a little anti-plasticizing effect, reducing the more abundant population. The combination of both ingredients, in particular at higher moisture content, seemed to limit the changes into physico-chemical properties of ready to eat pasta. But these samples exhibited similar rheological properties as samples with added gluten while they showed similar proton mobility as samples with added glycerol. Changes in retrograded amylopectin content were not well defined, thus it resulted very difficult to attribute it to a specific ingredient or even to reduced starch. Thus it will be necessary to explore the changes in retrograded amylopectin content in samples with similar starch content.

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Table 1

Hardness, retrograded amylopectin, population F (popF) and relaxation time (T_{2F}) of pasta with 56% of moisture during storage. Standard deviations are given in parenthesis following the means values; different letters close to numbers indicate significant difference among samples ($p \leq 0.05$), where the small letters referred the difference due to storage and capital letters to formulation.

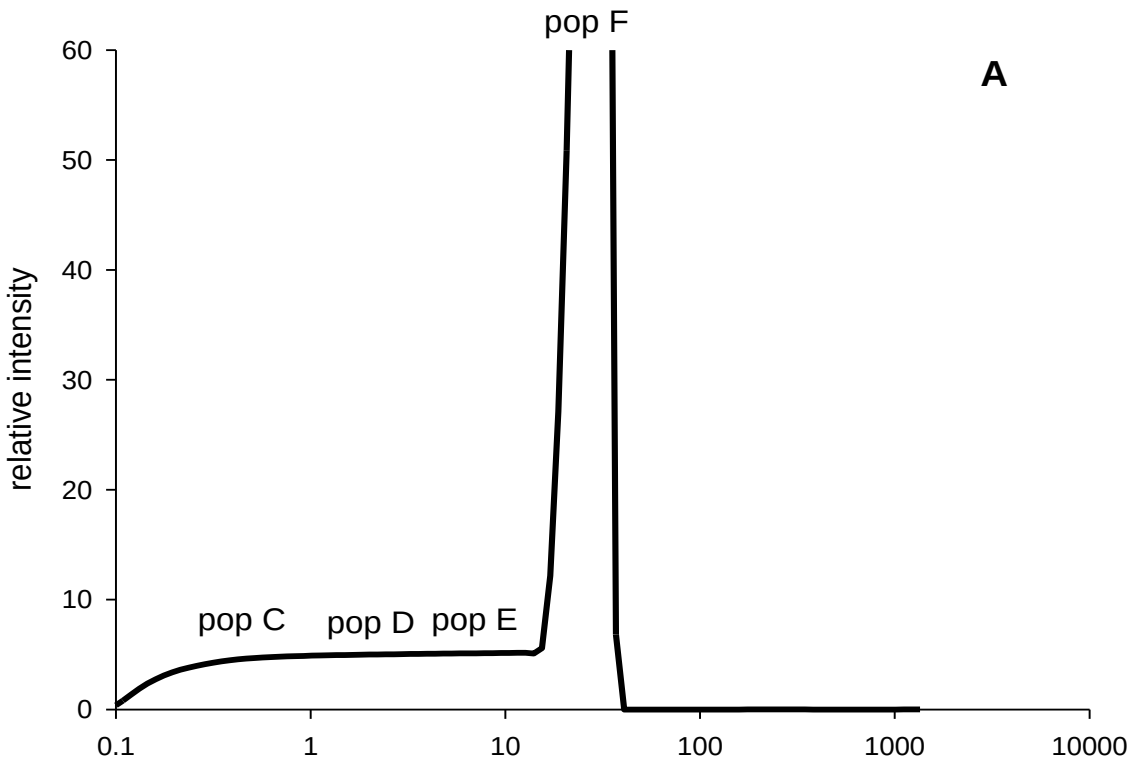
	Days	0	7	14	21	28	42	70
Hardness (N)	STD-56	11.1(0.9)d/B	11.9(1.4)cd/C	13.4(0.9)bc/B	14.8(0.4)ab/B	16.5(1.4)a/A	16.0(1.0)a/AB	16.6(1.1)a/A
	GLY7-56	11.7(0.9)d/B	12.5(0.7)cd/BC	13.6(0.7)bc/B	13.9(0.9)b/B	14.6(0.9)ab/B	15.3(0.4)a/B	15.4(0.3)a/B
	GLU7-56	13.4(0.6)c/A	13.7(0.7)c/AB	15.1(1.0)b/A	16.7(0.5)a/A	16.6(0.6)a/A	16.6(1.1)a/AB	16.5(0.4)a/A
	GLU7GLY7-56	13.5(1.0)d/A	14.8(0.6)c/A	15.3(0.8)bc/A	16.4(0.8)ab/A	16.4(0.6)ab/A	17.0(0.8)a/A	17.2(0.5)a/A
Amylopectin (J/ g starch)	STD-56	-	1.8(0.4)c/A	2.2(0.5)bc/A	1.9(0.6)bc/A	3.0(0.4)ab/A	3.5(0.1)a/A	3.4(0.4)a/AB
	GLY7-56	-	1.9(0.1)bc/A	2.4(0.7)abc/A	2.7(0.4)abc/A	1.6(0.2)c/B	3.5(0.7)ab/A	3.8(0.4)a/A
	GLU7-56	-	1.7(0.2)abc/A	1.3(0.1)c/A	2.1(0.3)abc/A	1.6(0.3)bc/B	2.9(1.0)a/A	2.6(0.2)ab/B
	GLU7GLY7-56	-	1.4(0.4)c/A	1.9(0.4)bc/A	2.9(0.3)abc/A	2.4(0.3)abc/AB	3.0(0.9)ab/A	3.7(0.6)a/AB
popF (%)	STD-56	71.9(1.0)a/A	62.5(1.9)b/C	71.1(0.9)a/B	71.6(2.1)a/B	71.1(1.9)a/B	71.2(1.9)a/C	69.2(1.9)a/C
	GLY7-56	72.3(1.9)c/A	76.6(0.6)b/A	80.9(0.3)a/A	80.9(0.4)a/A	80.2(0.5)a/A	80.9(0.5)a/A	79.4(0.7)a/A
	GLU7-56	68.4(0.7)c/B	73.8(1.2)ab/B	63.8(2.4)d/C	71.4(1.4)b/B	72.1(1.0)b/B	75.3(0.9)a/B	72.1(1.1)b/B
	GLU7GLY7-56	70.8(1.2)c/A	76.9(1.1)b/A	78.8(0.8)a/A	78.8(0.7)a/A	78.5(1.0)ab/A	79.0(0.6)a/A	78.9(0.5)a/A
T_{2F} (ms)	STD-56	28.8(0.2)a/B	29.6(0.3)a/B	26.5(0.1)a/B	26.9(0.2)a/B	26.9(0.2)a/B	26.3(0.2)a/B	25.8(0.2)a/B
	GLY7-56	27.7(0.4)a/C	25.0(0.2)cd/D	26.3(0.1)b/B	25.4(0.1)c/C	24.6(0.3)d/D	25.4(0.1)c/C	24.7(0.2)d/D
	GLU7-56	34.3(0.3)a/A	30.6(0.2)c/A	31.9(0.4)b/A	29.0(0.9)d/A	28.9(0.1)d/A	28.1(1.2)e/A	28.7(0.2)d/A
	GLU7GLY7-56	26.1(0.3)b/D	26.8(0.3)a/C	25.3(0.2)c/C	25.3(0.3)c/C	25.6(0.3)bc/C	24.1(0.3)d/C	25.1(0.1)c/C

Table 2

Hardness, retrograded amylopectin, population F (popF) and relaxation time (T_{2F}) of pasta with 62% of moisture during storage. Standard deviations are given in parenthesis following the means values; different letters close to numbers indicate significant difference among samples ($p \leq 0.05$), where the small letters referred the difference due to storage and capital letters to formulation.

Hardness (N)	Days	0	7	14	21	28	42	70
	STD-62	8.7(0.5)d/AB	9.0(0.8)cd/A	10.5(0.6)ab/A	10.2(0.4)bc/A	11.6(0.8)a/A	11.6(0.3)a/A	11.8(0.1)a/AB
	GLY7-62	7.3(0.5)c/C	8.7(0.2)b/A	9.0(0.4)b/B	8.9(0.1)b/B	10.4(0.9)a/B	10.6(0.8)a/B	10.9(0.4)a/B
	GLU7-62	9.3(0.4)e/A	9.9(0.8)de/A	10.8(0.8)bcd/A	10.4(0.5)cd/A	11.1(0.5)bc/AB	11.7(0.7)b/A	13.0(0.5)a/A
	GLU7GLY7-62	8.3(0.8)c/B	10.0(1.0)b/A	10.5(0.8)ab/A	10.5(0.8)ab/A	11.6(0.8)a/A	11.6(0.7)a/A	11.8(0.2)a/B
Amylopectin (J/ g starch)	STD-62	-	-	1.0(0.7)c/A	1.1(0.1)c/B	3.0(0.6)ab/A	1.7(0.2)bc/A	3.8(1.1)a/A
	GLY7-62	-	0.8(0.5)b	1.3(0.2)b/A	1.0(0.1)b/B	1.9(0.3)ab/B	2.0(0.7)ab/A	2.6(0.4)a/A
	GLU7-62	-	-	0.9(0.2)b/A	1.0(0.1)b/B	1.9(0.3)ab/B	2.0(0.3)ab/A	2.6(0.7)a/A
	GLU7GLY7-62	-	-	-	1.6(0.2)a/A	1.8(0.2)a/B	1.9(0.1)a/A	2.2(0.6)a/A
popF (%)	STD-62	76.8(0.4)d/A	80.7(0.5)b/A	78.4(0.4)c/A	78.8(0.5)c/A	80.4(0.3)b/A	81.8(0.4)a/A	80.7(0.4)a/A
	GLY7-62	64.9(1.1)a/C	62.7(1.6)a/B	47.9(1.3)c/C	54.6(2.2)b/C	46.6(4.0)c/C	61.5(3.6)a/B	36.7(2.6)b/C
	GLU7-62	74.1(0.4)e/B	76.8(1.5)d/A	76.9(0.3)cd/A	79.3(0.6)ab/A	78.6(1.0)ab/AB	78.4(0.9)bc/A	80.1(0.6)a/A
	GLU7GLY7-62	73.4(1.1)a/B	66.1(5.7)bc/B	58.8(2.2)d/B	69.7(2.8)ab/B	60.9(2.2)cd/B	60.5(2.6)cd/B	67.0(0.2)abc/B
T_{2F} (ms)	STD-62	43.7(0.4)a/C	43.5(0.2)a/A	40.2(0.2)b/C	38.5(0.2)d/C	39.2(0.1)c/C	38.0(0.1)e/C	38.1(0.2)e/C
	GLY7-62	45.7(0.2)a/B	40.6(0.2)b/B	41.1(0.2)b/B	39.4(0.3)c/B	40.33(0.6)bc/B	38.9(0.6)c/B	41.0(0.8)b/A
	GLU7-62	43.9(0.1)a/C	40.4(0.2)b/B	36.8(0.1)e/D	37.6(0.5)c/D	37.6(0.2)d/D	34.5(0.2)f/D	36.9(0.1)e/D
	GLU7GLY7-62	46.3(0.4)a/A	44.0(1.0)b/A	42.9(0.3)bc/A	41.9(0.5)c/A	42.3(0.4)cd/A	40.9(0.4)d/A	39.1(0.2)e/B

Figure 1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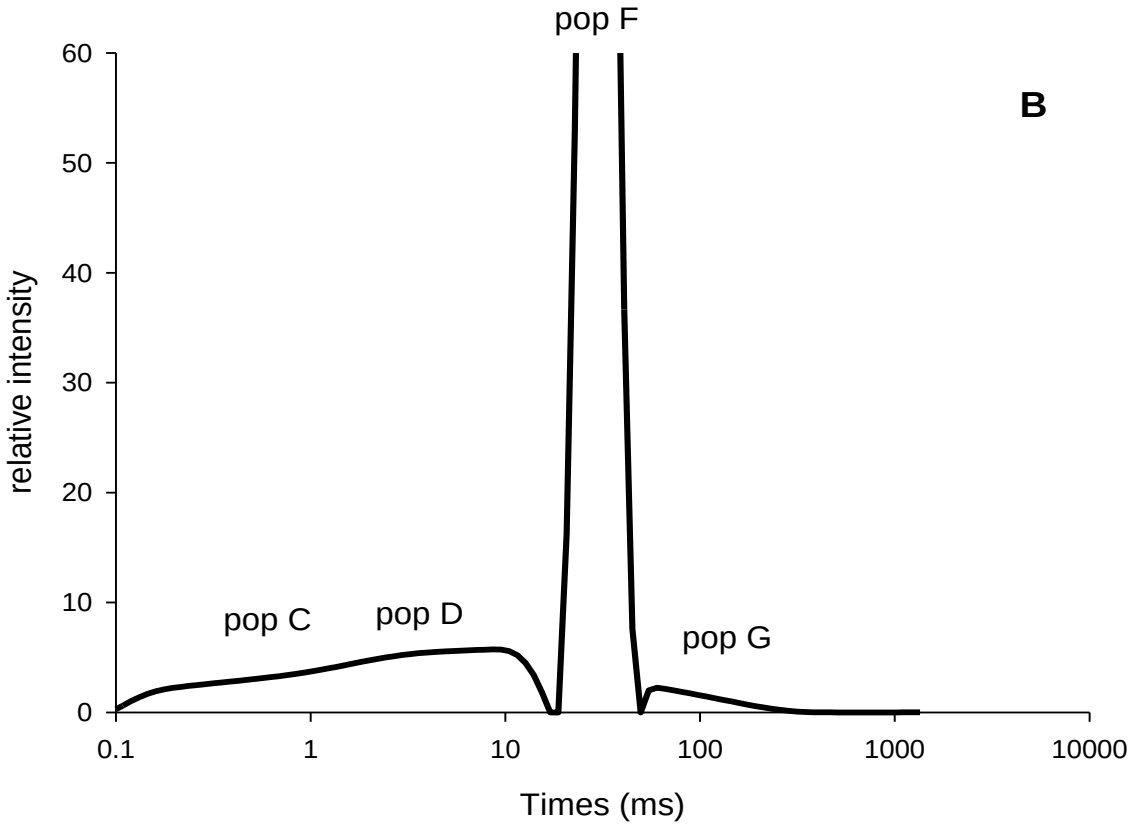
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Figure captions

Figure 1

Characteristic ^{1}H T_2 relaxation time distribution for cooked pasta with different formulation

Effect of water, glycerol and gluten on physico-chemical properties and water status during storage.

Abstract

This work aims to investigate the effect of water, glycerol and gluten on physico-chemical properties of ready to eat pasta with two levels of water (56%, 62%, 65% g water /100 g product). The macroscopic water status (moisture content, frozen water), proton molecular mobility indicators (^{1}H FID, ^{1}H T_2), rheological properties and retrograded amylopectin content were measured to characterize the product.

Neither glycerol nor gluten affected macroscopic waters status. The increase of moisture and glycerol content softened samples and increased proton molecular mobility. Gluten hardened samples but it did not affect the proton molecular mobility. The combination of both ingredients resulted in harder samples ("gluten-effect") with high proton molecular mobility ("glycerol-effect"). Retrograded amylopectin content increased during storage, but its increase was not related to presence of different ingredients. However changes into amylopectin retrogradation might be related to the starch amount and not the effect of added ingredients

Keywords Pasta . Water, Gluten . Glycerol . Physico-chemical properties . ^{1}H NMR mobility

1- Introduction

Ready to eat pasta meals have been recently introduced by the pasta industry in the retail market to respond to an increasing demand of the consumers for convenience food. They are subjected to a decrease in quality and consumers' acceptability during storage (Olivera & Salvadori, 2011). A deep understanding of the causes leading to quality loss will help pasta industry to introduce the necessary modifications in product formulation, processing and/or packaging to extend its shelf-stability and to produce a food item with the best sensory attributes at the time of consumption. Ready to eat pasta meals may be sold with pasta and sauce (mostly tomato based) assembled or packed in separated containers. The dynamics at the base of storage driven modifications are different in these two types of products. In the case of pasta and sauce assembled, pasta softening was reported and related to water migration between pasta and sauce phases detectable only at a molecular level (^{1}H T_1 and T_2) as water activity was at equilibrium between the two phases very shortly after production (Carini et al., 2013). When, on the contrary, pasta and sauce are kept separated during storage an increase in pasta hardness and retrograded amylopectin content as well as an increased molecular rigidity as measured by ^{1}H NMR (increased ^{1}H FID steepness and decreased ^{1}H T_2 relaxation times) were reported in pasta (Carini et al., 2014), strongly resembling the dynamics at the bases of bread staling (Hallberg & Chinachoti, 2002; Lin & Lineback, 1990; Imberty & Perez, 1988; Slade & Levine 1991; Vittadini & Vodovotz, 2003; Sereno et al., 2007). Pasta textural quality decrease may be reduced and controlled increasing moisture and/or gluten content in the pasta (Diantom et al, 2015), suggesting that formulation may have an important role in controlling and modulating cooked and stabilized pasta shelf-stability. Glycerol and gluten are known to have an important role in bread staling (Berkowitz & Oleksyk, 1991; Hallberg & Chinachoti, 1992; Taub et al., 1994; Eliasson, 1983a, 1983b; Ottenhof & Farhat, 2004; Callejo et al., 1999; Curti et al., 2014) and their effect on cooked pasta properties was recently explored (Curti et al., 2015). Glycerol in the formulation was reported to soften pasta texture and enhance proton

molecular mobility, while gluten hardened pasta but did not influenced the proton molecular mobility.

The aim of this work was, therefore, to verify the effect of moisture content, glycerol, and gluten alone or in combination, on physico-chemical properties (water status, rheological properties, retrograded amylopectin) of ready to eat pasta during storage. A pasta sample was also produced using an inert filler (titanium dioxide) in substitution of gluten and glycerol amounts to verify the effect of "starch dilution" and/or ingredient action in the pasta matrix on starch retrogradation and physico-chemical properties.

2- Materials and Methods

2.1- Materials

2.1- Ready to eat shelf stable pasta production

Dry pasta (penne shaped) was produced by a local pasta maker using a standard formulation (semolina and water, STD) and was cooked into boiling water (pasta/water ratio 1:10) to reach 59%, 62% and 65% moisture (g H_2O /100 g product, STD-56, STD-62 and STD-65, respectively). Dry pastas was also produced with a formulation modified by the addition of glycerol, gluten and their mixture, and titanium dioxide at a 15% level (g added ingredient /100 product) (Table 1). These pastas were cooked into boiling water to reach moisture contents of 59% (GLU15-59, GLY715-59 and GLU3GLY12-59, respectively), 62% (GLY15-62, TiO2-62), and 65% (GLU15-65, GLY15-65 and GLU12GLY3-65, respectively). About 60 g of cooked pastas were packed into multi-layer (polypropylene-PP, polyethylene terephthalate-PET, and polyamide-PA) pouches and sterilized in autoclave ($F_0 \geq 7$) to obtain ready to eat shelf-stable pasta (RTE pasta). RTE pasta pouches were then kept at 22.5 °C for 90 days, and analyzed within 24 h from production (day 0) and after 7, 14, 28, 42, 63 and 90 days of storage.

2.2- Methods

2.2.1- Moisture content

Moisture content (MC, % g water/100 g product) of RTE pasta was determined by weight loss by drying in a forced-air oven (ISCO NSV 9035, ISCO, Milan, Italy) at 105 °C to constant weight. At least ten pasta pieces of each RTE pasta sample at each storage time were analyzed.

2.2.2- Texture

Pasta texture was measured using a TA.TX2 Texture Analyzer equipped with a 25 kg load cell (Stable Micro systems, Goldalming, UK). Single pasta pieces were cut with a flat blade (speed of 2 mm/s; trigger force 0.1 N). The maximum height of the cutting peak was taken as ‘‘Hardness’’. 15 pasta pieces for each sample at each storage time were analyzed.

2.2.3- Thermal properties

Frozen water content and amylopectin melting were measured using a Differential Scanning Calorimeter (DSC Q100, TA Instruments, New Castle, DE, USA) calibrated with indium (T = 156.6 °C; H = 28.71 J/g) and mercury (T = -38.83 °C, H = 11.40 J/g). About 5–10 mg of RTE pasta were placed into hermetic stainless steel pans (Perkin Elmer, USA). Samples were heated from -80 °C to 100 °C at 5 -C/min. DSC thermograms were analyzed using an Universal Analysis Software, version 3.9A (TA Instruments, New Castle, DE). Frozen water content (at the select experimental conditions; FW) was calculated from the endothermic peak around 0 °C (ice melting) using the following equation:

*FW = Enthalpy Ice Fusion * (1/Latent Heat Ice Fusion)* (1/MC)*100*

where FW is frozen water (%, g frozen water/g water), ice fusion enthalpy (J/g product), latent heat of ice fusion is 334 J/g ice, and MC is moisture content (g water/1 g product).

The occurrence of an endothermic peak in the 50–80 °C range was taken as recrystallized amylopectin melting. Enthalpy of this peak was measured (J/g product) and normalized to the grams of starch in the sample (J/g starch). At least triplicated samples of each product were analyzed at each storage time.

2.2.4- Proton molecular mobility

A low resolution (20 MHz) ^{1}H NMR spectrometer (the Minispec, Bruker Biospin, Milano, Italy) operating at 25.0 ± 0.1°C was used to study proton molecular mobility by measuring the free induction decay (FID) and transverse relaxation times (T_2). About 2 g of RTE pasta were placed into a 10 mm NMR tube that was then sealed with Parafilm to avoid moisture loss during the NMR experiment. ^{1}H FIDs were acquired using a single 90° pulse, followed by a dwell time of 7 µs, a recycle delay of 3 s and a 10 ms acquisition window. ^{1}H FIDs were analyzed in the time range 7–100 µs where the homogeneity of magnetic field was assured. The curves were fitted with a two components model (exponential and gaussian; Le Grand, Cambert, & Mariette, 2007; Sigmaplot, v6, Systat Software Inc., USA):

$f\ (t) = y0 + A*\exp(-t/T_A) + B*\exp[-(t/T_B)^2]$

where y0 is the FID decay offset, A and B are the intensities of each relaxation component, T_A and T_B are the apparent relaxation times.

T_2 relaxation curves were acquired with a CPMG pulse sequence with a recycle delay of 3 s (≥ 5 ^{1}H T_1), an interpulse spacing of 0.04 ms and 4000 data points. T_2 curves were analyzed as quasi-continuous distributions of relaxation times using a UPENWin software (Alma Mater Studiorum, Bologna, Italy). Default values for all UPEN parameters were used with the exception of one parameter (LoXtrap) that was set to 1 to avoid extrapolation of relaxation times shorter than the first experimental point. ^{1}H T_2 CPMG relaxation decays were also fitted with a discrete exponential model (Sigmaplot, v.6, Systat Software Inc., USA).

2.2.5- Statistical analysis

Statistical analysis was carried out with SPSS software (Version 22.0, SPSS Inc., Armonk, New York, USA). Significant differences ($p \leq 0,05$) among different samples were verified with by one-way-analysis of variance (ANOVA) with a Tukey-high and LSD significant difference test.

3- Results and Discussions

3.1 Moisture and Frozen water contents

Moisture content was found to be 59.1 ± 0.5; 61.8 ± 0.2 and 65.0 ± 0.3% (g H2O / 100 g product) in STD-59, STD-62 and STD-65, respectively, confirming that the desired moisture content was reached during cooking. All the other samples with glycerol and gluten also exhibited the desired moisture content, 58.4 ± 0.5; 57.6 ± 0.3 and 59.6 ± 0.5 % for GLU15-59, GLY15-59 and GLU3GLY12-59, respectively; 61.3 ± 0.2 and 61.4 ± 0.4% for GLY15-62 and TiO2-62, respectively, then 64.7 ± 0.2, 64.9 ± 0.3 and 64.8 ± 0.3 for GLU15-65, GLY15-65 and GLU12GLY3-65, respectively, and were statistically comparable to the controls (STD-56, STD-62 and STD-65). Neither formulation nor storage significantly affected moisture content in all samples. This was in agreement with previously reported data (Diantom et al., 2015; Carini et al., 2014).

The frozen water content (data not shown) of STD-56, STD-62 and STD-65 was found to be about 69.7 ± 2.3; 70.2 ± 6.0 and 72.4 ± 8.8% (g frozen water / 100 g water), respectively, showing that frozen water was not related to the sample's moisture content. The rest of samples exhibited similar frozen water content during storage. Frozen content was not significantly affected by formulation and storage.

3.2- Texture and retrograded amylopectin

Hardness of pasta with different composition is reported in Table 2. At day 0, STD pasta exhibited different hardness, that increased with increasing moisture content. STD-59 exhibited the highest hardness while STD-65 was the softest sample. At 59% of moisture, GLU15-59 was significantly harder than STD-59 while GLY15-59 and GLU3GLY12-59 were softer than STD-59. However GLU3GLY12-59 exhibited lower hardness than GLU15-59 and GLY15-59. At 62% moisture, GLY15-62 exhibited similar hardness to STD-62 but both samples were harder than TiO_2-62. At 65% moisture, GLU15-65 and GLU12GLY3-65 were harder than STD-65 while GLY15-65 had hardness comparable to STD-65.

Hardness significantly increased during storage in all samples, as expected (Carini et al, 2014; Diantom et al, 2015), except in the case of TiO_2-62 and GLU15-65 where the plateau was reached at day 28 and hardness remained almost constant over the storage. Increased moisture content

helped retaining softness in the samples during storage, as expected (Diantom et al, 2015), confirming that water played a plasticizing effect correlated to its amount. On the contrary, the presence of added gluten significantly increased samples' hardness, independently on the level of moisture content (Diantom et al., 2015). The combination of higher added gluten and higher moisture content (65%, g water / 100 product) resulted to strongly reduce the changes of pasta texture during storage. The hardness increased in the presence of gluten, might be related to a strengthening of the gluten network (Malcolmson et al., 1993, Feillet 1984). On the contrary, the presence of glycerol significantly softened pasta (similarly to what observed in cooked pasta, Curti et al., 2015) as compared to the control at all moisture levels, even if at higher moisture content (65%, g water / 100 g product) the glycerol exhibited a significant effect only after 28 days. It might be speculated this to be due to the plasticizing effect of glycerol that became evident only when some of the water had lost some of its mobility and was, therefore, replaced by the glycerol. Hallberg & Chinachoti, (1992) and Taub et al., (1994) reported that added glycerol to white bread softened during storage. The combination of glycerol and gluten affected pasta hardness, depending on moisture content and ingredient substitution. In fact at 59% moisture, GLU3GLY12-59 was softer than GLU15-59 and GLY15-59, confirming the plasticizing effect of glycerol. While at 65% moisture, GLU12GLY3-65 showed higher hardness as compared to samples with only the single ingredient. This suggests that the interactions between gluten and glycerol are influenced by moisture content, where the three ingredients interact together resulting in a particular texture of the sample.

The presence of titanium dioxide significantly softened pasta as compared to the control formulation. This might be associated to an eventual alteration of gluten network, which induced an impoverishment of the structure generating a disintegration of the system. The softness observed in the presence of TiO2 might be correlated to the reduced amount of starch. In fact it's known that gelatinized starch was trapped by coagulated gluten to form a strengthened network (Resmini &

Pagani, 1983). Thus in this case starch was replaced by an inert material, which did not increase the viscosity and induced the formation a weak gluten network.

Retrograded amylopectin content of all samples is reported in Table 3. At day 0, no amylopectin was observed in all samples as expected, and confirming the complete starch gelatinization achieved during cooking and sterilization. All control samples, unregarding their moisture content, started to show retrograded amylopectin at day 7. Similarly, at 59% moisture, GLU15-59 and GLU3GLY12-59 showed retrograded amylopectin at day 7, while in the glycerol containing sample it was detected only after 14 days in GLY15-59. At 62% moisture content GLY15-62 showed retrograded amylopectin at day 14 and TiO_2 after 28 days. At 65% moisture, GLU15-65 and GLY15-65 showed retrograded amylopectin at day 28 but in the GLU12GLY3-65 amylopectin retrogradation was recorded at day 7. The retrograded amylopectin content increased during storage in all samples as expected (Diantom et al., 2015), except in GLU15-65 where it resulted to be almost constant during storage. The moisture content alone did not show an evident effect on amylopectin retrogradation, and gluten or glycerol added alone did not exhibit a relevant effect on retrograded amylopectin content. But the combination of higher moisture content (65%, g water / 100 g product) with higher amount of gluten or glycerol (15%, g added ingredient / 100 g product) seemed to delay the amylopectin retrogradation (Diantom et al., 2015). The presence of TiO_2 significantly amylopectin retrogradation, retarding its beginning and resulting in a drastically reduced amount of crystals at the end of storage. Glycerol and gluten generally did not show any effect on amylopectin retrogradation, as did not significantly affect the, but the changes into retrograded amylopectin might be related to reduced amount of starch in samples with added ingredients.

3.2- Proton molecular mobility

^1H NMR investigation was used to explore the effect of formulation on proton molecular mobility. ^1H FID was used to evaluate the more rigid protons while ^1H T_2 to analyze the effect of formulation on the more mobile protons. ^1H FIDs were fit with a two components model (data not shown).

Populations were named population A and B, and the corresponding relaxation times, T_A and T_B. At day 0, the more rigid protons population A was represented according to the moisture content, with increasing moisture content, 34.1%, 31.8% and 28.4% for STD-59, STD-62 and STD-65, respectively. At 59% moisture, GLU15-59 showed higher value of population A (35.5%) than STD-59 (34.1%), while GLY15-59 was comparable to STD-59, with the lowest percentage in but the GLU3GLY12-59 (29.2%). At 62% moisture, both GLY15-59 (29.8%) and TiO2 (28.0%) exhibited lower population A than STD-62. Whereas at 65% moisture, population A of GLU15-65 was significantly higher (31.6%) than STD-65 while GLY15-65 exhibited a lower percentage (27.6%) , with GLU12GLY3-65 showing the highest rigid protons (36.0%). The more rigid proton percentage increased during storage in all samples, suggesting an increase of rigidity during storage (Diantom et al., 2015).

The increase of moisture content resulted in a reduction of population A, that was in accordance to the pasta softening (Diantom et al., 2015). The presence of gluten increased molecular rigidity at all moisture contents, that also resulted in a higher hardness of the gluten samples. The presence of glycerol enhanced molecular mobility (decrease in population A), in accordance to the softening effect of glycerol (lower hardness). Samples with the mixture of gluten and glycerol showed changes that were related to the ingredient that was present in a greater amount.

The corresponding relaxation times, T_A and T_B, of both STDs were found to be about 0.016 ms and 0.620 ms, respectively, indicating that the more rigid relaxation time did not depend on moisture content. Similar relaxation times were observed for the rest of samples, indicating that neither glycerol nor gluten affected the mobility of the more rigid protons. Both relaxation times did not depend on storage time.

Representative ^1H T_2 distributions of pasta with different formulation were analyzed with UpenWin and indicated the presence of four protons populations that were named population C, D, E and F from the fastest to the slowest relaxing one (Figure 1). These populations were unresolved in all samples, and ^1H T_2 curves were therefore fit to quantify the percentage of protons belonging to each

population and their relaxation times. Population E was the more represented in all samples while population F resulted to be the more mobile in all samples. Even if the population were four, in this study, the discussion was focused on the more abundant population E and the more mobile population F.

At day 0, the population E percentage in STD-59 was the highest followed by STD-62 and then STD-65 which resulted to be the lowest percentage (Table 4), while the more mobile population, population F, resulted to be higher in STD-65, followed by STD-62 and then STD-59. Both populations were affected by the presence of added ingredient at day 0. GLU15-59 exhibited a lower percentage of population E than STD-59 while GLY15-59 had similar value to STD-59, but GLU3GLY12-59 exhibited the highest percentage of population E as compared to STD-59, GLU15-59 and GLY15-59. At 62% moisture, GLY15-62 had the highest representation of population E, followed by STD-62 and TiO2-62 with the lowest value. At 65% moisture, GLY15-65 had the highest value of population E, followed by GLU12GLY3-65, GLU15-65 and STD-65 with the lowest value. Concerning population F (Table 5), GLU15-59 showed the higher percentage, followed by STD-59, GLU3GLY12-59 and GLY15-59 with the lowest value. At 62% moisture, GLY15-62 had lower amount of population F than STD-62 while TiO2-62 exhibited the highest amount. Whereas at 65% moisture, GLU15-65 and GLY15-65 had lower amount of population F than STD-65, but the GLU15-65 had higher amount than GLY15-65 and similar as GLU12GLY3-65.

Changes in population E did not showed any clear trend relatable to storage and/or moisture content, as a strong fluctuation of values was detected in all samples over storage. Conversely, it was possible to observe a significant increase of population F relatable to storage (more pronouncedly higher moisture content 65%). The presence of glycerol alone in pasta formulation induced an increase of the more abundant population E, more evident at higher moisture content (65%), while it significantly reduced the more mobile population F.

The presence of gluten alone did not significantly affect neither population E nor population F. The simultaneous presence of gluten and glycerol affected proton distribution in a different manner according to the amount of each ingredients. Population E was higher in GLU3GLY12-59 than GLU15-59 and GLY15-59, suggesting an eventual interaction between gluten and glycerol, which conferred a lower molecular rigidity to pasta. At higher moisture content, Population E was lower in GLU12GLY3-65 than GLY15-65 and GLU15-65 over storage, but it seemed to be similar to GLU15-65 however fluctuations were observed. This might be correlated to the higher amount of gluten in this case, which generated the increase of matrix rigidity, reducing the proton mobility. The addition of TiO_2 did not induce strong changes in proton distribution during storage, however significant difference was observed at day 0.

The corresponding relaxation time of the observed populations were named $T_{2C,}$ T_{2D}, T_{2E} and T_{2F} for population C, D, E and F, respectively. At day 0, the relaxation times of the more abundant population (T_{2E}) (Table 4) and the more mobile population (T_{2F}) (Table 5) resulted to be the same in all STDs. At 59% moisture, GLY15-59 had higher T_{2E} and T_{2F} than STD-59 while GLU15-59 showed lower T_{2E} and T_{2F} than STD-59, but GLU3GLY12-59 exhibited higher T_{2E} and T_{2F}. At 62% moisture, GLY15-62 showed higher T_{2E} and T_{2F} than STD-62, while TiO_2-62 had similar values as STD-62. At 65% moisture, GLU15-65, GLY15-65 and GLU12GLY3-65 showed higher T_{2E} and T_{2F} than STD-62. The relaxation times of more abundant and more mobile populations significantly decreased in all samples during storage. T_{2E} significantly increased with increasing moisture content, confirming that pasta structure become less rigid, inducing higher mobility, and this was in agreement with the softening observed at macroscopic level, while T_{2F} did not depend to the moisture content. The presence of glycerol significantly increased both proton relaxation time (T_{2E} and T_{2F}), while added gluten did not significantly affect both relaxation times.

The combination of 12% of glycerol and 3% of gluten significantly increased the relaxation time of the more abundant population, while T2F was higher than STDs and gluten samples but lower than glycerol samples.

Proton molecular mobility was influenced by the presence of moisture content, as expected, with proton molecular mobility increasing with increasing moisture content. This might be associated to the plasticizing effect of water, which softens samples and increasing the proton mobility (Diantom et al. 2015). The presence of added gluten did not significantly affect proton molecular mobility, even if it significantly induced hardness increase. This might suggest that the changes at macroscopic level, is not correlated to those occurred at molecular level. In fact there was no correlation between the more abundant proton and the hardness (data not shown). The presence of glycerol significantly increased the proton molecular mobility (Curti et al. 2015), and softened pasta structure while acting as plasticizer, and allowing proton to move easily. However it was observed that the relaxation time of the more mobile proton of glycerol samples significantly decreased with increasing moisture content. This might suggest that there was an interaction between water and glycerol inducing an increase of the rigidity into pasta, anti-plasticizing effect, which reduces proton mobility. Lourdin et al. (1997) reported that the effective role of glycerol and water, which coexisting in a water-glycerol-starch system, was related to their respective concentration.

4- Conclusions

The effect of water, glycerol and gluten on physico-chemical properties of ready to eat pasta with was explored during storage. Both moisture content and frozen water content were not affected neither by storage time nor by formulation. Water softened pasta structure and increased the proton molecular mobility, but it did not affect amylopectin retrogradation. The presence of gluten hardened samples, at all level of moisture content, but it did not affect the proton molecular mobility and amylopectin retrogradation. In fact the effect of gluten was more highlighted at macroscopic level than at molecular level. In contrast, the presence of glycerol softened pasta and induced strong changes at molecular level, affecting the proton molecular mobility. Glycerol effect seemed to be related to the moisture content into the samples, exhibiting the higher plasticizing effect at lower moisture content while at higher moisture content it seemed to play a little anti-plasticizing effect. Samples with both ingredients (gluten and glycerol) exhibited similar rheological properties as samples with added gluten while they showed similar proton mobility as samples with added glycerol. The presence of Titanium dioxide significantly affect the rheological properties (softening), but it did not significantly induce changes in other parameters.

Changes in retrograded amylopectin content were not well defined, but according to these results we might conclude that changes in amylopectin retrogradation were related to amount of starch into the formulation.

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Table 1

Formulation of different pasta used in this study (g)

	Semolina	Gluten	Glycerol	Titanium dioxide
STDs	100	-	-	-
GLU15	85	15	-	-
GLY15	85	-	15	-
GLU3GLY12	85	3	12	-
GLU12GLY3	85	12	3	-
$TiO_2$15	85	-	-	15

Table 2

Hardness (N) of RTE pasta meals over storage. Standard deviations are given in parenthesis following the means values; different letters indicate significant difference among samples ($p \leq 0.05$); small letters indicate differences during storage; capital letters indicate differences among the samples.

Days	0	7	14	28	42	63	90
STD-59	10.1(0.7)c/B	10.4(0.5)c/A	12.0(0.8)b/A	12.5(0.9)b/B	14.3(0.7)a/B	14.4(0.6)a/B	14.5(0.5)a/B
GLU15-59	11.5(0.9)d/A	14.4(0.8)c/A	15.2(0.3)c/A	15.2(0.3)bc/A	16.1(0.6)ab/A	16.1(0.4)a/A	17.0(0.6)a/A
GLY15-59	8.7(0.7)d/C	10.0(0.6)c/B	11.7(0.6)b/B	11.7(0.6)b/B	12.1(0.8)ab/C	12.5(0.8)ab/C	12.8(0.7)a/C
GLU3GLY12-59	6.8(0.6)d/D	7.3(0.6)c/C	8.0(0.4)bc/C	8.2(0.5)bc/C	8.4(0.6)bc/D	8.4(0.5)ab/D	8.9(0.7)a/D
STD-62	8.0(0.9)e/A	8.8(0.6)d/A	10.5(0.7)c/A	10.5(0.4)bc/A	11.2(0.6)b/A	12.0(0.8)a/A	10.1(0.7)a/A
GLY15-62	7.4(0.7)e/A	7.6(0.6)e/B	8.9(0.4)d/B	9.4(0.6)cd/B	9.7(0.8) bc/B	10.1(0.6)ab/B	10.5(0.6)a/B
TiO2-62	6.7(0.8)c/B	7.5(0.5)b/B	8.4(0.6)a/B	8.6(0.6)a/C	9.0(0.6)a/C	9.0(0.6)a/C	9.1(0.9)a/C
STD-65	6.9(0.6)c/C	7.2(0.5)c/C	8.3(0.7)b/C	8.5(0.7)b/C	8.7(0.6)b/C	8.8(0.5)a/C	10.0(0.8)a/C
GLU15-65	8.5(0.8)c/B	9.9(0.6)b/B	11.1(0.4)a/B	11.1(0.1)a/B	11.1(0.3)a/B	11.2(0.6)a/B	11.8(0.8)a/B
GLY15-65	6.3(0.6)c/C	6.8(0.5)c/C	7.7(0.6)b/C	7.5(0.6)b/D	7.8(0.6)b/D	8.0(0.6)ab/D	8.5(0.6)a/D
GLU12GLY3-65	12.5(0.8)d/A	13.7(0.9)c/A	14.4(0.8)bc/A	14.7(0.9)bc/A	14.8(0.5)bc/A	14.9(0.8)ab/A	15.9(0.7)a/A

Table 3:

Retrograded amylopectin (J/g of starch) of RTE pasta meals over storage. Standard deviations are given in parenthesis following the means values; different letters indicate significant difference among samples ($p \leq 0.05$); small letters indicate differences during storage; capital letters indicate differences among the samples.

Days	0	7	14	28	42	63	90
STD-59	-	0.9(0.3)c/A	0.9(0.1)c/A	1.3(0.1)bc/B	1.7(0.4)b/B	1.7(0.2)b/BC	3.1(0.2)a/AB
GLU15-59	-	0.8(0.1)c/A	1.2(0.2)c/A	1.5(0.4)bc/B	2.0(0.4)bc/B	4.4(0.5)a/A	3.2(0.1)ab/AB
GLY15-59	-	-	1.3(0.1)b/A	2.4(0.7)ab/AB	3.7(0.6)a/A	3.1(0.5)ab/B	3.9(0.1)a/A
GLU3GLY12-59	-	0.3(0.1)b/A	1.4(0.5)b/A	3.5(0.6)a/A	1.0(0.1)b/B	1.3(0.5)b/C	3.2(0.3)a/B
STD-62	-	1.4(0.1)abc	0.6(0.1)c	1.2(0.3)bc/AB	2.0(0.2)ab/B	2.6(0.9)a/A	2.6(0.2)ab/B
GLY15-62	-	-	1.3(0.3)b	1.3(0.2)b/A	1.4(0.3) b/A	1.8(0.2)ab/A	3.1(1.0)a/A
TiO2-62	-	-	-	0.5(0.1)b/B	1.1(0.4)ab/C	2.2(0.8)a/A	1.5(0.3)ab/C
STD-65	-	0.5(0.2)b	0.9(0.2)b	1.2(0.2)A	0.7(0.1)b/B	1.3(0.3)b/BC	2.6(0.6)a/A
GLU15-65	-	-	-	1.0(0.3)a/A	1.0(0.4)a/B	1.1(0.3)a/C	1.7(0.9)a/A
GLY15-65	-	-	-	0.9(0.2)b/A	0.9(0.2)b/B	3.8(0.4)a/A	2.8(0.5)a/A
GLU12GLY3-65	-	0.5(0.1)c	0.8(0.1)c	0.8(0.1)c/A	2.1(0.1)b/A	2.6(0.8)ab/A	3.1(0.2)a/A

Table 4

Population and relaxation time of the more abundant protons of RTE pasta meals over storage. Standard deviations are given in parenthesis following the means values; different letters indicate significant difference among samples ($p \leq 0.05$); small letters indicate differences during storage; capital letters indicate differences among the samples.

Days	0	7	14	28	42	63	90
STD-59	75.5(0.5)a/B	77.4(0.3)a/B	79.7(0.8)a/B	80.1(1.8)a/AB	63.4(3.1)b/B	60.5(4.8)b/C	59.4(2.1)b/B
GLU15-59	66.2(1.6)c/C	72.9(1.9)b/A	77.7(1.6)a/C	78.1(1.4)a/BC	77.4(1.8)a/A	76.2(1.5)a/B	47.1(2.5)d/C
GLY15-59	76.1(0.4)c/B	74.5(2.4)bc/BC	78.3(1.0)ab/B	76.9(2.6)bc/C	74.6(2.1)c/A	80.2(1.0)a/A	80.6(1.0)a/A
GLU3GLY12-59	80.3(1.1)ab/A	82.1(1.3)a/A	82.1(1.6)a/A	82.7(1.1)a/A	73.2(3.6)c/A	77.2(0.9)b/AB	80.7(2.7)a/A
STD-62	61.8(3.6)b/B	78.4(1.6)a/A	77.5(0.9)a/B	81.3(0.8)a/A	80.7(0.9)a/A	56.1(2.6)c/C	56.9(3.5)c/C
GLY15-62	80.3(0.6)bc/A	74.9(2.8)d/B	79.8(0.8)c/A	82.1(1.4)ab/A	82.0(1.1)abc/A	82.1(1.3)ab/A	83.6(0.7)a/A
TiO2-62	36.8(6.0)c/C	72.3(1.6)b/B	80.0(1.4)a/A	80.3(2.1)a/A	78.3(0.7)a/B	70.4(1.6)b/B	68.3(2.7)b/B
STD-65	53.4(1.1)e/D	69.5(3.9)d/B	77.3(0.9)bc/C	76.3(3.1)bc/B	81.8(1.0)a/A	73.3(3.7)cd/A	79.9(1.3)ab/B
GLU15-65	69.4(0.4)c/C	72.5(2.0)abc/B	69.8(3.6)bc/B	69.7(3.2)bc/C	78.2(2.8)a/B	75.8(3.3)ab/A	77.0(0.7)a/C
GLY15-65	80.3(0.7)b/A	80.5(1.2)b/A	82.5(0.9)a/A	81.8(1.2)ab/A	82.6(1.4)a/A	76.0(0.7)c/A	83.1(0.9)a/A
GLU12GLY3-65	75.8(0.8)b/B	77.3(1.5)ab/A	79.6(1.1)a/B	78.5(2.2)ab/AB	78.3(1.7)ab/B	71.9(1.6)c/A	57.7(2.4)d/D

T_{2E}

Days	0	7	14	28	42	63	90
STD-59	32.8(0.3)a/C	32.4(0.3)a/C	32.3(0.1)a/C	31.2(0.3)a/B	27.8(0.9)b/C	23.1(1.7)d/D	25.0(0.9)c/C
GLU15-59	20.0(0.8)f/D	31.5(0.4)b/D	33.2(2.1)a/B	29.4(0.1)c/C	30.5(0.2)bc/B	26.2(0.5)d/C	22.0(1.5)e/D
GLY15-59	37.7(0.3)a/B	36.3(0.7)b/B	33.7(0.5)c/B	32.3(0.8)d/B	30.9(0.5)e/B	31.4(0.3)ef/B	30.3(0.5)f/B
GLU3GLY12-59	48.9(0.5)a/A	45.8(0.1)b/A	45.6(0.3)b/A	43.1(0.4)c/A	40.1(1.1)d/A	38.7(0.2)de/A	39.5(0.4)e/A
STD-62	30.1(5.7)b/B	37.6(1.6)a/B	33.0(0.4)ab/C	32.1(0.2)b/C	31.0(0.5)b/B	21.2(1.0)c/B	24.8(1.2)c/B
GLY15-62	43.2(0.2)a/A	39.4(0.4)b/A	39.2(0.1)b/A	37.9(0.5)c/A	35.7(0.5)e/A	36.6(0.2)d/A	34.7(0.2)f/A
TiO2-62	29.7(3.9)d/B	38.1(0.1)a/B	36.0(0.3)ab/B	33.7(0.6)bc/B	31.2(0.4)cd/B	15.0(1.1)e/C	16.5(0.8)e/C
STD-65	35.9(0.3)ab/C	22.1(2.0)d/D	37.2(0.4)a/B	33.2(0.6)c/B	34.0(0.3)bc/AB	21.9(1.3)d/C	13.3(0.6)e/D
GLU15-65	47.3(0.1)a/A	37.6(2.0)b/B	33.7(0.1)bc/D	27.0(3.2)c/C	27.7(6.3)c/C	18.0(2.3)d/D	19.1(0.5)d/C
GLY15-65	47.5(0.2)a/A	44.8(0.3)b/A	43.7(0.4)c/A	40.6(0.8)d/A	39.4(0.4)e/A	40.6(0.2)d/A	37.1(0.4)f/A
GLU12GLY3-65	38.7(0.2)a/B	34.9(0.2)b/C	35.3(0.3)b/C	34.4(0.5)b/B	30.4(0.3)c/BC	28.5(0.5)d/B	20.9(1.0)e/B

Table 5

Population and relaxation time of the more mobile protons of RTE pasta meals over storage. Standard deviations are given in parenthesis following the means values; different letters indicate significant difference among samples ($p \leq 0.05$); small letters indicate differences during storage; capital letters indicate differences among the samples.

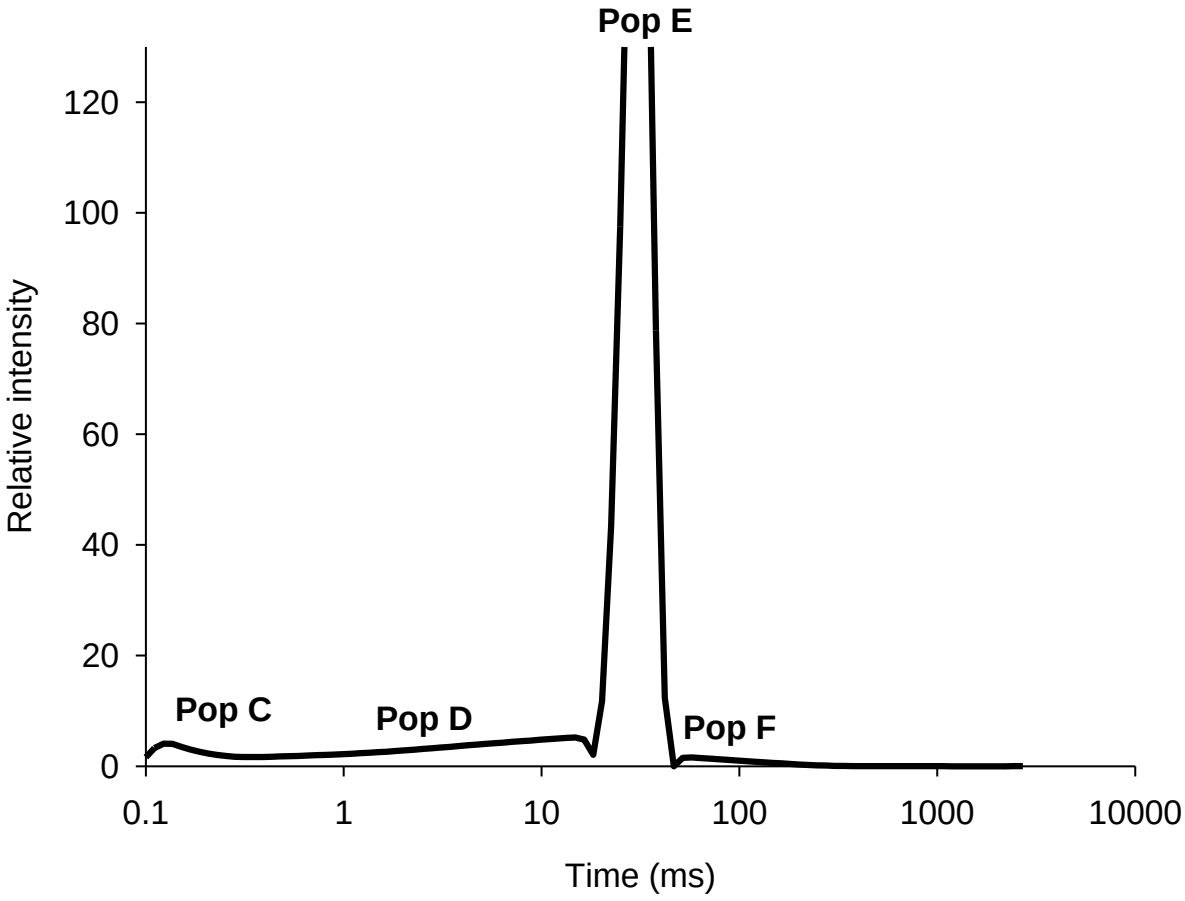
Population F

Days	0	7	14	28	42	63	90
STD-59	5.8(0.8)b/B	4.0(0.5)b/AB	2.5(0.1)b/B	3.8(1.8)b/B	23.6(3.1)a/A	26.6(4.3)a/A	27.4(2.2)a/B
GLU15-59	20.5(1.5)b/A	4.5(0.9)d/A	4.1(0.7)d/A	7.0(1.7)cd/A	3.7(1.0)cd/B	8.6(1.7)c/B	39.7(2.5)a/A
GLY15-59	3.1(0.1)a/C	3.1(0.2)a/B	2.9(0.1)ab/B	3.0(0.2)a/B	3.0(0.2)ab/B	2.8(0.1)b/C	3.1(0.2)a/D
GLU3GLY12-59	4.9(0.8)c/B	3.3(0.8)c/B	3.9(0.7)c/A	3.1(0.8)c/B	14.8(4.7)a/A	11.8(1.2)ab/B	9.3(1.9)b/C
STD-62	24.4(3.2)b/B	3.5(0.6)d/AB	8.1(0.5)c/A	4.9(0.4)cd/A	5.1(0.6)cd/B	32.4(2.4)a/A	32.5(3.4)a/A
GLY15-62	2.4(0.1)c/C	3.8(0.4)a/A	2.5(0.2)c/C	2.4(0.3)c/B	2.4(0.1)c/C	2.4(0.1)c/C	2.9(0.2)b/C
TiO2-62	36.8(6.0)a/A	2.9(0.2)c/B	4.5(0.8)c/B	4.3(0.8)c/A	7.4(2.0)c/A	18.0(1.1)b/B	20.8(2.2)b/B
STD-65	34.6(1.4)a/A	21.7(3.6)b/A	9.3(1.6)cd/B	12.2(4.1)c/B	5.8(1.9)d/B	17.3(3.5)b/A	9.2(0.8)cd/C
GLU15-65	5.0(0.1)c/B	13.3(2.8)ab/B	19.8(2.8)ab/A	19.7(3.6)a/A	10.3(4.3)bc/A	13.7(3.1)ab/A	12.1(0.7)b/B
GLY15-65	2.8(0.2)b/C	2.8(0.4)b/C	2.6(0.3)b/C	2.4(0.3)b/C	2.3(0.4)b/C	2.4(0.5)b/B	4.9(1.1)a/D
GLU12GLY3-65	5.8(0.2)cd/B	4.7(0.6)cd/C	4.3(0.7)d/C	4.5(0.8)d/C	6.8(1.1)c/B	14.2(1.6)b/A	28.5(2.4)a/A

T_{2F}

Days	0	7	14	28	42	63	90
STD-59	79.7(5.6)b/C	87.4(7.4)b/C	108.4(6.5)a/C	103.4(2.9)a/C	48.2(1.5)c/D	38.3(3.1)d/D	41.6(1.8)cd/C
GLU15-59	41.8(0.4)e/D	95.7(3.7)b/C	101.9(4.7)b/C	65.6(4.6)c/D	96.5(4.1)ab/B	53.7(2.1)d/C	35.7(1.1)f/D
GLY15-59	228.1(3.6)c/A	251.4(6.9)b/A	248.9(6.9)b/A	269.2(7.2)a/A	266.1(6.2)a/A	233.6(6.5)c/A	182.7(4.3)d/A
GLU3GLY12-59	124.2(4.2)c/B	142.6(4.7)b/B	138.2(2.5)b/B	151.6(4.2)a/B	67.8(3.6)e/C	71.7(1.5)de/B	76.6(5.0)d/B
STD-62	51.6(6.7)d/B	116.7(4.9)a/C	73.3(2.8)c/C	85.0(3.6)b/C	82.9(3.2)b/B	38.6(0.9)e/B	39.2(1.5)e/B
GLY15-62	232.4(4.6)b/A	218.7(4.5)c/A	230.5(4.8)b/A	253.9(5.6)a/A	234.8(4.8)b/A	219.5(7.3)c/A	154.3(1.1)d/A
TiO2-62	49.9(4.8)d/B	130.4(4.7)a/B	97.8(2.6)b/B	98.8(3.5)b/B	75.8(7.0)c/B	34.4(0.6)e/B	36.0(0.3)e/C
STD-65	57.5(0.5)d/D	48.6(1.0)e/D	80.0(3.7)b/C	63.4(5.9)c/C	85.1(3.6)a/B	40.4(0.9)f/C	36.6(0.2)f/C
GLU15-65	117.7(2.4)a/B	61.6(3.6)b/C	56.0(4.0)b/D	47.7(3.2)c/D	48.1(5.8)c/D	41.3(0.8)c/C	43.3(0.3)c/B
GLY15-65	180.3(4.9)cd/A	176.2(5.5)d/A	188.3(5.5)bc/A	205.2(4.0)a/A	195.4(4.2)ab/A	162.8(6.3)e/A	101.3(0.4)f/A
GLU12GLY3-65	102.4(4.5)b/C	102.6(4.5)b/B	103.5(4.9)b/B	111.3(2.5)a/B	69.8(1.7)c/C	54.8(1.9)d/B	36.3(1.1)e/C

Figure 1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80
60
40
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Relative intensity
Pop C
Pop D
Pop F
0.1
1
10
100
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10000
Time (ms)

Figure caption

Figure 1

Characteristic ^{1}H T_2 relaxation time distribution for cooked pasta with different formulation

SECTION C

CHARACTERIZATION OF TOMATO SAUCE

Diantom et al., to be submitted

Effect of added hydrocolloids on physico-chemical properties of tomato sauce

Abstract

The effect of different hydrocolloids on physico-chemical properties of tomato sauce was explored. Guar, xanthan, carboxy methyl cellulose (CMC), locust bean gum, potato fiber, milk, potato and soy proteins were added to tomato sauce at different concentrations (0, 0.5%, 1% and 1.5%). The macroscopic water status indicators (water activity and moisture content), rheological properties (Bostwick consistency and apparent viscosity), color and proton molecular mobility indicators (^{1}H FID, ^{1}H T_2 and ^{1}H D) were investigated.

water activity significantly decreased in processed samples but it was not affected by the added hydrocolloids, except in the case of potato fiber which significantly decreased water activity. Bostwick consistency and apparent viscosity were significantly enhanced in the presence of xanthan, locust bean gum, guar and CMC. Changes were also observed in protons molecular mobility indicators, which significantly decreased with increasing hydrocolloid concentration.

Keywords Tomato sauce, hydrocolloids Physico-chemical properties . ^{1}H NMR mobility

1-Introduction

Tomatoes are an important agriculture crop which is great part of the human diet. Tomato can be used fresh but important quantities are processed to obtain different products, such as tomato juice, paste, puree, ketchup and sauce. Tomato sauce is a common ingredient used as a condiment, for example in pasta meals, both fresh and ready to eat (RTE) products. RTE pasta meals available in the market can be divided in two categories, with tomato sauce being in strict contact with pasta or placed in a separate compartment from the pasta phase. The presence of tomato sauce in strict contact with RTE pasta might compromise the quality of the product during storage throughout exchanges (mainly of water) occurring between the two product phases. In a recent study, Carini et al. (2013) studied physico-chemical properties and water status of a cooked and sterilized RTE "fusilli all'arrabbiata" pasta meal (pasta in contact with tomato sauce). Water migration between pasta and tomato sauce phases was observed by means of proton molecular mobility indicators but not of macroscopic water status descriptors (i.e. moisture content and water activity), highlighting the importance of regulating water migration between pasta and sauce with the aim to improve products quality and stability during storage.

Thus, to enhance RTE pasta meals quality during storage, the improvement of tomato sauce performance and stability should be addressed, with a proper management of sauce formulation. To this aim, different actions are available, according to the good practice of tomato sauce production. For example, the use of additional ingredients, such as citric acid and sodium chloride (NaCl), in conjunction with high pressure treatment (Palaza et al., 2003) significantly enhanced colour stability in tomato sauce due to inhibition of enzymes responsible for the browning of tomato sauce. Carini et al. (2014) investigated the effect of flour, gelatin and salt on physico-chemical properties of tomato sauce, reporting that salt enhanced the colour of tomato sauce, while gelatin and flour increased its consistency and viscosity. Salt, gelatin, and flour also differently affected water status at a molecular level (proton NMR molecular) but they affected macroscopic water descriptors with

water activity, in particular, significantly dropping only in the presence of added salt (also in combination with flour and gelatin) and not of only gelatin or flour.

Long chain polymers, such as hydrocolloids (polysaccharides and proteins), are extensively used in the food industry, in different food systems as soups, beverages, desserts, ketchups, sauces, salad dressings due to their functional properties (Saha & Bhattacharya, 2010), as thickening (Philips et al., 1986; Philips & Williams, 2000; Gibinski et al., 2006; Sikora et al., 2007; Kok et al. 1999; Wang et al., 2000; Dunstan et al., 2001; Alexander, 1999 a, b; Casas et al., 2000; Kulicke et al., 1996; Murray, 2000; Sahin & Ozdemir, 2004; Koocheki et al., 2009; Saha & Bhattacharya, 2010), gelling (Aguilera 1992; Oakenfull, 1987, Philips and Williams, 2000; Williams, 2006; Saha & Bhattacharya, 2010), emulsifying and stabilizing (Milani & Maleki, 2012) agents. The investigation of the effect of different hydrocolloids on tomato sauce properties may help in the selection of ingredients that might be used in a pasta meal recipe to improve its shelf-stability.

This study aims, therefore, to explore the effect of different hydrocolloids on physico-chemical properties of tomato sauce, investigating macroscopic water status indicators, rheological properties, color as well as proton molecular mobility indicators.

2. MATERIALS AND METHODS

2.1 Materials

Tomato pulp without salt (Siccagno di Valledolmo, Soc. Coop. Rinascita s.r.l., Palermo, Italy, STD1) was purchased from a local supermarket. All the tomato pulp used in this study originated from the same industrial production.Xanthan (Xan), Guar (G), Locust bean gum (LBG) and Carboxy Methyl Cellulose (CMC) gums were provided from Chimab, S.p.a (Campodaresego, Italy), soy proteins (SP), potato proteins (PP) and milk proteins (MP) from Solae Belgium N.V (Ieper, Belgium), and potato fiber (PF) from HI-FOOD S.p.a (Collecchio, Parma, Italy).

Tomato pulp (control, STD1) and tomato pulp added with 0.5, 1 and 1.5% of the hydrocolloids were mixed using an Osterizer® blender (Sunbeam, USA?) for 2 min at low speed (2). 400 g of the

mixed tomato sauces were inserted in sealed glass jars, placed into a water bath at 100°C for 30 min to simulate a traditional tomato sauce production. The jars were then cooled at room temperature for 12 hours before analysis. Tomato pulp was also analysed prior to heating (STD2).

2.2 Methods

2.2.1 Moisture content

Moisture content (MC, % g water / 100 g product) of tomato sauces was determined by weight loss by drying in a forced-air oven (ISCO NSV 9035, ISCO, Milan, Italy) at 80°C to constant weight. At least triplicate tomato sauce samples were analysed.

2.2.2 Water activity

Water activity of tomato sauces were measured at 25°C with an Aqualab 4TE (Decagon Devices, Inc. WA, USA). At least triplicate tomato sauce samples were analysed.

2.2.3 Color

The a^* value (degree of redness) and b^* value (degree of yellowness) were measured (Colorimeter Minolta CM 2600d, Minolta Co., Osaka, Japan) equipped with D65 at 10° position of the standard observer (CIE, 1978). The a^*/b^* ratio was used to describe tomato sauces redness (Barreiro, Milano, Sandoval, 1997; Batu, 2004; Weatheral & Lee, 1991; Yang & Chinnan, 1988). At least twelve measurements were taken for each tomato sauce sample.

2.2.4 Bostwick consistency

Bostwick consistency was measured with a Bostwick Consistometer (LS 100, Laboscientifica, Parma, Italy). The Bostwick consistometer sample chamber was filled with 100 ml of tomato sauce, and then the gate of the chamber was released to allow for tomato sauce to flow. The distance (cm) travelled by the sample was recorded after 30 s (Barringer, Azam, Heskitt, & Sastry, 1998*)*. At least three measurements were taken for each tomato sauce sample.

2.2.5 Apparent viscosity

Tomato sauces apparent viscosity was measured with a Brookfield digital viscometer (MODEL LVDV-I PRIME) using spindle S64 at different speeds (1.5, 3, 6, 10, 12, 20, 30, 50, 60 and 100 rpm). Sauce samples (about 300 ml) were inserted in 300 ml beaker. The apparent viscosity value (Pa*s) was recorded after 60 seconds. The recorded data were used to calculate the shear rate (γ) (equation 1) and shear stress (τ) (equation 2). The non-Newtonian model (equation 3) was used to fit the shear stress vs shear rate curves to obtain the flow index *n* which represents the fluid behavior, with 0 < n < 1 indicative of a pseudoplastic behaviour, and K which represent the consistency coefficient.

$$\gamma = C * \omega \qquad \textbf{equation 1}$$

where C is the probe constant and ω is the speed (rpm)

$$\tau = \gamma * \eta \qquad \textbf{equation 2}$$

where η is the viscosity

$$\tau = K * \gamma^{n} \qquad \textbf{equation 3}$$

where K is the consistency coefficient and n is the flow index

2.2.6 Protons Nuclear Magnetic Resonance (^{1}H NMR)

A low resolution (20 MHz) ^{1}H NMR spectrometer (the MiniSpec, Bruker Biospin, Milano, Italy) operating at 25.0 ± 0.1 °C was used. About 4 grams of tomato sauce (10 mm high) were placed into a 10 mm NMR tube that was then sealed with Parafilm® to prevent moisture loss during the NMR experiment. ^{1}H FIDs were acquired using a single 90° pulse, followed by a dwell time of 7 μs, a recycle delay of 5 s and a 8 ms acquisition window. ^{1}H FIDs were analysed only in the time range 7–100 μs where the homogeneity of magnetic field was assured. The curves were fitted with a two components model (exponential and gaussian; Le Grand, Cambert, & Mariette, 2007; Sigmaplot, v6, Systat Software Inc., USA):

$$f(t) = y0 + A*\exp(-t/T_A) + B*\exp[-(t/T_B)^2]$$

where y0 is the FID decay offset, A and B are the intensities of each relaxation component, T_A and T_B are the apparent relaxation times.

T_2 relaxation time was measured with a CPMG pulse sequence with a recycle delay of 3 s (≥5 ^{1}H T_1), an interpulse spacing of 0.04 ms and 10000 data points. ^{1}H T_2 curves were analysed as quasi-continuous distributions of relaxation times using a UPENWin software (Alma Mater Studiorum, Bologna, Italy). Default values for all UPEN settings were used with the exception of one parameter (LoXtrap) that was set to 1 to avoid extrapolation of relaxation times shorter than the first experimental point. ^{1}H T_2 CPMG relaxation decays were also fitted with a discrete exponential model (Sigmaplot, v.6, Systat Software Inc., USA).

Proton self-diffusion coefficient (^{1}H D) was measured at 25°C with a pulsed-field gradient spin echo (PFGSE) pulse sequence and a 30% gradient. The instrument was calibrated with water at 25°C ($D = 2.29*10^{-9}$ m^2 /s).

2.2.7 Statistical analysis

Statistical analysis was carried out with SPSS software (Version 22.0, IBM SPSS Statistics, Armonk, New York, USA). Significant differences ($p \leq 0.05$) among different samples were verified with by one-way-analysis of variance (ANOVA) with a Tukey-high and LSD significant difference test.

3- Results and Discussion

3.1 – Moisture content and water activity

Moisture content of STD1 and STD2 was 91% (g water / 100g product), indicating that moisture content was not affected by heating, as expected since it was carried out in sealed jars. On the contrary, water activity was significantly reduced by the heating process (Table 1), possibly because of the establishment of stronger interactions between water and macromolecules during heating. Even if the changes induced by hydrocolloids were very small, moisture content significantly

decreased with increasing hydrocolloids concentration in all samples, as expected, except in CMC where no changes were observed between 1% and 1.5% of hydrocolloids, while SP and PP showed changes in moisture content only at higher concentration. The ability to bind water was correlated to the concentration and type of hydrocolloid. In fact at 0.5% of hydrocolloids, LBG showed the highest capacity to hold water, followed by PP and CMC gum, while Xan and MP showed the lowest ability to bind water. The interactions between water and hydrocolloids were correlated to the type and concentration of hydrocolloids. This might be associated to the fact that these hydrocolloids present different molecular structure.

The addition of hydrocolloids did not affect the water activity, independently of their nature and concentration, except in the case of potato fiber, which significantly reduced water activity in the same manner at different concentrations, suggesting that potato fiber was able to bind water more strongly than the other hydrocolloids used in this study.

3.4 Color

The effect of added hydrocolloids and processing on tomato sauce color was investigated with Minolta Colorimeter, and the redness index, a*/b* ratio, was carried out (Table 1). Heated tomato sauce (STD1) showed lower value of redness index than the unheated tomato sauce (STD2), indicating a reducing effect of heating on the redness of tomato saucePresence of hydrocolloids drastically changed redness index of the sauce. At 0.5% of hydrocolloids, the redness index ratio significantly increased in all samples, except in the case PF and MP, which seemed to not affect the color of tomato sauce. Thus hydrocolloids exalted the color of tomato sauce. This might be associated to a probable effect of hydrocolloids on burning reaction, with an inhibition of Maillard reaction, possibly decreasing water availability and reducing the reaction rate. Further increase in hydrocolloids concentration further decreased redness of all samples, except in the presence of CMC, Xan, SP and MP. The redness index significantly decreased in the presence of 1% of CMC while it increased in the presence of 1% of SP, but no changes was observed in the presence of

these hydrocolloids at 0.5% and 1.5%. In Xan changes in redness index did not depend on hydrocolloid concentration.

3.5 – Bostwick consistency

The running distance on a inclined plane of heated tomato sauce (STD1) significantly decreased as compared to STD2, indicating an enhanced consistency resulting from processing. Heating probably allows the main components of tomato sauce to aggregate, interactions between water and carotenoids were promoted, and a more rigid network was formed conferring a high resistance to flow to the sample.

Changes in Bostwick consistency were related to the type of hydrocolloid and concentration. At 0.5% hydrocolloid addition, Bostwick consistency was significantly increased in the presence of PF, LBG, PP, and SP, significantly reduced in the presence of CMC and Guar, while it did not significantly change with the addition of Xan and MP. Bostwick consistency significantly increased with increasing hydrocolloids concentration, more markedly in Xan (from 7.1 ±0.3 cm at 0.5% to 2.5 ± 0.1 at 1.5%) and PF (6.8 ± 0.3 at 0.5% to 2.8 ± 0.3 at 1.5%).

The changes in the consistency might be probably attributed to the formation of a network following water binding by hydrocolloids molecules, inducing samples to resist to the flow (Gujral et al., 2002) Sahim & Ozdemir (2004) reported that ketchup fluidity was significantly affected by the type and concentration of hydrocolloids: xanthan caused the maximum increase of Bostwick consistency of ketchup, followed by guar and locust bean gums and carboxy methyl cellulose, respectively. Carini et al. (2014) reported a strong increase in Bostwick consistency due to the addition of gelatin and flour while the NaCl reduced the consistency.

3.6 Apparent viscosity

Apparent viscosity decreased with increasing probe speed in all samples, which is the typical behavior of pseudoplastic systems, as tomato sauce (Figure 1). Tomato sauce apparent viscosity was affected by formulation, and was strongly increased in the presence of all hydrocolloids. The increase in viscosity was more marked in the presence of Guar, LBG, Xan and CMC, and did not

allow to measure viscosity at higher concentration (1.5%) and higher speed (100rpm for Guar, 50, 60 and 100 rpm for Xan and CMC, 20, 30, 50, 60 and 100 for LBG) due to exceeding of the maximum detectable value for the instrument. Apparent viscosity increased with increasing hydrocolloids concentration (data not shown), except for PP and MP where viscosity was reduced at higher concentration.

These results were in agreement with the Power law (equation 3) fitting results, consistency coefficient and flow behavior index, that are reported in Table 2. The model was able to properly describe the flow behavior of tomato sauce, with R^2 values in the range 0.901-0.999. The flow behavior index behavior (n) was < 1 in all samples, indicating the pseudoplastic (shear thinning) nature of tomato sauce. The flow index and consistency coefficient were comparable between tomato pulp before (STD2) and after (STD1) heating, indicating no effect of processing on flow behavior. The addition of hydrocolloids significantly affected the flow model parameters. For all hydrocolloids the flow behavior index decreased, more markedly in PP, where it was about 0.08 at 0.5%. The addition of different hydrocolloids induced changes in consistency coefficient (K). At 0.5% the consistency coefficient increased in all samples, except for SP where K was reduced as compared to STD1. The consistency coefficient increased with increasing concentration in all samples (Table 2) except for SP where it was approximately constant. Similar changes in viscosity parameters, consistency coefficient and flow behavior index, were reported by Dervisogh & Kokin (1986), Rani & Bains (1987), Bottiglieri et al. (1991), Sanchez et al. (1995), Sahim & Ozdemir (2004), Sharoba et al. (2005), Koockeki et al. (2009) and Carini et al. (2014), and they correlated those changes to the hydrocolloids capacity to bind water, which might generate the formation of new network.

3.7 Protons Nuclear Magnetic Resonance (*^{1}H NMR)*

To better investigate the effect of hydrocolloids on physico-chemical properties at a molecular level, proton mobility was studied with low resolution ^{1}H NMR (20 MHz) through different

parameters, Free Induction Decay (^{1}H FID), rotational spin-spin transverse relaxation time (^{1}H T_2) and self-diffusion coefficient (D).

^{1}H FIDs were fit with a two component model and two proton populations were named population A and B (data not shown). The more rigid protons population (population A) was ~3% relaxing at ~ 0.01 ms while population B, the more represented (~97%) relaxed at ~ 1.2 ms, for both standards (STD 1 and STD 2), indicating that heating did not affect the more rigid FID protons mobility. The protons observed in the ^{1}H FIDs were not affected by hydrocolloids and concentration (data not shown), except for PF where relevant changes were observed at 1.5%. In fact the more mobile protons (population B) was about 51% and relaxed at about 0.22 ms, indicating a reduced mobility in this sample. This might be correlated to stronger interactions between water and hydrocolloids which have generated the formation of a rigid network, and this was in agreement with the strong increase of Bostwick consistency.

^{1}H T_2 representative distributions of tomato sauce with different hydrocolloid are showed in Figure 2. Two unresolved populations were observed in all samples, except for Xan which exhibited three unresolved populations. According to the distributions, ^{1}H T_2 curves were fitted using a bi-exponential equation for all samples and tri-exponential for Xan. Populations were named population C (pop C) and population D (pop D) and their corresponding relaxation times T_{2C} and T_{2D}, respectively. The three populations observed in Xan were named population C, population D and population E and their corresponding relaxation times T_{2C}, T_{2D}, T_{2E}, respectively. The fitting results are showed in Table 3.

The more abundant and more mobile population D was significantly larger in STD1 as compared to the untreated sample STD2, and consequently the less mobile protons population C was less represented in STD1. The corresponding relaxation times, T_{2C} and T_{2D} significantly decreased in processed sauce (STD1) (Table 3). The observed changes have been associated to an effect of processing, which might have promoted the formation of a network of interactions between water and hydrocolloids and, consequently, a decreased molecular mobility. At the same time, processing

increased the more mobile protons (population D), although they also became more mobile (higher T_{2D}). Similar relaxation times in tomato sauce were reported in a recent paper, Carini et al. (2014).. ^{1}H T_2 mobility in raw tomato was also previously investigated (Musse et al., 2009), and protons with different mobility were observed, in relation to the cell compartmentalization, and attributed to exchangeable solutes (< 100 ms), cell walls (≈100ms), cytoplasm (400-600 ms) and vacuole (900-1600 ms) protons. A very different mobility was observed as, in the present study, cell compartments were altered by processing, resulting in the observation of an "averaged" mobility originating from all protons into tomato pulp matrix, that were not located in separated compartments.

Hydrocolloids significantly affected tomato sauce proton mobility, in a manner dependent on hydrocolloids type. At 0.5 % the more abundant population, pop D, was significantly decreased in all samples as compared to STD1, except for CMC and LGB, where it was significantly increased (Table 3). These changes in protons mobility might be related to the interactions of each specific hydrocolloid with water, with a modification of the sauce structure which became more rigid. The increase in hydrocolloids concentration induced a significant decrease of the more abundant and more mobile protons abundance, except for CMC where population D significantly increased with increasing concentration. Consequently population C significantly increase in all samples while it decreased in the presence of CMC. These changes in proton distribution were in agreement with those observed in Bostwick consistency, except in the case of Guar, which showed a lower Bostwick consistency and a decrease in the more abundant and more mobile proton populations.

A reduced mobility was also indicated by the decrease in T_{2C} and T_{2D}. Hydrocolloid concentration generally decreased molecular mobility (T_{2D} and T_{2C} considerably decreased significantly decreased) in all samples.

Xan exhibited a very different mobility, with an additional more mobile population (population E) and the splitting of the correspondent population C observable in the other samples in two

populations, indicating a higher molecular mobility and a different structure of this sample (Table 3).

Proton self-diffusion coefficient (^{1}H D) values are reported Table 3. Both standards showed similar D values (1.898 ± 0.003*10^{-9} m^2 /s in STD1 and 1.901 ± 0.004 *10^{-9} m^2 /s STD2) , indicating that processing did not affect translational mobility. The presence of hydrocolloids induced changes in ^{1}H D values. At 0.5% of hydrocolloids ^{1}H D was significantly decreased in LBG and PP, and it significantly increased in Xan and SP, while it seemed to not be significantly affected by the rest of added hydrocolloids. D significantly decreased with increasing hydrocolloids concentration in all samples, except for LBG and SP. This decrease might be correlated to the strong interactions with tomato sauce components/water with a matrix structure that hindered protons translational mobility. Similar results were reported by Carini et al., (2014), due to the addition of salt, flour and gelatin to tomato sauce. In the case of LBG tomato sauce D significantly decreased after the addition of 1% then it remained constant, while SP induced significant changes in ^{1}H D only at higher concentration (1.5%). However there was no linear correlation between the proton molecular mobility and the proton self-diffusion coefficient.

4- Conclusions

The effect of eight added hydrocolloids and processing on physico-chemical properties of tomato sauce was explored at different time-space levels. Processing affected macroscopic water status indicator (water activity), rheological attributes (Bostwick consistency), color and molecular mobility parameters. Moisture content decreased with increasing amount of added hydrocolloid, as expected. Whereas water activity was not affected by the added hydrocolloids except in the case of potato fiber, which significantly reduced water activity. Xan, LBG, Guar and CMC induced strong changes in rheological properties (Bostwick consistency and apparent viscosity). Hydrocolloids also induced changes in the molecular mobility indicators.

These results suggested that the addition of hydrocolloids into tomato sauce formulation strongly affects its physico-chemical at different time-space levels but the effect of single hydrocolloid might be carefully considered during the production of tomato sauce. In the same way, hydrocolloids might allow to control the changes in physico-chemical properties of RTE pasta /sauce meals.

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Table 1

Physico-chemical properties of tomato sauces with different formulations: MC (Moisture Content), a_w (Water Activity), Bostwick consistency, Self-diffusion coefficient (D) and a*/b* (colorimetric index). Standard deviations are given in parenthesis following the means values; different letters close to numbers indicate significant difference among samples ($p \leq 0.05$), where the small letter referred the difference due to the concentration and the big one to the type of hydrocolloids. the "a" and “A” letters were assigned to the highest value.

		Moisture content (%, g H_20/100g sample)	a_w	Bostwick consistency (cm)	a*/b*
STD 1		90.93 (0.03) A	0.996 (0.001) A	7.3 (0.3) C	1.07(0.01)E
STD 2		90.85 (0.07) A	0.982 (0.003) B	8.6 (0.3) B	1.10(0.01)F
Guar	0.5%	90.42 (0.07) a/CD	0.997(0.001) a/A	9.0 (0.1) a/A	1.14(0.02) a/C
	1%	90.05(0.09) b/C	0.996 (0.001) a/A	8.8 (0.42) a/A	1.14(0.01) a/C
	1.5%	89.13(0.08) c/DE	0.996 (0.001) a/A	8.0(0.41) b/B	1.10(0.01) b/C
LBG	0.5%	89.77(0.10) a/F	0.992 (0.002) b/A	6.5(0.1) a/D	1.21(0.02) a/A
	1%	89.63(0.05) b/E	0.995(0.003) ab/A	5.5(0.1) b/D	1.17(0.01) b/B
	1.5%	89.12(0.05) c/DE	0.997(0.001) a/A	5.8(0.3) b/DE	1.06(0.01) c/D
Xan	0.5%	90.59(0.06) a/B	0.996 (0.003) a/A	7.1(0.3)a/BC	1.13(0.01)ab/CD
	1%	89.67(0.05) b/DE	0.999 (0.001) a/A	4.4(0.3) b/E	1.14(0.01) a/C
	1.5%	89.05(0.03) c/E	0.995 (0.004) a/A	2.5(0.1) c/G	1.13(0.01) b/B
CMC	0.5%	90.04 (0.07) a/E	0.996 (0.003) a/A	8.9(0.3) a/A	1.15(0.01) b/C
	1%	89.28 (0.05) b/F	0.996 (0.003) a/A	8.3(0.3) b/A	1.20(0.01) a/A
	1.5%	89.23 (0.04) b/CD	0.997 (0.004) a/A	7.1(0.3) c/C	1.16(0.03) b/A
PP	0.5%	89.87(0.15) a/EF	0.996 (0.004) a/A	4.5(0.1) b/F	1.17(0.01) a/B
	1%	89.80(0.09) a/D	0.995 (0.005) a/A	4.9(0.3) a/E	1.16(0.01) a/B
	1.5%	89.19(0.03)b/CDE	0.998 (0.001) a/A	4.5(0.1) b/F	1.12(0.01) b/B
MP	0.5%	90.47(0.06) a/BC	0.997 (0.002) a/A	6.9(0.3) a/BCD	1.07(0.01) a/F
	1%	89.96(0.08) b/C	0.996 (0.002) a/A	6.4(0.3) b/C	1.06(0.01) a/E
	1.5%	89.28(0.07) c/C	0.996 (0.002) a/A	5.5(0.1) c/E	1.07(0.01) a/D
SP	0.5%	90.28(0.04) a/D	0.993 (0.002) a/A	6.6(0.3) b/CD	1.12(0.01) a/D
	1%	90.28(0.03) a/B	0.994 (0.002) a/A	7.5(0.1) a/B	1.07(0.01) b/E
	1.5%	89.47(0.07) b/B	0.994 (0.003) a/A	6.1(0.3) c/D	1.13(0.01) a/B
PF	0.5%	90.29(0.06) a/D	0.986(0.001) a/B	6.8(0.3) a/CD	1.07(0.01) a/F
	1%	89.65(0.09) b/DE	0.986 (0.001) a/B	4.4(0.3) b/E	1.06(0.01) b/E
	1.5%	89.14(0.09) c/DE	0.985 (0.001) a/B	2.8(0.3) c/G	1.07(0.01) b/D

Table 2

Consistency coefficient (K) and flow behaviour index (n) of tomato sauce with different formulation

		Consistency coefficient (K)	Flow behavior index (n)
STD 1		12.39	0.315
STD 2		12.88	0.306
Guar	0.5%	17.25	0.365
	1%	29.53	0.303
	1.5%	41.35	0.339
LBG	0.5%	32.66	0.285
	1%	50.08	0.293
	1.5%	81.08	0.283
Xan	0.5%	26.32	0.202
	1%	56.47	0.175
	1.5%	94.50	0.136
CMC	0.5%	19.70	0.284
	1%	31.26	0.328
	1.5%	51.58	0.357
PP	0.5%	33.51	0.080
	1%	29.51	0.113
	1.5%	33.33	0.096
MP	0.5%	21.21	0.178
	1%	22.20	0.194
	1.5%	18.45	0.236
SP	0.5%	11.74	0.371
	1%	11.23	0.369
	1.5%	11.12	0.399
PF	0.5%	18.25	0.256
	1%	17.74	0.259
	1.5%	18.55	0.277

		T_{2C} (ms)	Pop C (%)	T_{2D} (ms)	Pop D (%)	T_{2E} (ms)	Pop E(%)	Self-diffusion coefficient D 10^{-9} m^2 /s)
STD 1		93.4(0.6) C	6.3(0.1) E	303.0 (0.1) B	93.7(0.1) B	-	-	1.898(0.003) BC
STD 2		115.8(1.1) A	11.5(0.1) A	322.6(0.1) A	88.5(0.1) F	-	-	1.901(0.004) B
Guar	0.5%	97.8(1.8) a/B	9.5(0.3) c/B	294.1(4.5) a/C	90.5(0.3) a/E	-	-	1.894(0.002) a/BC
	1%	85.4(0.8) b/E	12.6(0.3) b/A	250.0(0.1) c/E	87.4(0.3) b/F	-	-	1.848(0.004) b/G
	1.5%	96.7(1.4) a/C	13.7(0.5) a/C	270.3(0.1) b/D	86.3(0.5) c/F	-	-	1.825(0.004) c/F
LBG	0.5%	84.1(0.9) a/E	5.4(0.1) b/F	301.8(3.4) a/B	94.3(0.1) a/A	-	-	1.850(0.005) a/E
	1%	76.0(0.5) c/G	5.8(0.1) b/F	268.2(3.5) b/C	94.2(0.1) a/A	-	-	1.831(0.003) b/E
	1.5%	80.7(0.5) /F	8.1(0.2) a/F	270.3(0.1) b/D	91.9(0.2) b/C	-	-	1.834(0.003) b/E
Xan	0.5%	18.4(1.0) b	0.9(0.1) c	212.0(1.8) a	76.8 (1.6)b	128.9(4.9) c	22.3(2.6) b	1.919(0.005) a/A
	1%	22.5(1.0) b	1.3(0.1) b	127.4(1.7) b	b 64.5(3.5) c	194.2(3.2) b	34.2(3.4) a	1.898(0.005) b/B
	1.5%	21.6(0.4) a	1.5(0.1) a	116.8(1.8) c	84.8(1.8) a	207.5 (3.6) a	13.8(1.8) c	1.880(0.003) c/B
CMC	0.5%	83.7(2.0) a/C	6.0(0.3) a/F	277.8(0.1) a/E	94.1(0.3) b/A	-	-	1.894(0.004) a/BC
	1%	72.0(0. 6) c/H	6.0(0.1) a/F	250.0(0.2) c/E	94.0(0.1) b/A	-	-	1.839 (0.002) b/F
	1.5%	73.8(0.6) b/G	5.4(0.1) b/H	270.3(2.4) b/D	94.6(0.1) a/A	-	-	1.831(0.004) c/EF
PP	0.5%	91.1(1.0) a/D	11.8(0.2) c/A	238.1(0.1) a/F	88.2(0.2) a/F	-	-	1.882(0.003) a/D
	1%	79.7(0.6) c/F	10.9(0.2) b/C	197.1(2.0) b/F	89.1(0.2) a/D	-	-	1.869(0.002) b/D
	1.5%	86.3(2.1) b/E	15.9(1.3) a/B	183.2(1.8) c/F	84.1(1.3) b/G	-	-	1.855(0.004) c/D
MP	0.5%	90.8(1.0) c/D	7.7(0.1) c/C	285.7 (0.1) a/D	92.4(0.1) a/D	-	-	1.893(0.002) a/C
	1%	88.2(0.8) b/D	9.0(0.2) b/D	263.2(0.1) b/D	91.0(0.3) b/C	-	-	1.879(0.002) b/C
	1.5%	148.4(1.2) a/A	48.5(0.1) a/A	285.7(0.1) a/C	51.5(0.1) c/H	-	-	1.852(0.002) c/D
SP	0.5%	85.6(1.3) a/E	7.0(0.1) b/D	277.8(0.1) a/E	93.0(0.1) b/C	-	-	1.923(0.003) a/A
	1%	76.5(0.7) b/G	5.9(0.1) c/F	270.3(0.1) b/C	94.1(0.1) a/A	-	-	1.926(0.003) a/A
	1.5%	68.0(0.4) c/H	7.4(0.1) a/F	232.6(0.1) c/G	92.6(0.1) c/C	-	-	1.903(0.004) b/B
PF	0.5%	90.3(1.9) a/D	8.9 (0.3) c/B	285.7(0.1) a/D	91.1(0.3) a/E	-	-	1.891(0.002) a/C
	1%	89.9(1.1) a/C	11.2(0.2) a/C	270.3 (0.1)b/C	88.8(0.2) c/D	-	-	1.883(0.001) b/C
	1.5%	80.3(1.0) c/F	10.5(0.2) b/E	263.2(0.1) c/E	89.5(0.2) b/D	-	-	1.872(0.002) b/C

Table 3

^{1}H T_2 relaxation times and populations of tomato sauce with different formulation. Standard deviations are given in parenthesis following the means values; different letters indicate significant difference among samples ($p \leq 0.05$); small letters indicate differences in the same sample at different concentrations; capital letters indicate differences among the samples, including STD1 and STD 2

Figure 1

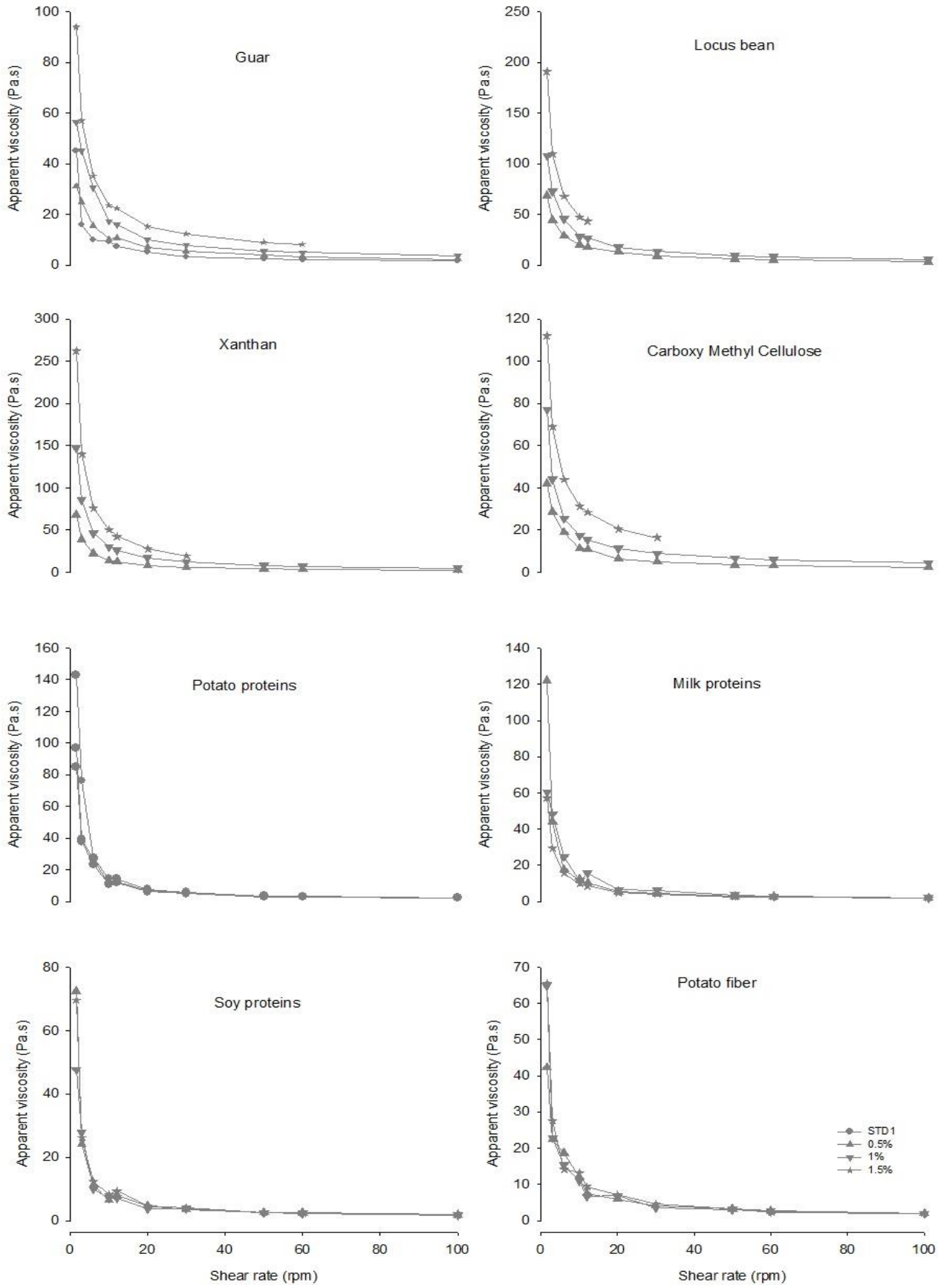
Guar
Locus bean
Xanthan
Carboxy Methyl Cellulose
Potato proteins
Milk proteins
Soy proteins
Potato fiber
Apparent viscosity (Pa.s)
Shear rate (rpm)
STD1
0.5%
1%
1.5%

Figure 2

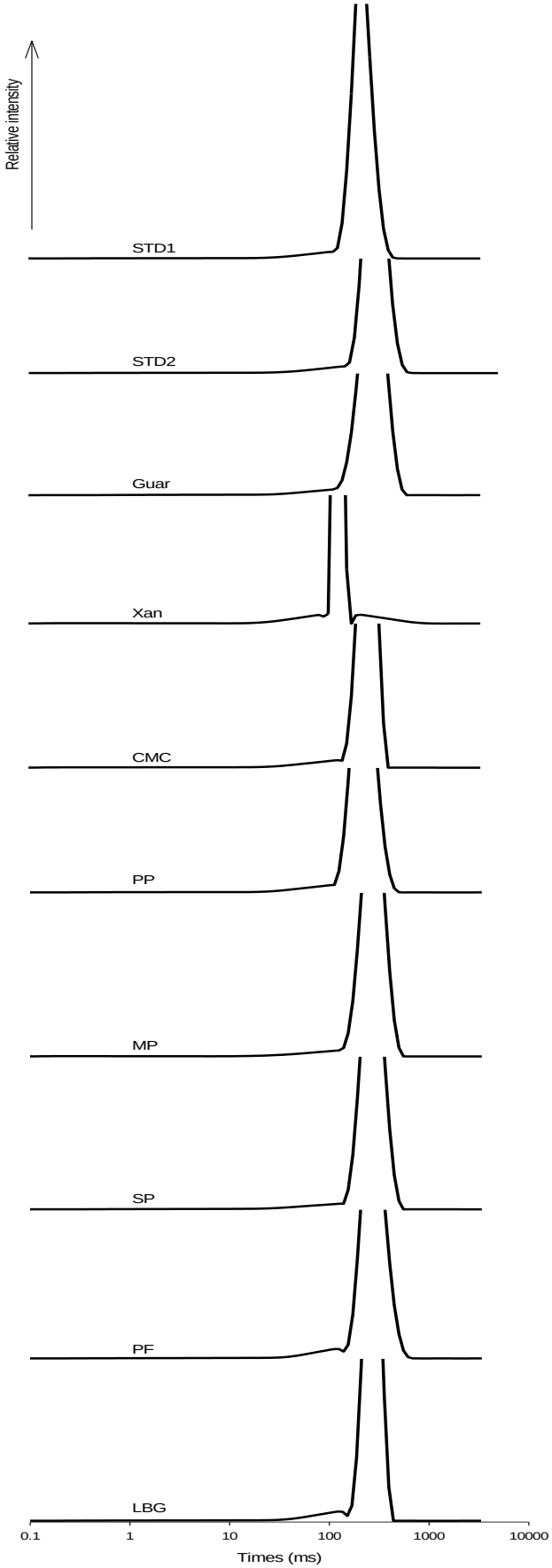
Relative intensity
STD1
STD2
Guar
Xan
CMC
PP
MP
SP
PF
LBG
0.1
1
10
100
1000
10000
Times (ms)

Figure captions

Figure 1

Apparent viscosity of tomato sauce with different formulations

Figure 2

Characteristic ^{1}H T_2 relaxation time distribution for tomato sauce with different formulation