

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Evaluation of physico-chemical changes and FT-NIR spectra in fresh egg pasta packed in modified atmosphere during storage at different temperatures

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Zardetto S., Pasini G., Romani S., Rocculi P., Dalla Rosa M. (2021). Evaluation of physico-chemical changes and FT-NIR spectra in fresh egg pasta packed in modified atmosphere during storage at different temperatures. *FOOD PACKAGING AND SHELF LIFE*, 28(June 2021), 1-10 [10.1016/j.fpsl.2021.100648].

Availability:

This version is available at: <https://hdl.handle.net/11585/815900> since: 2021-03-18

Published:

DOI: <http://doi.org/10.1016/j.fpsl.2021.100648>

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).
When citing, please refer to the published version.

(Article begins on next page)

Evaluation of physico-chemical changes and FT-NIR spectra in fresh egg pasta packed in modified atmosphere during storage at different temperatures

Stefano Zardetto^{1*}, Gabriella Pasini², Santina Romani^{3,4}, Pietro Rocculi^{3,4}, Marco Dalla Rosa^{3,4}

¹ Department of Quality Assurance, Research & Development, VOLTAN SPA, Via Dosa 24, 30300 Olmo di Martellago (VE), Italy

² DAFNAE, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy

³ Interdepartmental Centre for Agri-Food Industrial Research, *Alma Mater Studiorum*, University of Bologna, Via Quinto Bucci 336, 47521 Cesena (FC), Italy

⁴ Department of Agricultural and Food Sciences, *Alma Mater Studiorum*, University of Bologna, Campus of Food Science, Piazza Goidanich 60, 47521 Cesena (FC), Italy

*Corresponding author: tel. +39-041-546421; fax: +39-041-5464294 e-mail: stefanozardetto@voltan.biz

Keywords: fresh egg pasta, physicochemical stability, DSC, FT-NIR

Abstract

Physico-chemical properties (i.e. water activity, colour, water absorption index and hardness) influence stability, quality and consumer acceptability of fresh pasta. The aim of this study was to investigate the effect of storage time on physico-chemical properties of fresh pasta stored at different temperatures (0, 5 and 10 °C). Storage time significantly affected the macroscopic properties (cooking loss and hardness) of fresh pasta and its water status (water activity, freezable water). During storage at all temperatures, water activity and

freezable water decreased, confirming a reduction in water mobility in the product. Storage time increased pasta hardness and caused a reduction in the water absorption index. Fresh egg pasta retained its yellow colour, while lipid oxidation indices were only slightly affected. With the increase of storage temperature, the kinetic rate of water activity and gelatinisation degree decreased. In contrast, the kinetic rate of pasta hardness increased with the temperature. The Arrhenius relationship well explained the temperature dependence of reaction constants, while FT-NIR analysis showed a structural reorganisation of pasta matrix during storage. Generally pasta matrix reorganisation involved water, starch and proteins interactions, and storage temperature was an important factor affecting these modifications with specific effects on physico-chemical pasta properties (e.g. hardness).

37

38 **1. Introduction**

Pasta is a traditional cereal-based food of the Italian tradition that is popular worldwide because of its convenience, palatability and nutritional quality (Carini, Curti, Minucciani, Antoniazzi & Vittadini, 2014). Fresh pasta can also be produced using flour from *Triticum aestivum* (soft wheat), a grain type mainly used for bread making. Several other ingredients could be added to improve the colour, texture, and nutritional value of fresh pasta such as egg, following the Italian legislation (DPR 2001).

"Fresh egg pasta" is a traditional Italian product with remarkable commercial success in many countries, such as UK, Germany, France and USA. According to Italian law, the denomination of "fresh pasta" is given to products having a water activity below 0.97, be submitted to one heat treatment equivalent to pasteurisation, and stored at a maximum temperature of 6 °C in all its commercial steps (storage and the distribution chain until its final destination). Even if for fresh pasta refrigerated storage is compulsory, temperature abuse at about 10 °C can be found under real conditions (Zardetto, 2017). In the industrial process, fresh egg pasta is made using a dough machine by mixing durum wheat semolina,

53 soft wheat flour, egg and water. The product is extruded or rolled into various shapes and
54 then pasteurised, packed in modified atmosphere packaging (MAP) and subsequently
55 refrigerated (Carini et al., 2014; Zardetto & Dalla Rosa, 2007).

56 MAP is a well-established technique in which the gas composition surrounding a product is
57 altered, resulting in an atmosphere different from air. The two major gases used in
58 commercial MAP are nitrogen (N₂) and carbon dioxide (CO₂) and the optimal carbon dioxide
59 concentration for fresh filled pasta vary from 25 to 40%, while N₂ as filling gas is used to
60 prevent packages from collapsing due absorption of the CO₂ from the product (Zardetto,
61 2005b). As reported by Zardetto (2005b), the reliability of MAP in fresh pasta depends on
62 rigorous temperature control. Temperature is often not constant during distribution chain,
63 characterised by different processes such as transport, warehouse storage, picking, and
64 house storage, each with their typical conditions (Zardetto, 2017).

65 It is well known that temperature is one of the most critical factors affecting the shelf life of
66 foods. As most degradation reactions are Arrhenius-like, the higher the temperature, the
67 faster products achieve high degradation (Pedro & Ferreira, 2006). Nevertheless, some
68 relevant food reactions involved in matrix phase changing can significantly cause deviations
69 from Arrhenius behaviour with temperature (Labuza & Riboh, 1982). As Taoukis, Labuza &
70 Saguy (1997) reported, bread retrogradation shows a negative temperature effect between
71 4 °C and 40 °C, having a maximum rate at 4 °C. This temperature dependence determines
72 a reduction of the rate constant (k) with an increase of the storage temperature and a
73 negative value of activation energy (E_a) calculated from the Arrhenius plot's slope. Moreover
74 starch reorganisation in several food matrices has not shown obey the typical Arrhenius
75 response. The recrystallisation of gelatinised starch during the storage was lowest at 4 °C
76 and increased at the other temperatures (Baik, Kim, Cheon, Ha & Kim, 1997; Labuza, 1982;
77 Huang et al. 1994).

78 Fresh egg pasta has a long shelf-life, from 30 to 70 days (Pagani, Lucisano & Mariotti, 2007),
79 but very few published data are available on this important aspect. Several studies have
80 shown that microbiological stability of the industrial pasteurised product packed in MAP is
81 not the most critical factor limiting its durability (Costa, Lucera, Mastromatteo, Conte &
82 Nobile, 2010; Sanguinetti et al., 2011; Lucera et al., 2014). The available studies suggest
83 that the sensorial quality played a significant role in determining the product acceptability.
84 However, understanding the essential modifications of macroscopic quality in terms of
85 related physico-chemical index linked to the sensory quality and microstructural phenomena
86 is still scarce.

87 During the fresh egg pasta manufacturing processes, the physical and chemical
88 characteristics of the pasta can be modified by different technologies, which have different
89 effects on pasta macroscopic properties (Zardetto, Dalla Rosa, Placucci & Capozzi, 2005;
90 Alamprese, Casiraghi & Rossi, 2008; Carini, Vittadini, Curti & Antoniazzi, 2009). Fresh pasta
91 colour, cooking properties and texture are essential quality attributes (Carini et al., 2009;
92 Zardetto & Dalla Rosa, 2009), but to the best of our knowledge, no data are available in the
93 literature on how these properties change during fresh egg pasta storage. Specific
94 properties of the fresh pasta such as water amount and mobility (in terms of moisture
95 content, water activity, and freezable water content) have been investigated only concerning
96 the effect of mixing, shaping and pasteurisation phases (Zardetto et al., 2005; Carini,
97 Vittadini, Curti, Antoniazzi & Viazani, 2010).

98 In recent years, NIR spectroscopy has become a useful tool supporting chemical methods
99 for food analysis. Several studies reported that NIR spectroscopy is a rapid and cost-
100 effective analytical tool to determine food structure and properties, both for fundamental
101 research, and as an on-line sensor for process monitoring (Ozaki, McClure & Chist, 2006;
102 Nicolai et al., 2007). NIR spectroscopy has been applied to egg pasta, indicating its high
103 potentiality for discriminating fresh pasta submitted to different processing steps. Zardetto

(2005) reported that FT-NIR could be used to rapidly determine the $F_{70/10}$ value in fresh egg pasta submitted to different pasteurisation processes. Zardetto & Dalla Rosa (2006) also reported NIR spectroscopy's potential application in the discrimination of fresh egg pasta obtained using two different production methodologies, extrusion and lamination. Bevilaqua, Bucci, Materazzi & Marini (2013) studied the effects of drying processes on pasta spectra. NIR analysis was also used to determine egg content and moisture in dried pasta (Fodor, Woller, Turza & Szigi, 2011; Temmerman, Saeys, Nicolai & Ramon, 2007) and the prediction of the starch gelatinisation index in fresh egg pasta (Zardetto, 2004). Nevertheless, only a few studies have addressed the NIR spectroscopy application to monitor chemical and physical indices evolution during food storage. Cattaneo, Giardina, Sinelli, Riva & Giangiacomo (2005) used NIR and MIR spectroscopy to study the shelf-life of Crescenza cheese stored at different temperatures, while Sinelli, Barzaghi, Giardina & Cattaneo, (2005) applied NIR spectroscopy to monitor the shelf-life of packed industrial ricotta cheese. However, few studies have been focused on using these techniques to monitor changes in fresh egg pasta during storage. The aim of this study was twofold: 1) to assess the main modifications occurring in physical and chemical characteristics monitored with traditional analytical methods during storage at different temperatures and 2) to evaluate the suitability of FT-NIR spectroscopy as a rapid non-invasive approach for monitoring the main changes occurring during storage in fresh egg pasta.

2. Materials and methods

2.1 Fresh egg pasta preparation

Industrial fresh egg pasta samples were produced in a pasta factory (VOLTAN SPA, Olmo di Martellago -Venezia, Italy) using commercial durum wheat semolina (35% (w/w)), soft wheat flour (35% (w/w)), fresh pasteurised eggs 19% (w/w) and 11% (w/w) water. The

dough, preformed in an industrial mixer (mod. II450, IMI, Padova, Italy) at 94 rpm for 120 sec, was formed into sheets by passage through a 10- and then 0.9-mm roller-sheeter. The pasta sheets were subjected to heat treatment at 78°C for 8 min. Then, they were air-cooled to 45°C, cut with a blade into 15 x 25 cm sheets to obtain the "lasagna" pasta type, and packed in a modified atmosphere (100% N₂; < 2% O₂) using an orientated polyamide (OPA) film composed by OPA (20 µm) and polypropylene (PP-60 µm, SDR PACK SpA, Rosà, Vicenza, Italy). The film has an Oxygen Transmission Rate (OTR) of 20 cc/(m² day) and Carbon Dioxide Transmission Rate (COTR) of 150 cc/(m² day), both at 23°C and 50% relative humidity. The packages, each containing six sheets, were submitted to a second heat treatment at 100°C for 20 min and chilled to 5 °C. A total of 140 packed samples from different batches have been stored at three different temperatures (0, 5 and 10 °C) for 75 days and analysed after 0,15, 35, 56 and 75 days.

2.2 Chemical and microbiological analysis

The samples collected at different sampling times were analysed for moisture and water activity. Microbiological assessment of the industrial samples was also performed. Total viable count (TVC) was evaluated on Plate Count Agar (Merck, VWR, Germany) (ISO, 4833:1 2013) by pouring plates and aerobically incubating them at 30°C for 48 h. Total viable count (TVC) was used as an overall quality indicator for microbial organisms (Ricci et al., 2017), which did not change during storage at the different temperatures, remaining below 10³ CFU/g (data not reported).

Moisture content was determined gravimetrically at 130 °C for 1 h according to D.M. 11/09/1967 and water activity (a_w) was measured using a dew point instrument (mod. Aqualab CX3, Decagon Devices Inc., Pullman, WA. USA) (Zardetto et al., 2005).

154 In the industrial pasta samples at the beginning of storage (t_0) proteins, lipids and NaCl were
155 determined by the Kjeldahl method, Soxhlet lipid extraction and atomic absorption
156 spectrometry (AAS), respectively, according to the methods of ISTISAN 96/34.

157

158 2.3 Differential scanning calorimetry (DSC)

159 For DSC analysis, a differential scanning calorimeter (mod. DSC-6, equipped with a cooling
160 unit, Perkin–Elmer Corp., Wellesley, USA) was used (Zuliani, 1998). Heat flow and
161 temperature calibration were performed with indium ($T_m=156.6\text{ }^{\circ}\text{C}$; $\Delta H_m=28.71\text{ J/g}$) and tin
162 ($T_m=231.93\text{ }^{\circ}\text{C}$; $\Delta H_m=60.46\text{ J/g}$) under a dry nitrogen flow of 20 mL/min, using the same
163 scanning rate applied in the sample analysis. Samples of approximately 10 mg were
164 weighed into 50 μL aluminium pans, and covers were hermetically sealed in place. At least
165 three replicates per sample were analysed using the following temperature programme:
166 cooling to $-70\text{ }^{\circ}\text{C}$, held for 30 min and heating to $100\text{ }^{\circ}\text{C}$ at $10\text{ }^{\circ}\text{C/min}$. Obtained peaks were
167 integrated with Pyris 6.0 software (Perkin–Elmer Corp., Wellesley, USA). From the melting
168 and gelatinisation endothermic curves, the freezable water and starch gelatinisation peaks
169 were identified. The total amount of freezable water was determined using the following
170 equation:

$$171 \quad \text{FW} = \frac{\Delta H}{\Delta H_{ice}} (1)$$

172

173 Where ΔH (J/g fw) is the heat of fusion of water for one gram of sample and ΔH_{ice} is the heat
174 of fusion of pure water at $0\text{ }^{\circ}\text{C}$, considered equal to 334 J/g ice (Carini et al., 2010). The
175 amount of starch gelatinisation (J/g fw) was evaluated by integrating the peak with an onset
176 temperature of about 45°C . The peak temperatures (T_{peak}) for gelatinisation and the freezing
177 of water were also determined.

178 2.4 Determination of gelatinisation degree and damaged starch

179 For the determination of gelatinisation degree, the chemical method proposed by Dalla
180 Rosa, Lerici, Cencic & Pinnavaia (1989) was used to calculate the colourimetric
181 measurement of starch–iodine complexes in an aqueous suspension of the sample, before
182 and after the complete gelatinisation of starch by alkali. The damaged starch was also
183 determined in the wheat mix and fresh uncooked pasta using a Megazyme Starch Damage
184 Assay Kit (K-SDAM; Megazyme International, Wicklow, Ireland; AACCC 76-31).

185

186 2.5 Oxidative stability

187 Fat was extracted from the fresh egg pasta with hexane-isopropanol (3+2 v/v) solvent
188 containing 0.01% butylated hydroxytoluene, followed by washing with sodium sulphate
189 solution, as described by Zardetto, Barbanti & Dalla Rosa (2014).

190 One aliquot of the extract was used for peroxide value (PV) determination, according to
191 Shantha & Decker (1994). This method is based on peroxides ability to oxidise ferrous ions
192 to ferric ions, which react with xylenol orange sodium and give rise to a coloured complex.
193 PV was evaluated at 560 nm with a double-beam Unicam mod. UV2 UV-VIS
194 spectrophotometer (Thermo Fisher, San Jose, CA, USA). For the quantitative determination
195 of PV, expressed as meq O₂/kg of lipid, an Fe(III) standard calibration curve was used with
196 a concentration range of 2.5–30 µg/ml. Other aliquots were used to determine the
197 thiobarbituric acid reactive substances (TBARs), according to the method described by
198 Decker (2004). Briefly, 5 g of previously extracted fat was placed into a coded polyethylene
199 stomacher bag. An additional empty stomacher bag was prepared as a blank. Then, 2.5 mL
200 of antioxidant solution (0.5 g of propyl gallate and 0.5 g of ethylenediaminetetraacetic acid
201 (EDTA) and disodium salt dihydrate in ethanol/water solution) and 50 mL of cold 20%
202 trichloroacetic acid solution (4 ± 2 °C) were added. Samples were blended in a Stomacher
203 400 blender (Seward, PBI International, Milan, Italy) for 2 min. Then, 50 mL of cold distilled
204 water (4 ± 2 °C) was added to each bag for a second blending for 30 sec, and the slurry was

205 filtered. Freshly prepared 0.02 M 4,6-dihydroxypyrimidine-2-thiol solution (5 mL) was added
 206 to each tube and mixed for 4-5 s. The tubes were heated for 35 min in boiling water and
 207 then cooled under running tap water for 10 min. The colour was measured with a Unicam
 208 UV2 UV-VIS spectrophotometer (Thermo Fisher, San Jose, CA, USA) at a wavelength of
 209 530 nm. The TBARs (mg of MDA/kg lipids) were calculated by multiplying the absorbance
 210 values by a constant coefficient K. This value was calculated from a standard curve
 211 generated by reacting TBARs with 1,1,3,3 -tetra-ethoxypropane (TEP) and dilution factor
 212 (DF) as follow:

$$213 \quad K = \left[\left(\frac{1}{S} \times 72.03 \times DF \times 10^6 \times \frac{100}{R} \right) \right] / m \quad (2)$$

214 where S is represented by the standard curve slope, 72.03 the molecular weight of
 215 malonaldehyde, R is the recovery and m is the sample mass (g).

216

217 2.6 Colour measurement and hardness evaluation on uncooked fresh pasta

218 The colour of pasta samples at different storage times was measured using a colourimeter
 219 (mod. CR-200 colourimeter, Minolta, Japan), applying a D65 illuminant and a 10° standard
 220 observer. The instrument was calibrated with a black and white standard tile before each
 221 set of measurements. The parameters L* (lightness), a* (red/green index) and b (yellow/blue
 222 index) were measured (MINOLTA, 1993). Hue angle values ($h^\circ = (\arctan ((b^*/a^*)/2\pi) \times 360)$)
 223 were also calculated (CIE, 1987).

224 The fresh egg pasta's hardness was determined by using a texture analyser (mod. CT3,
 225 Brookfield, Middleboro, USA) equipped with the software Texture Expert Exceed v. 2.61
 226 (Stable Micro Systems, UK) and a 50 kg load cell. The probe used was a Warner–Bratzler
 227 blade (Bourne, 2002). The test mode used was a force in compression with pre-test, test
 228 and post-test speeds of 1.5, 2.0 and 10.0 mm/sec, respectively. By the acquired curves of

229 force (N) versus time (sec), the parameter hardness (N) was extrapolated as the force
230 registered at the highest peak.

231

232 2.7 Cooking properties

233 The water absorption index (WAI) of cooked pasta was measured as follows (Zardetto, Di
234 Fresco & Dalla Rosa, 2002):

235

$$236 \quad \text{WAI} = \frac{\text{weight of cooked pasta (g)} - \text{weight of uncooked pasta (g)}}{\text{weight of uncooked dry matter (g)}} \quad (3)$$

237

238 Total organic matter (TOM), the amount of organic matter released from cooked pasta
239 during exhaustive rinsing, was determined by the method described by D'Egidio et al.
240 (1976). Briefly, the method is based on washing drained pasta with 500 mL of tap water at
241 room temperature to remove the substance coating the surface of the cooked pasta. An
242 aliquot of the washing water is then evaporated at 80 °C. The organic matter in the residue
243 was determined by titration with ferrous ammonium sulphate in excess of potassium
244 dichromate.

245

246 2.8 Near-infrared spectroscopy (FT-NIR)

247 FT-NIR spectra were recorded using a NIRLAB N-200 (FT-NIR Buchi, Switzerland) in
248 reflectance mode (Zardetto, 2005a). Spectra were recorded using an ISI ring cup; data were
249 recorded from 1000 to 2500 nm at 2 nm intervals and saved using three scans for each
250 sample. Measurements and the chemometric interpretation of the FT-NIR spectral data were
251 performed using the chemometric software NIRCAL ver. 4.21 (Buchi, Switzerland).

252 Partial least squares (PLS) regression analysis was the method selected to establish the
253 relationship between storage time values and FT-NIR absorbance values, with no outlier

elimination. The results of the PLS calibration process provided the number of PLS factors used in the model equation, the standard error of estimation (SEE), the coefficient of correlation (r), the standard error of performance (SEP), the slope of the linear regression of the predicted FT-NIR values versus the original values and the BIAS.

SEE is the standard deviation of differences between FT-NIR reflectance and reference values in the calibration sample set. The formula for its calculation, which incorporates BIAS, is as follows (Williams & Norris 2001):

$$SEE = \sqrt{\left(\frac{\sum(x_n - y_n - BIAS)^2}{N}\right)} \quad (4)$$

SEP was computed from the results of the prediction of the validation sample set.

SEE, and SEP provide the magnitude of the standard deviation for the calibration set and the independent validation set. The two values should be as small as possible, yet as the FT-NIR standard deviation includes the standard deviation of reference analysis used to calculate the linear regression of predicted FT-NIR, the SEE and SEP values are comparable with the standard deviation of the conventional reference analysis. With a good calibration technique, the two values are also roughly equal.

The BIAS value provides information on the average deviation between the reference analysis and FT-NIR value. BIAS was calculated from the FT-NIR reflectance predictions of data of the validation sample as follows:

$$BIAS = \frac{\sum(x_n - y_n)}{N} \quad (5)$$

The BIAS calibration set value was close to zero as requested by definition.

2.9 Mathematical model fitting for quality parameters

The experimental data were plotted against time for each selected temperature, and the kinetics of quality loss was determined as described by Singh (1994).

278 The temperature dependence of deterioration rate constant, k, was modelled using the
279 Arrhenius equation (Tsironi & Taoukis, 2011; Vaikousi, Biliaderis & Koutsoumanis, 2009):

$$280 \quad \ln(k) = \ln(k_{ref}) - \frac{E_a}{R} \left[\frac{1}{T} - \frac{1}{T_{ref}} \right] \quad (6)$$

281 where k_{ref} is the constant rate of the degradation of the respective quality index at a reference
282 temperature, T_{ref} is the reference temperature (273 K), T is the absolute temperature in K,
283 E_a is the activation energy of the deterioration (J/mol), and R is the universal gas constant
284 (8.314 J/mol·K or 1.98 cal/mol K). E_a values were estimated from the slope of Arrhenius
285 plots of $\ln(k)$ versus $(1/T - 1/T_{ref})$ by linear regression (Taoukis et al., 1997).

286

287 2.10 Statistical analyses

288 Results are averages of three measurements at least and reported as means \pm SD (standard
289 deviation). Means and standard deviations were calculated with the Microsoft Office Excel
290 program. Analyses of variance (ANOVA) was performed with a significance level set to $P <$
291 0.05. Statistical analysis was performed considering both storage time (0 to 75 days) and
292 different temperatures tested (0, 5 and 10 °C). Data were statistically analysed using the
293 Statgraphics program (Statistical Graphics Corp., STSC Inc., Englewood Cliffs, NJ, USA).

294

295 **3. Results and discussion**

296 *3.1 Fresh egg pasta composition*

297 The protein (8.80 ± 0.20 g/100 g fw), lipid (2.68 ± 0.42 g/100 g fw), starch (52.8 ± 0.50 g/100
298 g fw) and salt (0.047 ± 0.007 g/100 g fw) contents of the fresh egg pasta were measured at
299 the beginning of storage (t_0). These values were in the typical range for this kind of product,
300 and differences in product formulation can affect mechanical and cooking pasta properties
301 (Alamprese et al., 2009).

302 The damaged starch content in the wheat mix used to formulate the pasta samples and final
303 products (fresh pasteurised pasta) was determined. In the wheat mix, the damaged starch
304 value was 4.80 ± 0.89 g/100 g fw, while in the pasteurised pasta samples it was 11.1 ± 1.0
305 g/100 g fw. Damage starch can be used as an index of the degree of starch gelatinisation.
306 Its evaluation is based on α -amylase and amyloglucosidase attack on the polysaccharide
307 chains. Pasteurisation process determines the partially starch granules gelatinisation with
308 more accessibility to enzyme attack. The results obtained agree with Alamprese et al.
309 (2008), confirming that damage starch content was limited in the wheat mix and pasta before
310 the thermal treatment, and it increases in the pasteurised samples.

311

312 *3.2 Moisture content, water activity (a_w), and freezable water assessed by DSC*

313 At the beginning of storage (t_0), samples moisture content was 30.6 ± 0.43 g/100 g fw.
314 Moisture content did not change significantly ($p>0.05$) during the storage period at the three
315 different temperatures or among samples (data not reported). In contrast, in all the samples,
316 a_w values decreased during refrigerated storage (**Table 1**), starting from 0.9747 ± 0.0012 at
317 time zero and reaching the lowest value of 0.9647 ± 0.0017 in the sample stored at 0 °C at
318 the end of the storage period.

319 As shown in Table 1, freezable water (FW) ranged from approximately 8.9 to 14.2% on a
320 fresh weight basis for fresh samples, while the T_{peak} for this parameter did not show
321 significant changes during storage, ranging from -0.82 to 0.38 °C (data not reported), in
322 agreement with the findings of previous research (Carini et al., 2010). Earlier studies
323 (Cencic, Franca & Dalla Rosa, 1995; Carini et al., 2009, Carini et al., 2010) showed a total
324 FW of pasteurised fresh egg pasta in the range of 22-28 g FW/100 g water and a total FW
325 of unpasteurised fresh egg pasta in the range of 35-39 g FW/100 g water. Alamprese,
326 Iametti, Rossi & Bergonzi (2005) reported that this difference is caused by heat treatment,
327 which increases the starch-protein matrix's interactions, with an increase in water bonds and

consequently a decrease in water mobility. Considering an average water content value of 30 g/100 gfw and a FW of 11.6 g/100 gfw, our results agree with values in the literature, with an average FW equal to approximately 38 g/100 g water. This parameter showed decreasing values during storage, confirming a reduction in water mobility in the system.

Considering the different storage temperatures, both a_w and FW showed lower values at the lowest temperatures adopted. According to the variance analysis, significant differences ($p<0.05$) in the FW value were found for all the storage temperature used, whereas there were significant differences for water activity among the sample stored at 0 °C and the ones stored at the other different temperatures (5 °C and 10 °C) (Table 1). While the water amount in the samples did not change significantly, the water mobility decreased, in terms of both a_w and FW, which seemed to suggest an increase in the physico-chemical interactions between water and hydrophilic compounds, mainly represented by egg proteins and starch (Pagani et al., 2007), which became increasingly consistent with a decrease in storage temperature.

Water activity is a simple physical index of the product directly related to the typology of degradative reactions that can cause the end of its shelf-life (Labuza, McNall, Gallagher, Hawkes & Hurtado, 1972). An apparent pseudo-zero-order reaction adequately modelled the kinetic behaviour of a_w during storage, and the rate constants (k) are reported in **Table 2**. Many studies have used the apparent pseudo-zero-order reaction to model the evolution of different parameters in different food products (Strecker, Cavalieri, Zollars & Pomeranz, 1995; Zardetto, Dalla Rosa & Di Fresco, 2003; Shin et al., 2001). The a_w reduction rate increased significantly ($p<0.05$) from 0.000799 at a 10 °C storage temperature to 0.00124 at 0 °C. The Arrhenius kinetics adequately described the temperature dependence of the a_w reduction rate in the studied temperature range. By applying the Arrhenius model to a_w rate data at the three different temperatures and performing linear regression analysis ($R^2= 99.0$,

353 $r = -0.99$, $p < 0.05$), the activation energy (E_a) was calculated, and the result was equal to -
354 28 kJ/mol.

355

356 3.3 Gelatinisation degree and starch gelatinisation assessed by DSC

357 Starch gelatinisation degree at the different temperatures increased during storage, as
358 reported in **Table 3**. These values were found to be adequately modelled by an apparent
359 first-order reaction, and rate constants (k) were calculated ($r > 0.98$; $R^2 > 97$; $p < 0.01$). The
360 calculated rate constants (k) (days^{-1}) decreased with an increase in storage temperature
361 (0.00870 ± 0.00066 at 0°C , 0.00735 ± 0.00066 at 5°C and 0.00610 ± 0.00032 at 10°C),
362 suggesting that the gelatinisation index of starch increased as the storage temperature
363 decreased. Notably, the chemical method used to determine starch's gelatinisation index
364 was a spectrophotometric assay of the amylose complex-formed iodine. The iodine atoms
365 are included in the space inside the helix axis, forming a complex of intense blue colouring.
366 Amylopectin, the other component of the starch, reacts less intensively with the iodine
367 atoms, binding at a frequency less than 0.2% (Salisbury & Ross, 1995). As reported by Dalla
368 Rosa et al. (1989), in the case of retrogradation in bread, the variation in the gelatinisation
369 index determined by the iodometric method was related to a lower or greater "availability" of
370 amylose in the matrix, rather than a true decrease or increase in the starch gelatinisation
371 degree. Therefore, the higher gelatinisation degree of starch in samples stored at 0°C could
372 be related to less interrelation between starch and gluten in the pasta matrix, determining
373 an increase in amylose availability. The activation energy (E_a) was determined from the
374 slope of the Arrhenius plot ($R^2 = 99.2$; $r = 0.998$), and it was found to be -4.9 kcal/mol. This
375 result is similar to the one obtained by Baik et al. (1997) for rice starch during storage at
376 various temperature.

377 The enthalpy energy (J/g) involved in the gelatinisation process (Table 3) disagrees with the
378 results obtained for the chemical gelatinisation index, not showing a significant trend or

379 differences among temperatures during storage ($p>0.05$). The peak gelatinisation
380 temperature (T_{peak}) increased significantly ($p<0.05$) during storage in the analysed
381 samples (Table 3), in agreement with the observations of the gelatinisation index. In contrast
382 to enthalpy energy, T_{peak} seemed to be influenced by storage temperature with a
383 significantly difference between sample stored at 0 °C and 10 °C. Similar findings were
384 reported by Liu et al. (2020). This study conducted in heat-gelatinised wheat starch showed
385 that the peak gelatinisation temperature increased with gelatinisation index. The increasing
386 peak gelatinisation temperature was determined by less stable crystallites, which were
387 disrupted first, followed by more stable crystallites at high temperature.

388 In our experimental conditions, the differences in the partial restructuration/retrogradation of
389 starch and starch complexes (Ciésła & Eliasson, 2003) promoted by the different storage
390 temperatures seemed to mainly affect the gelatinisation index and the temperature of
391 gelatinisation/melting of starch as evaluated by DSC, rather than the enthalpy of
392 gelatinisation/melting.

393

394 *3.4 Physical-chemical changes*

395 The oxidative stability of the fresh egg pasta was monitored during storage at different
396 temperatures (data not reported). The primary oxidation products of lipids (peroxide value,
397 PV) and the secondary products of the oxidation processes (TBARs) were assessed. As
398 expected, PVs increased during storage, from an initial value of 1.88 ± 0.21 meq O₂/kg lipids
399 to approximately 3.0 meq O₂ /kg lipids after 75 days of storage, but the storage temperature
400 increase did not influence the PV. The same behaviour was observed for TBARs analysis,
401 which showed an increase from 0.17 ± 0.10 to approximately 0.63 ± 0.01 mg of MDA/kg
402 lipids at the end of the storage time for all the samples. The obtained data were in
403 accordance with those of Zardetto et al. (2014), who reported a PV equal to 2.69 meq O₂
404 /kg lipids and a TBARs value of 0.133 mg of MDA/kg lipids in fresh egg pasta.

405 A statistically significant modification ($p < 0.05$) of the colour parameters luminosity (L^*) and
406 hue angle (h°) of fresh pasta samples was measured from t_0 to t_{15} , independent of the
407 storage temperature adopted (**Table 4**). Specifically, the L^* values revealed a slight
408 increase, while the chromatic parameter h° decreased by approximately two points for all
409 the temperatures adopted. In terms of overall colour, during the first 15 days of storage, the
410 samples became slightly clearer and displayed a more visible yellow component. After this
411 initial change, all the samples maintained similar values until the end of the storage period.
412 Figure 1 shows the hardness values measured for fresh egg pasta samples stored at
413 different temperatures. The hardness values increased significantly ($p < 0.05$) during storage
414 and showed a significant positive correlation ($p < 0.05$) with storage temperature for all
415 samples. Samples stored at 5 and 10 °C showed increases in hardness during storage,
416 while at 0 °C, this parameter was almost stable until the end of storage. The pasta samples
417 stored at 10 °C showed higher values than the other samples. Alamprese et al. (2005)
418 reported that, for fresh lasagne, this toughness was attributable to the formation of a more
419 compact protein network, which offered more excellent resistance to the application of
420 tensile force.

421 Hardness modelling showed zero-order kinetics (the calculated rate constants (k) (N/day)
422 were 0.086 ± 0.067 at 0 °C, 0.214 ± 0.024 at 5 °C and 0.266 ± 0.054 T 10 °C), and the estimated
423 activation energy (E_a), as calculated by the Arrhenius equation, was 28 kJ/mol ($R^2 = 99.9$,
424 $r = 0.999$; $p < 0.01$).

425

426 3.5 Changes of cooking properties

427 The water absorption index (WAI) and the cooking organic matter loss (TOM) of pasta
428 samples were assessed. The WAI, evaluated in terms of the increase in pasta weight after
429 cooking, decreased during storage in all samples, as reported in **Figure 2**. Previous studies
430 have indicated that the WAI increase during cooking is an index of starch–protein matrix

quality. During pasta cooking, the protein network limits water diffusion and the swelling of the starch granules in the central zone of the pasta (Fardet et al., 1998). The WAI decrease in pasta samples during storage was probably related to an increasingly closed starch-protein matrix during storage, which prevented water absorption during cooking. There was a significantly ($p<0.05$) time-dependent decrease of WAI during all samples storage. On the other hand, no effect of storage temperature on WAI was observed.

At all the storage temperatures, the matter lost in the rinsing water (TOM) showed a decrease during the first two weeks of storage, after which this parameter did not change significantly until the end of the storage period (data not reported). The samples stored at 10 °C showed a smaller TOM amount at all the sampling times than the samples stored at 5 and 0 °C, even if they absorbed more water during cooking. Zardetto & Dalla Rosa (2009) reported that the WAI was inversely correlated with TOM in sheet-rolled pasta, attributing this phenomenon to the presence of a stronger protein network, which improved the trapping of starch granules. Therefore, rolled pasta achieved better product hydration without increased matter loss in boiling water (Zardetto & Dalla Rosa, 2009). The TOM index is also considered to estimate the stickiness of cooked pasta indirectly, and it is usually negatively correlated with hardness. A less sticky cooked pasta (with a lower TOM index) presents a better molecular structure of the starch-protein network on the surface, and as a consequence, better overall quality (Cubadda, Carcea, Marconi & Trivisonno, 2007). In our experiments, the pasta samples average TOM value ranged from 0.6 to 0.8 g /100 gdm and did not change significantly during storage. This value was higher than the average value for sheet-rolled pasta (0.487 g/100 gdm) reported by Zardetto & Dalla Rosa (2009).

3.6 Near-infrared spectroscopy and changes assessed by FT-NIR

455 The spectroscopic data are shown in **Figure 3**. The FT-NIR spectra of pasta samples
456 collected during storage were dominated by peaks attributed mostly to water (1450 and 1940
457 nm).

458 Using PLS regression, a NIR model was obtained to predict the values across storage in
459 fresh pasta samples at the different storage temperatures (0, 5 and 10 °C). A total of 120,
460 102 and 108 samples were considered during the calibration and validation procedure for
461 samples stored at 0, 5 and 10 °C, respectively, using a randomised procedure. A total of
462 167 samples were assigned to the calibration set, and the remaining 163 samples
463 constituted the validation set. The statistical results for calibration and validation are
464 summarised in **Table 5**, and as an example, the FT-NIR predicted values compared with
465 observed values for storage times of fresh pasta samples stored at 0 °C are shown in **Figure**
466 **4**. The standard error of estimation (SEE) ranged from 1.7 to 4.4, while the regression
467 coefficient of correlation (r) ranged from a minimum of 0.984 for samples stored at 10 °C to
468 a maximum of 0.997 for the samples at 0 °C. The validation samples were predicted with a
469 SEP and r of approximately 4 and 0.98, respectively, and the calculated BIAS was minimal
470 (0.02). The model developed used 12 PLS factors, which explained the spectral variation.

471 One PLS across only three spectral regions made positive contributions to the regression
472 equation for 1000-1282, 1514-1853 and 2082-2276 nm. PLS loading plots showed the
473 regression coefficients of each wavelength of the analysed spectra indicating which
474 wavelengths were essential for model development. Wavelengths corresponding to
475 significant variations in the loading plots were associated with areas of spectra of known
476 chemical origin. **Figure 5** shows the PLS loading spectra for the 1st (A) and 2nd (B) factors
477 containing the most spectral information. The spectra of fresh pasta samples show that the
478 main absorption bands involved were 1020, 1190, 1430, 1590, 1728, 1930, 2100 and 2280
479 nm, with different intensities depending on the adopted storage temperature of the samples.

480 The absorption bands at 1930, 1440 and 1190 nm are related to water and its interactions

with the other components. Miyazawa, Terazawa, Kawano & Maekawa (2005) investigated the hydration structures of gelatinised and retrograded wheat starch by NIR, finding that the absorbance peak at 1930 nm was ascribable to water and its bond with starch, while peaks at 2092 and 2280 nm were ascribable to the combination bands from starch molecules. Other studies on wheat flour reported that gluten hydration drives an increase in absorbance at 2280 nm, while starch gelatinisation corresponds to variation at approximately 1700 nm (Delwiche, Pitt & Norris, 1991; Millar, Robert, Devaux, Guy & Maris, 1996).

During the fresh egg pasta storage, all its main components (water, starch and gluten) were involved in structural modifications, with some overlapping bands detected by FT-NIR. These overlapping prevented the identification of each specific role of pasta component and their eventual interactions. In fact, as an example, the band at approximately 1450 nm corresponds to the first overtone of the O–H stretch vibrations for water, but that at 1460 nm corresponds to gluten, and that at 1450 nm corresponds to starch, both of which are biopolymers contained in wheat flour.

FT-NIR and physico-chemical results showed that the fresh egg pasta matrix changed during storage at different temperatures, and three main components were involved: water, starch, and gluten. These modifications seemed to be more evident at 0 and 10 °C.

In **Figure 6**, the values of the factor 1 loading plot obtained from the PLS analysis for samples stored at 0 and 10 °C are reported to understand the FT-NIR results deeply. From these results, the influences of water (1940 nm), starch (1440 and 2100 nm) and gluten (2310, 2280 nm) can be obtained. For samples stored at 0 °C, the starch and water bands are prevalent compared with those in the samples stored at 10 °C. In this sample, the absorption at 1020, 2280 and 2310 nm can be associated with proteins while the peak at 2310 nm could be attributed to proteins or lipids adsorption (Osborne, Fearn & Hindle, 1993). Importantly, the samples stored at 0 °C had lower water activity values and freezable water and a higher "availability" of amylose, resulting in less consistency and more water being

absorbed during cooking, while samples maintained at 10 °C showed the opposite characteristics. These results seemed to agree with the FT-NIR results. In fact, during storage, in the fresh egg pasta matrix, structural reorganisation involving water (different water activity values and freezing water amounts), starch (different available levels of amylose and peak temperature) and proteins (different cooking behaviours and textures) happened. This reorganisation did not involve the starch retrogradation phenomenon or starch gelatinisation because the gelatinisation enthalpy assessed by DSC and starch damage index did not show significant variations. However, they still seemed to involve the links between starch and water. This change in the mobility of the starch-water system was confirmed by the data for gelatinisation temperature (T_{peak}) assessed by DSC, where all the samples showed a significant increase during storage, particularly the one stored at 0 °C. The physico-chemical results obtained for the samples stored at 10 °C, in terms of high hardness and low water absorbed during cooking, could be due to significant protein network changes, with starch granules deeply embedded in a strong protein matrix. Several authors showed that a more linked protein network is responsible for increased hardness and a water absorption decrease during cooking (Zardetto et al. 2002; Alamprese et al., 2008). To highlight this finding, FT-NIR spectra at 1440, 2100 and 2300 nm, collected during storage for samples stored at 0 and 10 °C, are reported in **Figures 7A, B and C**, respectively. The absorbance at 1440 nm decreased in both samples at approximately 56 days of storage, agreeing with the freezable water data that showed a significant decrease at this sampling time. At 1440 nm, the band corresponds to the first overtone of the starch O–H stretch vibration and hydrogen bonding between starch and water. At the same time, the band at 2100 nm showed an increase in absorbance. This band is considered to be due to O–H bonds (stretch and deformation) and C–O bond absorption of starch. These data indicated re-arrangement of starch molecules gelatinised by heat treatment during storage, which involves hydrogen bonding and links between water and O-H groups. This process is

533 associated with decreased water mobility and lower water activity values and freezable
534 water.

535 The protein modification during storage can be followed using the 2300 nm band. The
536 selected regions contain the first overtone of CH_x stretching vibrations from amino acids in
537 rehydrated gluten, as reported by Bruun, Sondergaard & Jacobsen (2007). As shown in Fig.
538 7C, the absorbance tended to decrease during the last period of storage at 0 °C, in contrast
539 to the sample at 10 °C, which increased. For both samples, the absorbance increased at
540 the 15th day of storage, probably in association with the decrease in WAI value observed in
541 all samples at the same storage time, which was likely because of the modification of starch-
542 water-protein interactions.

543

544 **4. Conclusion**

545 This study demonstrated that some physico-chemical characteristics of fresh egg pasta,
546 such as starch gelatinization degree, water activity, freezable water and hardness, were
547 influenced by the different storage temperatures. The lowest used temperature (0 °C)
548 appeared to drive the formation of a less compact protein-starch network, impacting some
549 pasta characteristics.

550 Water activity and freezable water decreased significantly during storage time, showing a
551 reduction in water mobility in the system and suggesting an increase in physico-chemical
552 interactions between water and hydrophilic compounds, mainly represented by egg and
553 wheat grain proteins and starch. This result is significant in the light of fresh egg pasta
554 stability, showing that it depends not only on water content and its thermodynamic status,
555 but also on water compartmentation in the matrix and its mobility decrease.

556 FT- NIR analysis confirmed that several modifications involve the starch-water-protein
557 matrix, with the consequent change of some macroscopic pasta properties (e.g hardness,
558 absorbed water during cooking).

559 The approach used in this study can be applied, in combination with sensorial assessment,
560 to evaluate which of our studied parameters affects sensory acceptability, such as texture
561 and/or cooking behaviour.

562 Considering that consumer sensations often do not correlate adequately with physico-
563 chemical determinations, further researches are needed to carefully evaluate sensorial
564 aspects, in order to understand which of the studied parameters is the most influent to
565 determine the acceptability limits related to the shelf-life of fresh pasta, on the basis of
566 consumer dissatisfaction.

567

568 **References**

569 Alamprese, C., Iametti, S., Rossi, M., Bergonzi, D. (2005). Role of pasteurisation heat
570 treatment on rheological and protein structural characteristics of fresh egg pasta. *European*
571 *Food Research Technology*, 221, 759-767.

572 Alamprese, C., Casiraghi, E., Rossi, M.. (2008). Structural and cooking properties of fresh
573 egg pasta as a function of pasteurisation treatment intensity. *Journal of Food Engineering*,
574 89, 1-7.

575 Alamprese, C., Casiraghi, E., Rossi, M.. (2009). Modelling of fresh egg pasta characteristics
576 for egg content and albumen to yolk ratio. *Journal of Food Engineering*, 93, 302-307.

577 Baik, M.Y., Kim, K.J., Cheon, K.C., Ha, Y.C., Kim, W.S.. (1997). Recrystallisation Kinetics
578 and Glass Transition of Rice Starch Gel System. *Journal of Agricultural Food Chemistry*, 45,
579 4242-4248.

580 Bevilacqua, M., Bucci, R., Materazzi, S., Marini, F.. (2013). Application of near-infrared (NIR)
581 spectroscopy coupled to chemometrics for dried egg-pasta characterisation and egg content
582 quantification. *Food Chemistry*, 140, 726-734.

583 Bourne, M.C..(2002). *Food texture and viscosity: concept and measurement*. Elsevier
584 Science & Technology book. Accademic Press. Cambridge, Massachusetts, US.

585 Bruun, S.W., Søndergaard, L., Jacobsen, S. (2007). Analysis of Protein Structures and
 586 Interactions in Complex Food by Near-Infrared Spectroscopy. 1. Gluten Powder. *Journal of*
 587 *Agriculture and Food Chemistry*. 55, 7234-7243.

588 Carini, E., Vittadini, E., Curti, E., Antoniazzi, F. (2009). Effects of different shaping modes
 589 on physico-chemical properties and water status of fresh pasta. *Journal of Food Engineering*
 590 93, 400–406.

591 Carini E., Vittadini E., Curti E., Antoniazzi F., Viazzani P. (2010). Effect of different mixers
 592 on physicochemical properties and water status of extruded and laminated fresh pasta. *Food*
 593 *Chemistry*, 122, 462-469.

594 Carini, E., Curti, E., Minucciani, M., Antoniazzi, F., Vittadini, E.. (2014). Pasta. In R. P. Guiné
 595 & P.M. Reis Correia (Eds.), *Engineering Aspects of Cereal and Cereal-Based Products* (pp.
 596 211-231). CRC Press, Taylor & Francis Group, New York, US.

597 Cattaneo, T.M.P., Giardina, C., Sinelli, N., Riva, M., Giangiacomo, R.. (2005). Application of
 598 FT-NIR and FT-IR spectroscopy to study the shelf-life of Crescenza cheese. *International*
 599 *Dairy Journal*, 15, 693-700.

600 C.I.E. (1987). Colorimetry. Wien, A: Central Bureaux of the Commission Internationale de
 601 l'Eclairage (2nd ed.).

602 Cencic L., Franca C., Dalla Rosa M.. (1995). Studio su pasta fresca farcita (ravioli) a diverse
 603 attività dell'acqua. *Tecnica Molitoria*, 5, 449-464.

604 Ciésła K., Eliasson A.C., (2003). DSC studies of gamma irradiation influence on
 605 gelatinisation and amylose-lipid complex transition occurring in wheat starch. *Radiation*
 606 *Physics and Chemistry*, 68, 933-940.

607 Cubadda, R.E., Carcea, M., Marconi, E., Trivisonno, M.C. (2007) Influence of gluten proteins
 608 and drying temperature on the cooking quality of durum wheat pasta. *Cereal Chemistry*, 84,
 609 48-55.

610 Costa, C., Lucera, A., Mastromatteo, M., Conte, A., Del Nobile, M.A.. (2010). Shelf life
 611 extension of durum semolina-based fresh pasta. *International Journal of Food Science &*
 612 *Technology*, 45, 1545-1551

613 Dalla Rosa, M., Lerici, C.R., Cencic, L., Pinnavaia, G.. 1989. Sul grado di gelatinizzazione
 614 dell'amido in diversi alimenti. *Tecnica Molitoria*, 40, 692-699.

615 D'Egidio, M.G., Sgrulletta, D., Mariani, B.M., Galterio, G., De Stefanis, E., Fortini, S..
 616 (1976). Quantitative evaluation of stickiness and spaghetti quality. *Tecnica Molitoria*, 27, 89-
 617 93.

618 Decker, E., 2004. Spectrophotometric Measurement of Secondary Lipid Oxidation products.
 619 In R.E. Wrolstad et. al., (Eds.), *Handbook of Food Analytical Chemistry* (pp. 547-551). John
 620 Wiley & Sons, Inc. Hoboken, New Jersey, US.

621 Delwiche, S. R., Pitt, R.E., Norris, K.H.. (1991). Examination of starch-water and cellulose-
 622 water interactions with near infrared (NIR) diffuse reflectance spectroscopy. *Starch*, 3, 85-
 623 92.

624 Decreto Ministeriale (DM) 11/09/1967 Supplemento n°4. "Italian official methods for cereal
 625 analysis."

626 Decreto del Presidente della Repubblica (DPR) 9 febbraio 2001, n. 187, "Regolamento per
 627 la revisione della normativa sulla produzione e commercializzazione di sfarinati e paste
 628 alimentari, a norma dell'articolo 50 della legge 22 febbraio 1994, n. 146" Gazzetta Ufficiale
 629 n. 117, 22/05/2001, Italy.

630 Fardet, A., Baldwin, P.M., Bertrand, D., Bouchet, B., Gallant, D.J., Barry, J. (1998). Textural
 631 images analysis of pasta protein network to determine the influence of technological
 632 processes. *Cereal Chemistry*, 75, 699-704.

633 Fodor, M., Woller, A., Turza, S., Szigedi, T. (2011). Development of a rapid, non-destructive
 634 method for egg content determination in dry pasta using FT-NIR techniques. *Journal of Food*
 635 *Engineering*, 107, 195-199.

636 International Organization for Standardization ISO 4833-1 (2013). Microbiology of the food
637 chain - Horizontal method for the enumeration of microorganisms Colony count at 30
638 degrees C by the pour plate technique.

639 ISTISAN 96/34. (1996). Analytical methods used in food chemical control. Istituto Superiore
640 di Sanità (Rome, IT)

641 Labuza, T. P., McNally, L., Gallagher, D., Hawkes, J., & Hurtado, F. (1972). Stability of
642 intermediate moisture foods. *Journal of Food Science*, 37, 154–159.

643 Liu Y., Chao C., Yu J., Wang S., Wang S., Copeland L. (2020). New insights into starch
644 gelatinisation by high pressure: Comparison with heat gelatinisation. *Food Chemistry*, 318,
645 1-7.

646 Lucera, A., Costa, C., Padalino, L., Conte, A., Lacivita, V., Saccotelli, M. A., Esposto, D.,
647 Nobile, M. A.. (2014). Combination of process technology and packaging conditions to
648 improve the shelf life of fresh pasta. *Journal of Food Processing and Technology*, 5, 403-
649 408.

650 Lund, D. 1986. Influence of time, temperature, moisture, ingredients and processing
651 conditions on starch gelatinisation. *CRC Critical Reviews in Food Science and Nutrition*, 20,
652 249-273.

653 Miyazawa, M., Terazawa, Y., Kawano, S., Maekawa, T. (2005) Use of near infrared
654 spectroscopy to investigate the hydration structures of gelatinised and retrograded wheat
655 starch. NIR Proceedings of the 12th International Conference. 463-466.

656 Millar, S., Robert, P., Devaux, M.F., Guy, R.C.E., Maris, P.. (1996) Near Infrared
657 spectroscopic measurements of structural changes in starch-containing extruded products.
658 *Applied Spectroscopy*, 50, 1134-1139.

659 Nicolaï, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, K.I., Lammertyn, J.,
660 (2007). Nondestructive measurement of fruit and vegetables quality by means of NIR
661 spectroscopy: a review. *Postharvest Biology Technology*, 46, 99–108.

662 Osborne, B.G., Fearn, T., Hindle, P.H.. (1993). *Practical NIR spectroscopy with application*
663 *in Food and Beverage*. Longman group UK Limited. England.

664 Ozaki, Y., McClure, W.F., Christy, A.A. (Eds.), (2006). *Near-infrared Spectroscopy in Food*
665 *Science and Technology*. John Wiley & Sons, Inc., Hoboken, New Jersey, US.

666 Pagani, M.A., Lucisano, M., Mariotti, M.. (2007). Traditional Italian Products from Wheat and
667 Other Starchy Flours. In Y. H. Hui (Eds.), *Handbook of Food Products Manufacturing* (pp.
668 339-354). John Wiley & Sons, Inc., Hoboken, New Jersey, US.

669 Salisbury, F.B., Ross, C.W.. (1995) *Fisiologia Vegetale*. Zanichelli. Bologna. Italy.

670 Sanguinetti, A.M., Del Caro, A., Mangia, N.P., Secchi, P., Catzeddu, P., Piga, A.. (2011)
671 *Quality Changes of Fresh Filled Pasta During Storage: Influence of Modified Atmosphere*
672 *Packaging on Microbial Growth and Sensory Properties. Food Science and Technology*
673 *International*, 17, 23-29.

674 Shantha, N., Decker, E., (1994). Rapid, sensitive, iron-based spectrophotometric methods
675 for determination of peroxide values of food lipids. *Journal of AOAC*, 77, 421-424.

676 Shin, J.H., Chung, H.L., Seo, J.K., Sim, J.H., Huh, C.S., Kim, S.K., Baek, Y.J.. (2001).
677 Degradation kinetics of capsantin in paprika as affected by heating. *Journal of Food Science*,
678 66, 15-19.

679 Singh, R. P. (1994). Scientific principles of shelf life evaluation. In C. M. D. Man, & A. A.
680 Jones (Eds.), *Shelf life evaluation of foods* (pp. 3–26). London, UK: Blackie Academic &
681 Professional.

682 Sinelli, N., Barzaghi, S., Giardina G., Cattaneo, TMP.. (2005). A preliminary study using
683 Fourier transform near infrared spectroscopy to monitor the shelf life of packed industrial
684 ricotta cheese. *Journal Near Infrared Spectroscopy*, 13, 293-300.

685 Streker, T.D., Cavalieri, R.L., Zollars, R.L., Pomeranz, Y.. (1995). Polymerisation and
686 mechanics degradation kinetics of gluten and glutenina at extruder melt-section
687 temperatures and shear rates. *Journal of Food Science*, 60, 532-537.

688 Taoukis, P.S., Labuza, T.P., Saguy, I.S., (1997). Kinetics of Food Deterioration and Shelf-
689 Life prediction. In Valentas, K.J., Rotstein, E., Singh, R.P. (Eds.), *Handbook of Food*
690 *Engineering Practice*. CRC Press, Taylor & Francis Group, New York, US

691 Temmerman, D.J , Saeys, W., Nicolaï, B., Ramon, H. (2007). Near infrared reflectance
692 spectroscopy as a tool for the in-line determination of the moisture concentration in extruded
693 semolina pasta. *Biosystems Engineering*, 97, 313-321.

694 Tsironi, T.N., Taoukis, P.S., (2011). Shelf-life extension of gilthead seabream fillets by
695 osmotic treatment and antimicrobial agents. *Journal Applied Microbiology*, 112, 316–328.

696 Vaikousi, H., Biliaderis, C.G., Koutsoumanis, P., (2009). Applicability of a microbial time
697 temperature indicator (TTI) for monitoring spoilage of modified atmosphere packed minced
698 meat. *International Journal of Food Microbiology*, 133, 272–278.

699 Williams P., Norris, K.. (2001). Near-infrared technology in the agricultural and food
700 industries. American Association of Cereal Chemistry Inc.:St. Paul MN.

701 Zardetto, S. (2004). Measuring of the degree of starch gelatinisation in fresh pasta by near
702 infrared reflectance analysis. *Tecnica Molitoria*, 7, 662-668.

703 Zardetto, S., Di Fresco, S., Dalla Rosa, M. (2002). Effetto di trattamenti termici sulle
704 caratteristiche chimico-fisiche della pasta. *Tecnica Molitoria*, 2, 113-130.

705 Zardetto, S., M. Dalla Rosa, G. Placucci, F. Capozzi. (2005) Effect of extrusion process on
706 chemical and physical properties of fresh egg pasta. *Tecnica Molitoria*, 56, 505-514

707 Zardetto, S., Dalla Rosa, M. (2006) Study of the effect of lamination process on pasta by
708 physical chemical determination and near infrared spectroscopy analysis. *Journal of Food*
709 *Engineering*, 74, 402-409.

710 Zardetto, S., Dalla Rosa, M..(2007). Effect of heat treatment on the microbiology and quality
711 of fresh filled pasta. In P. Riley (Eds.), *New Issues in Food Policy, Control and Research*
712 (pp. 45-66). Nova Science Publishers, Inc , New York USA..

713 Zardetto, S., Dalla Rosa, M. (2009). Effect of extrusion process on properties of cooked,
 714 fresh egg pasta *Journal of Food Engineering*. 92, 70-77.

715 Zardetto, S., Dalla Rosa, M., Di Fresco, S. (2003). Effects of different heat treatments on the
 716 furosine content in fresh filled pasta. *Food Research International*, 36, 887-883.

717 Zardetto, S. (2005a). Potential applications of near infrared spectroscopy for evaluating
 718 thermal treatments of fresh egg pasta. *Food Control*, 16, 249-253.

719 Zardetto, S. (2005b). Effect of modified atmosphere packaging at abuse temperature on the
 720 growth of *Penicillium aurantiogriseum* isolated from fresh filled pasta. *Food Microbiology*,
 721 22, 367-371.

722 Zardetto, S., Barbanti, D., Dalla Rosa, M.. (2014) Formation of Cholesterol Oxidation
 723 Products (COPs) and loss of cholesterol in fresh egg pasta as a function of thermal treatment
 724 processing. *Food Research international*, 62, 177-182.

725 Zardetto, S. (2017). Foreseeable storage conditions to validate product shelf life.
 726 *Professional Pasta*. 6, 14-31

727 Zuliani, R. 1998. Studio sulle capacità di reidratazione di pasta farcita. Tesi di laurea.
 728 Dipartimento di Scienze degli Alimenti, Università degli studi di Udine.

729

730

731

732

733

734

735

736

737

738

739

740

741

Table 1. Water activity (a_w) and freezable water (FW) values in fresh egg pasta during 75 days of storage at three different temperatures (0, 5 and 10°C) (average value \pm standard deviation; n= 6)

Time (days)	Storage temperature (°C)		
	0 ^a	5 ^b	10 ^{c,b}
a_w			
0	0.9747 \pm 0.0012 ^a	0.9747 \pm 0.0012 ^a	0.9747 \pm 0.0012 ^a
15	0.9698 \pm 0.0030 ^b	0.9735 \pm 0.0017 ^b	0.9710 \pm 0.0028 ^b
35	0.9660 \pm 0.0021 ^c	0.9690 \pm 0.0029 ^c	0.9690 \pm 0.0020 ^c
56	0.9647 \pm 0.0035 ^{d,c}	0.9680 \pm 0.0025 ^{d,c}	0.9688 \pm 0.0021 ^{d,c}
75	0.9647 \pm 0.0017 ^{c,d}	0.9670 \pm 0.0013 ^{e,c,d}	0.9677 \pm 0.0022 ^{e,c,d}
	0 ^a	5 ^b	10 ^c
FW (J/gfw)			
0	0.142 \pm 0.006 ^a	0.142 \pm 0.006 ^a	0.142 \pm 0.006 ^a
15	0.108 \pm 0.005 ^b	0.122 \pm 0.002 ^b	0.129 \pm 0.006 ^b
35	0.107 \pm 0.006 ^{c,b}	0.120 \pm 0.002 ^{c,b}	0.124 \pm 0.005 ^{c,b}
56	0.089 \pm 0.006 ^d	0.116 \pm 0.000 ^d	0.117 \pm 0.000 ^d
75	0.091 \pm 0.004 ^{e,d}	0.116 \pm 0.004 ^{e,d}	0.124 \pm 0.002 ^{e,d}

^a Different superscript letters within the same column indicate significant differences ($p < 0.05$)

Table 2. Kinetic parameters for water activity in fresh egg pasta as a function of storage temperature

Storage temperature (°C)	a_w kinetic rate (a _w /day)	Intercept	r	R^2 (%)	p
0	0.0012391	0.97441	0.98	96	<0.01
5	0.0009617	0.97543	0.95	91	<0.01
10	0.0007993	0.97438	0.99	97	<0.01

Table 3. Gelatinisation degree, enthalpy (ΔH) (J/gfw) and peak temperature (T_{peak}) ($^{\circ}\text{C}$) of starch gelatinisation in the fresh pasta samples as a function of storage temperature and time (average value \pm standard deviation; n=3)

Time (days)	Storage temperature ($^{\circ}\text{C}$)		
	0 ^a	5 ^b	10 ^{c,b}
Gelatinisation degree			
0	7.85 \pm 0.99 ^a	7.85 \pm 0.99 ^a	7.85 \pm 0.99 ^a
15	8.75 \pm 0.81 ^{b,a}	8.19 \pm 0.65 ^{b,a}	8.24 \pm 1.03 ^{b,a}
35	10.17 \pm 0.54 ^c	9.57 \pm 0.48 ^c	9.39 \pm 0.54 ^c
56	11.96 \pm 1.81 ^d	11.14 \pm 0.04 ^d	10.90 \pm 0.95 ^d
75	15.55 \pm 0.73 ^e	13.51 \pm 0.76 ^e	12.16 \pm 0.72 ^e
	0 ^a	5 ^a	10 ^a
Δh_{starch} (J/gfw)			
0	0.425 \pm 0.052 ^a	0.425 \pm 0.052 ^a	0.425 \pm 0.052 ^a
15	0.304 \pm 0.036 ^a	0.480 \pm 0.222 ^a	0.445 \pm 0.017 ^a
35	0.601 \pm 0.145 ^b	0.800 \pm 0.074 ^b	0.559 \pm 0.106 ^b
56	0.474 \pm 0.062 ^a	0.583 \pm 0.398 ^a	0.561 \pm 0.271 ^a
75	0.332 \pm 0.202 ^{c,a}	0.435 \pm 0.229 ^{c,a}	0.436 \pm 0.066 ^{c,a}
	0 ^a	5 ^b	10 ^b
T_{peak} ($^{\circ}\text{C}$)			
0	53.81 \pm 0.76 ^a	53.81 \pm 0.76 ^a	53.81 \pm 0.76 ^a
15	53.96 \pm 1.20 ^a	54.70 \pm 0.96 ^a	54.86 \pm 1.12 ^a
35	55.41 \pm 0.84 ^b	57.03 \pm 0.60 ^b	55.87 \pm 0.48 ^b
56	60.13 \pm 1.09 ^c	54.88 \pm 1.40 ^c	57.13 \pm 0.67 ^c
75	57.95 \pm 0.52 ^{d,c}	55.99 \pm 0.49 ^{d,c}	57.51 \pm 1.26 ^{d,c}

^a Different superscript letters within the same column indicate significant differences ($p < 0.05$)

798
799
800
801

802
803
804
805
806
807
808
809
810

Table 4. Colour parameters (luminosity: L*; hue angle: h°) in the fresh egg pasta as a function of storage temperature and time (average value ± standard deviation; n=3)

Time (days)	Storage temperature (°C)					
	0 ^a	5 ^{b,a}	10 ^{c,a}	0 ^a	5 ^a	10 ^a
	L*			h°		
0	82.7±0.5 ^a	82.7±0.5 ^a	82.7±0.5 ^a	94.2±1.1 ^a	94.2±0.1 ^a	94.2±0.1 ^a
15	83.2±0.8 ^b	83.0±0.8 ^a	83.7±0.3 ^b	91.6±1.9 ^b	91.6±0.3 ^b	92.0±0.3 ^b
35	83.4±0.4 ^{c,b}	83.5±0.1 ^{b,a}	83.4±0.8 ^c	91.7±0.9 ^b	91.7±0.2 ^b	91.4±0.8 ^b
56	83.5±0.3 ^{d,c}	83.4±0.1 ^{c,a}	83.5±0.2 ^{d,b,c}	91.5±0.9 ^b	91.6±1.2 ^b	91.7±2.5 ^b
75	84.0±0.1 ^e	83.7±0.8 ^{d,b,c}	84.1±0.4 ^{e,b}	92.2±0.9 ^b	92.0±1.5 ^b	92.2±0.4 ^b

^a Different superscript letters within the same column indicate significant differences (p < 0.05)

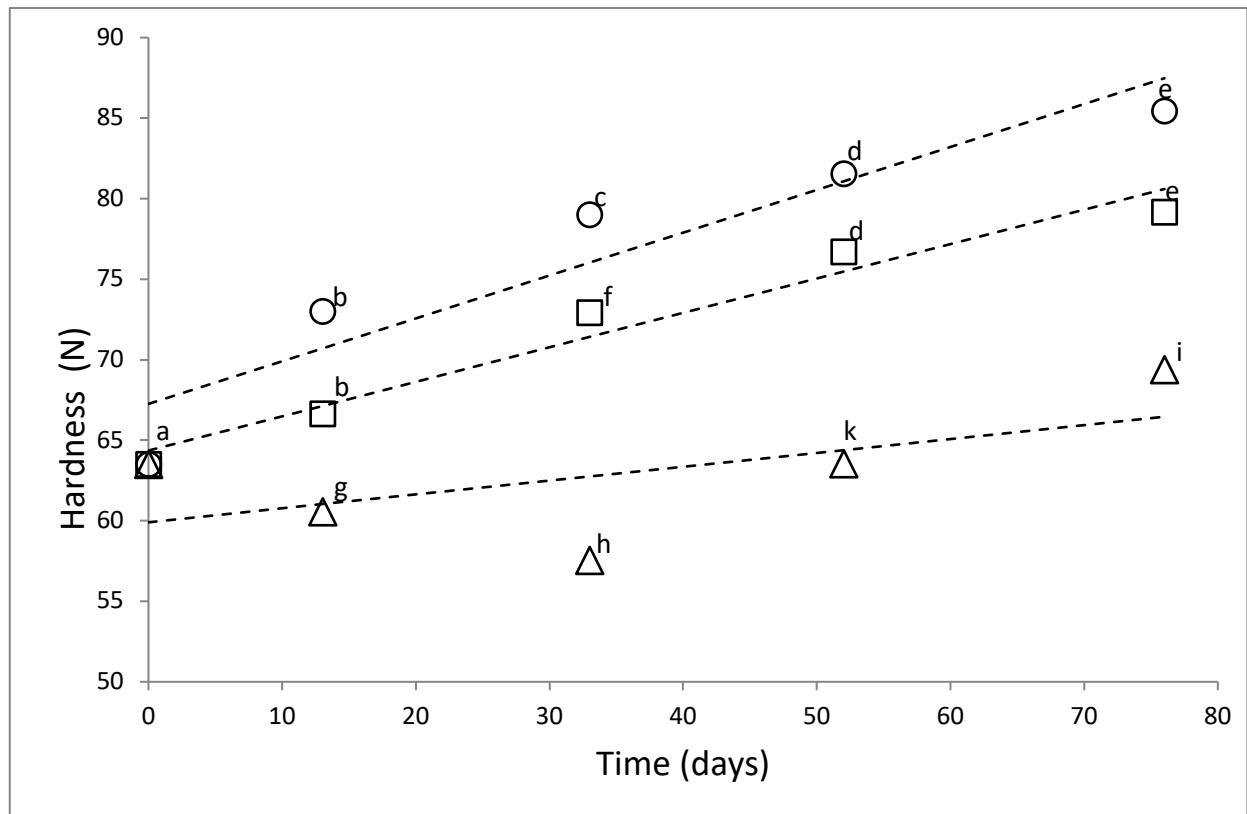


Figure 1. Hardness (N) of fresh egg pasta as a function of temperature* (10°C (circle), 5°C (square) and 0°C (triangle)) during storage (n= 5±SD). ^a Different letters indicate significant differences (p < 0.05) * Significant difference (p<0.05) were found with storage temperatures.

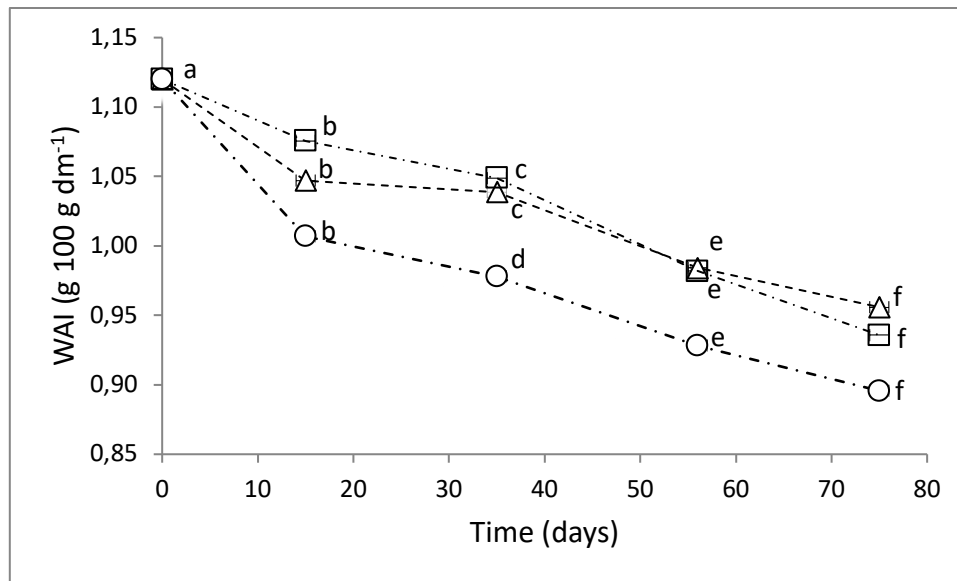
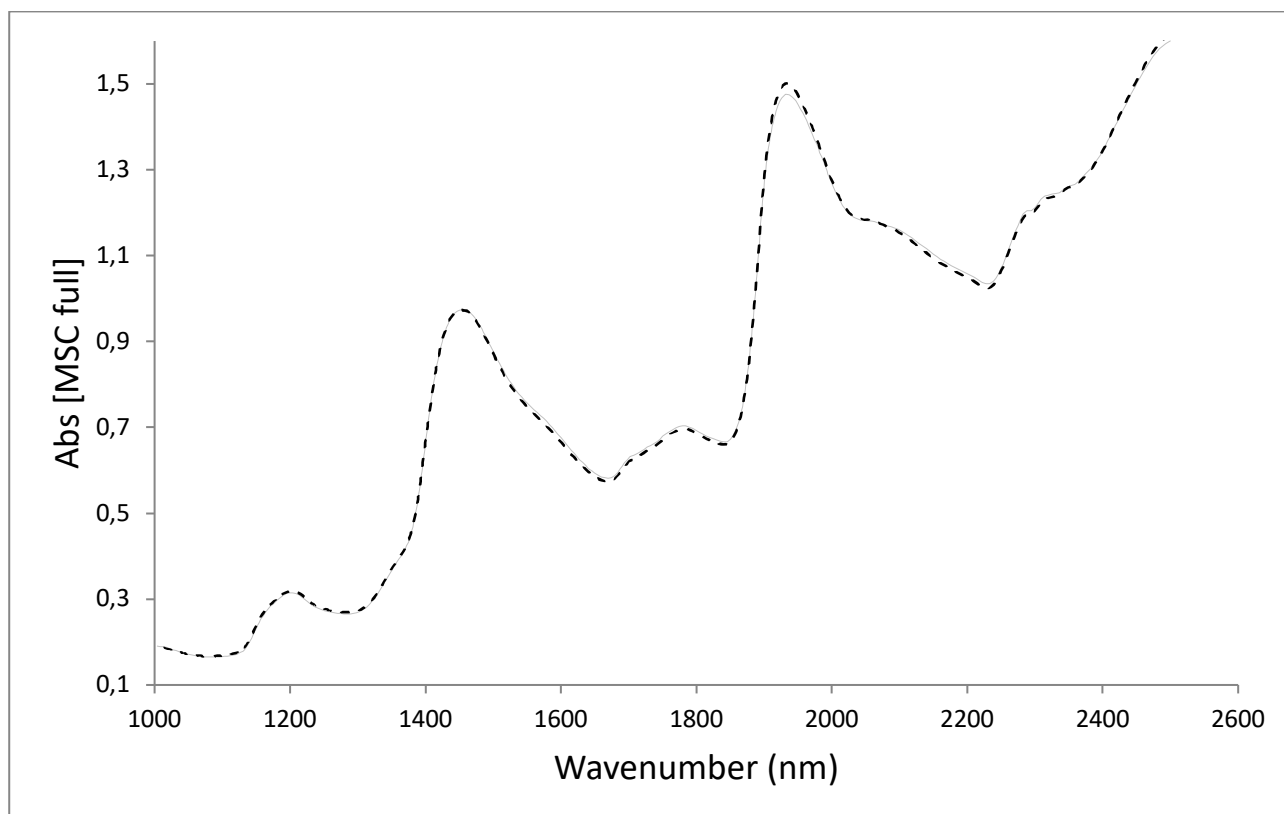


Figure 2. Water absorption index (WAI) in cooked pasta of samples stored at different temperatures* (10°C (circle), 5°C (square) and 0°C (triangle)) during storage (n= 5 ± SD). ^a Different letters indicate significant differences (p < 0.05) * No significant difference (p>0.05) were found with storage temperatures.

849
850



851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868

Figure 3: Average reflectance FT-NIR spectra of fresh egg pasta storage at 10 °C and collected at the start (dashed line) and the end of storage time (75 days)

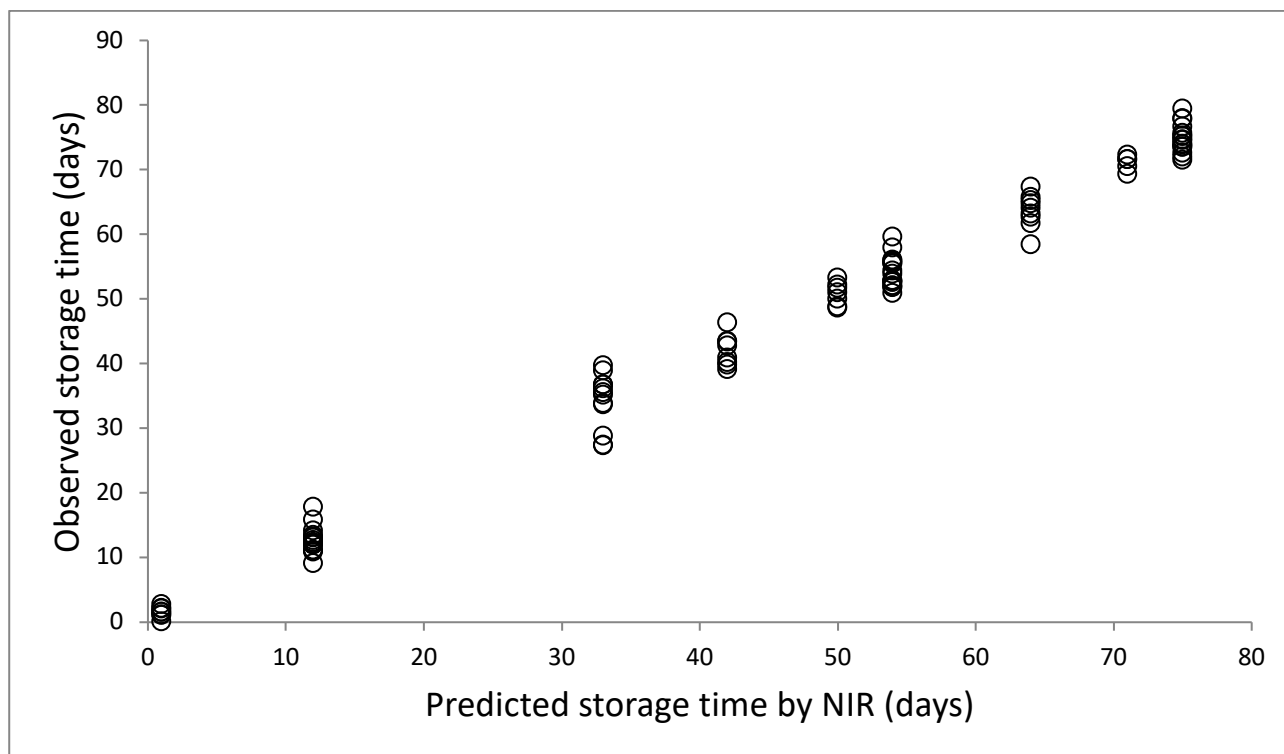
869
870
871
872
873
874
875
876

877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896

Table 5. Statistical parameters for calibration and validation procedures for the prediction of the storage time value of fresh pasta samples using FT-NIR spectroscopy (n = number of samples; r = coefficient of correlation; SD = standard deviation; SEE = standard error of estimation; SEP= standard error of prediction)

Storage temperature (°C)	Calibration				Validation				
	Statistical parameters								
	n	r	SD	SEE	n	r	SD	SD NIR	SEP
0	58	0.997	26.57	1.7	62	0.984	25.93	25.37	4.5
5	54	0.996	25.76	2.0	48	0.987	26.00	24.78	4.1
10	55	0.984	25.08	4.4	53	0.984	25.00	23.82	4.4

897
898
899
900



901
902
903
904
905
906
907

Figure 4. Regression curves of FT-NIR storage time of fresh pasta samples stored at 0°C

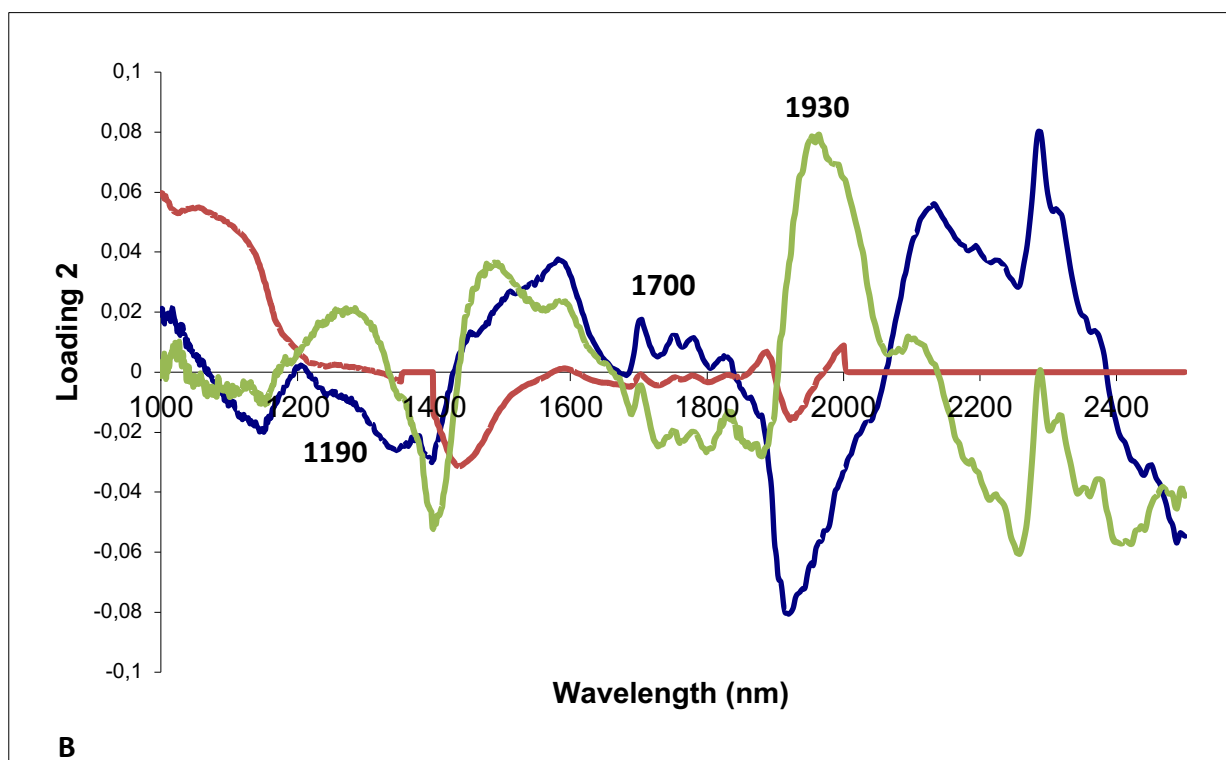
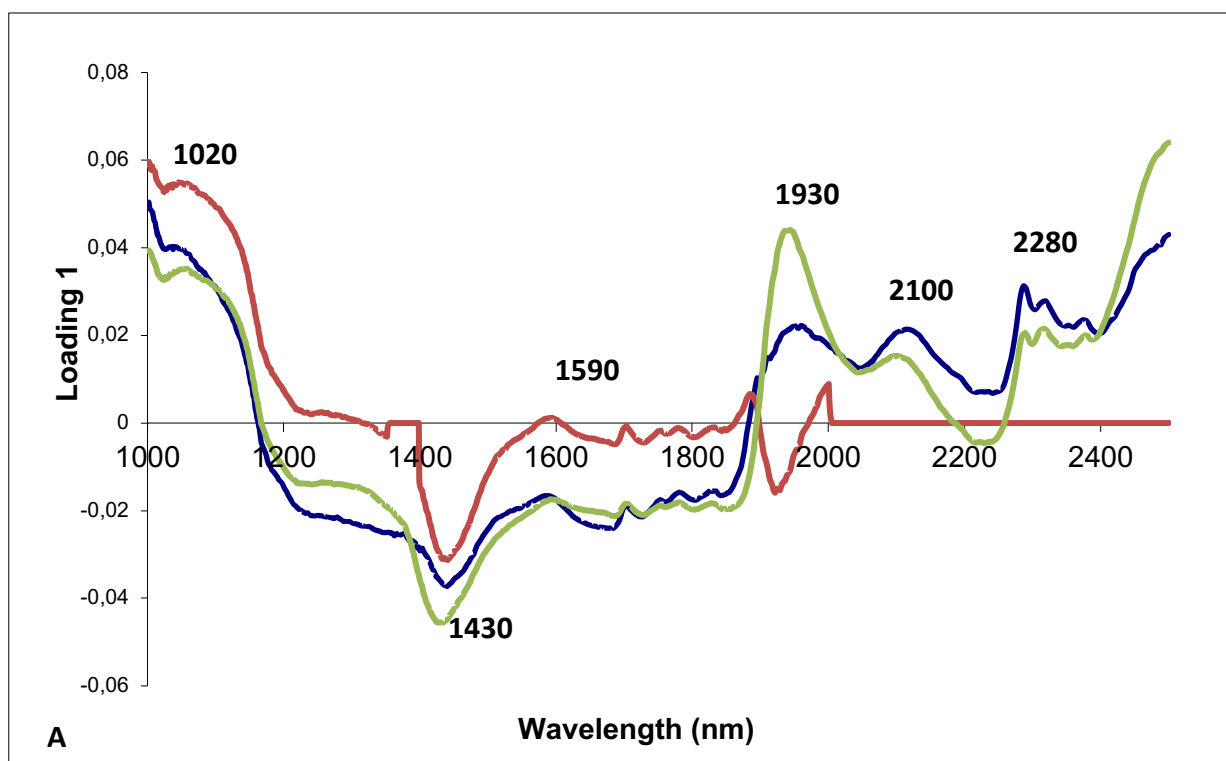
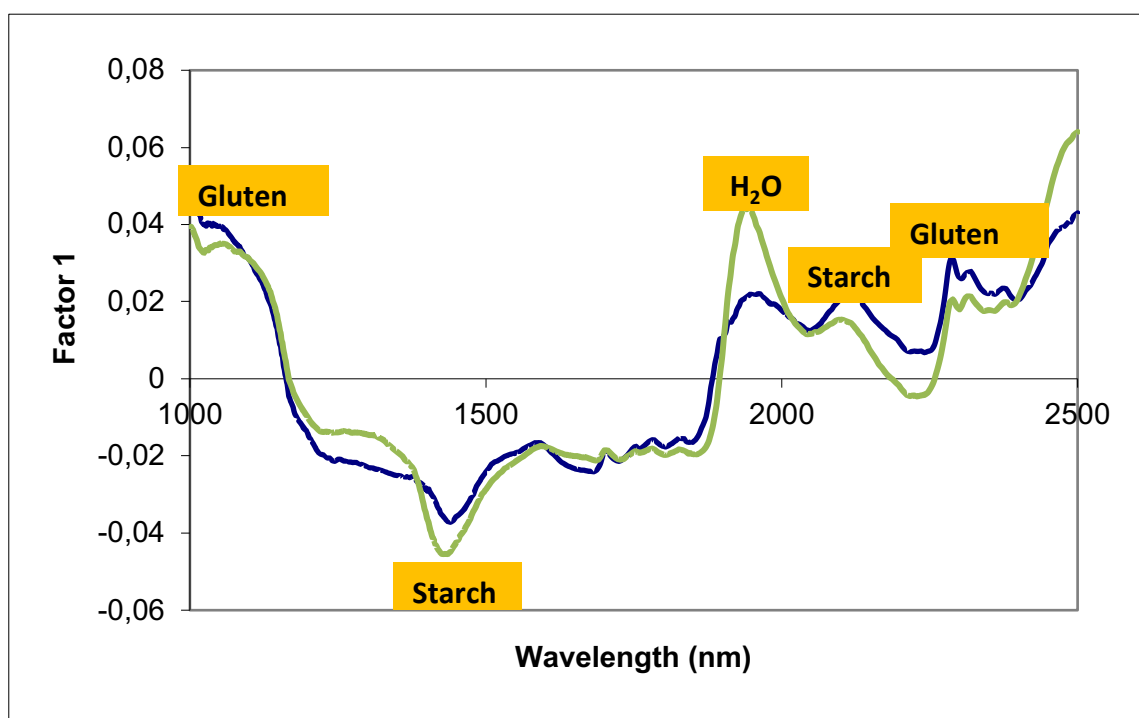


Figure 5. Loading plot for PLS1 (A) and PLS2 (B) of FT-NIR MSC FULL spectra of fresh pasta samples during storage (green samples: storage at 0°C, red: at 5°C and blue: at 10°C)

916



917

918 **Figure 6.** Loading plot of PLS factors for storage day prediction for fresh egg pasta stored
919 at 0 °C (green) and 10 °C (blue)

920

921

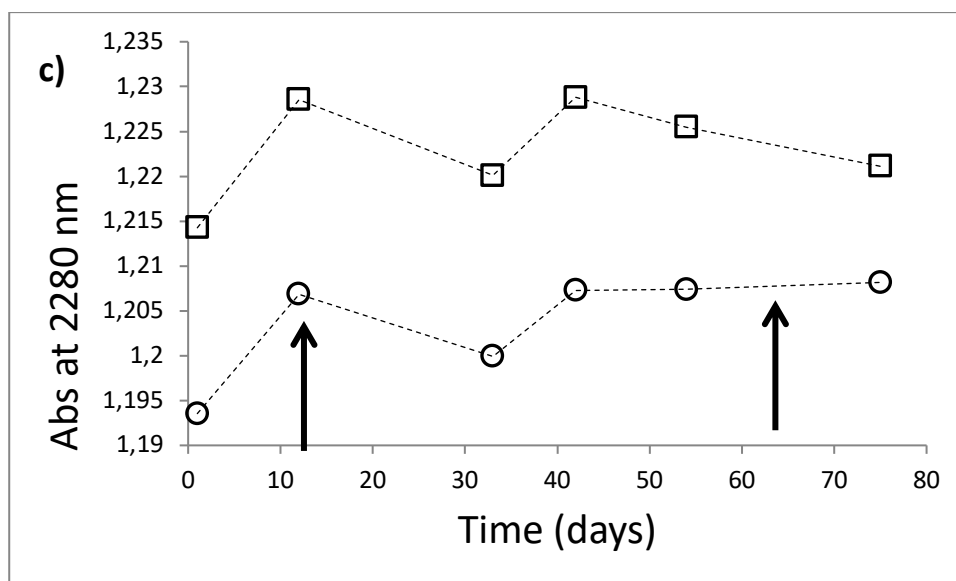
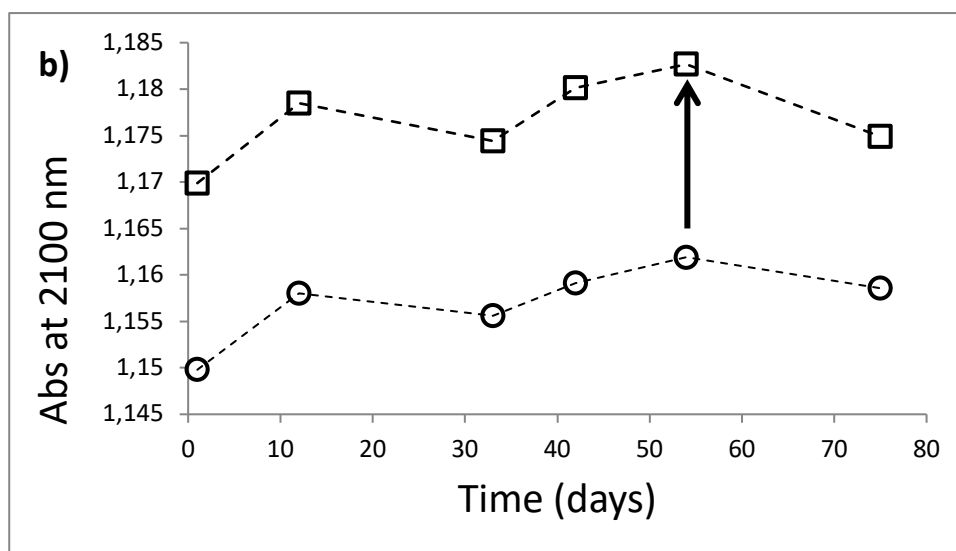
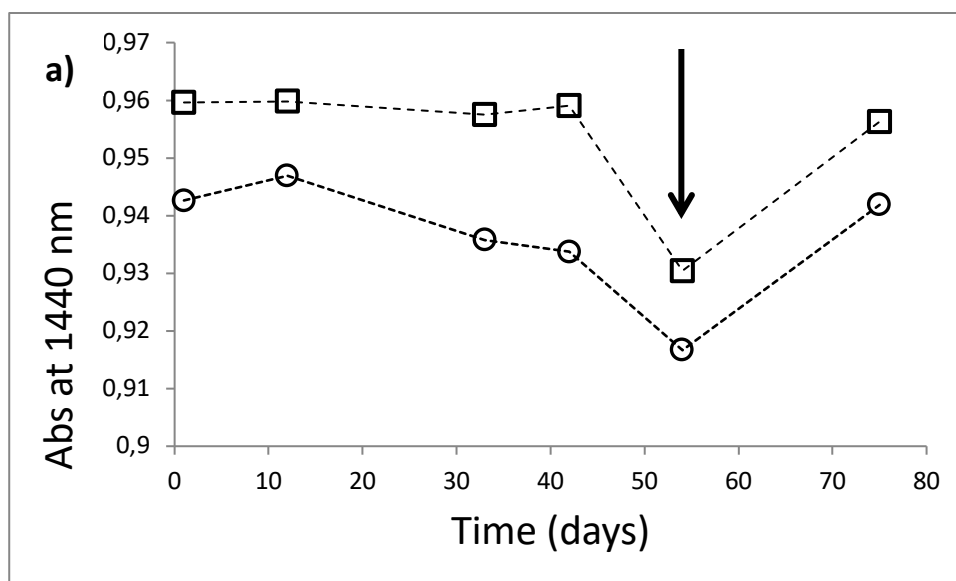


Figure 7. Loading plot of PLS factors for storage day prediction for fresh egg pasta stored at 0 °C (triangle) and 10 °C (circle) at 1440 nm (7a), 2100 nm (7b) and 2280 nm (7c)