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Improving the Health Quality of Fried Falafel (Middle Eastern Food) by Using Transglutaminase and/or Pectin Coating

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Abstract: In this study we investigated the effect of transglutaminase (TGase) addition to the falafel dough followed or not by dipping into pectin (PEC 1%) coating solution. Acrylamide (ACR), oil and water content of the fried falafel balls treated or not by TGase (5 or 20 U/g of chickpea proteins) and coated or not with PEC-containing film forming solutions were evaluated. Texture profile analyses and in vitro gastric digestion experiments were also carried out. We observed that the ACR content was reduced, compared to control sample, by 10.8% and 34.4% in the samples set up by adding 5 and 20 U TGase/g, respectively. In PEC-coated samples, ACR reduction was about 59%, 65.3% and 84.5%, in falafel balls prepared either without TGase or containing 5 or 20 U of the enzyme. However, TGase treatment does not affect oil content, while the PEC coating reduces the oil uptake by 23.5%. No difference was observed between the control sample and the one dipped in PEC regarding their texture properties while these properties were influenced in samples set up in the presence of the enzyme. Finally digestion studies demonstrated that the falafels prepared in the presence of TGase are digested in the gastric environment.

Keywords: Falafel; acrylamide; transglutaminase; pectin; oil uptake

1. Introduction

Falafel is traditionally fast and street food in Middle Eastern, also known as "ta`amiyya" in Egypt and Sudan, and it is a deep fried ball made of spiced fava beans and/or chickpeas [1,2]. Nowadays several countries like Palestine, Jordan, Lebanon, Syria and other Middle Eastern counters use chickpeas to prepare this most popular fast food that is eaten for breakfast and also for dinner. The chickpea was used as a food popular in the East since 4000 BC [3]. Falafel dough is made of a mixture of soaked ground chickpeas, parsley, onions, spices and leavened by sodium bicarbonate. The dough is shaped as balls just before deep-frying in vegetable oil until they become crusty and brown [2,4]. According to United States Department of Agriculture USDA [5], the homemade falafel contains 13.3% proteins, 17.8% total fat, 31.8% carbohydrate and it is a rich source of different minerals like calcium, magnesium, phosphorus, potassium, sodium and also vitamins such as folate, vitamin C, vitamin A.

In 2002 Swedish researcher discovered that exposure of starch containing foods to temperatures above 120 °C in a low moisture environment provokes formation of acrylamide (ACR,

H_2C=CH–CO–NH_2), that is highly soluble in water [6,7]. According to European Food Safety Authority (EFSA), ACR is produced in numerous baked and fried foods, including French fries, potato crisps, breads, biscuits, and coffee (roasted beans). EFSA scientists conclude that ACR is a health concern [8]. Moreover, the pathway of ACR formation probably involves Strecker degradation of amino acids, especially asparagine in the presence of dicarbonyl products from the Maillard reaction. Al-Dmoor et al. [4], indicated that ACR in Jordanian fried falafel (cooked for 6–8 min at 160–180 °C) have very high values ranging from 2700 to 4200 μg kg^{-1}, moreover, the same study demonstrated that the excessive use of frying oil in food preparation caused significant increases (~33%) in ACR content. Very little work has been done to decrease oil absorption in fried falafel balls and the only indications come from Abu-Alruz. [2], which assessed that increasing falafel ball size provokes a reduction of oil uptake together with a decrease of frying time.

Transglutaminases (TGase, EC 2.3.2.13) are a widely distributed groups of enzymes that crosslink protein through an acyl-transfer reaction resulting in a ε-(γ-glutamyl) lysine isopeptide bond. Recently, using TGase to improve the physiochemical properties of different food products and also edible films and coatings are rise up due to the ability of TGase to improve the crosslink network inside the food matrixes [9,10]. Due to the enzymatic cross-linking of milk proteins by TGase, yoghurt viscosity and yield stress were increased [11]. Moreover, TGase was successfully founded that is substrate for many proteins such as egg proteins [12], fish proteins [13], soy proteins [14,15], bitter vetch proteins [16], grass pea proteins [17].

Pectins (PEC) are plant cell wall structural polysaccharides composed mainly of galacturonic acid units with variations in composition, structure and molecular weight [18]. In general, PEC (E440) are used as food additive, known as thicker or stabiliser used to prepare different food products like jelly, jam, marmalades and other products, due to the their gelling properties [19]. PEC are also used in pharmaceuticals and cosmetics industry due to all these properties. Moreover, PEC applications are devoted to increase since these biopolymers have great potential and possibilities for future developments [20]. Coatings are one of the most important food preservation methods that are applied to protect highly perishable foods by creating a thin layer of edible materials onto surfaces of the products. For example, Yossef [21], find out that PEC coated strawberry fruits retained physico-chemical properties and visual quality significantly in comparison to the ones coated by soy proteins, gluten, or starch.

Healthy food is becoming the most interesting objective for several industries and for a large part of consumers since many health problems are correlated to the consumption food products. Recently Al-Asmar et al. [22], concluded that the use of PEC as a dip coating material for the French fried provokes reduction ACR formation of about 48% in comparison to uncoated samples. Moreover, Suyatma et al. [23], have studied the synergistic effect of blanching and PEC coating of fried banana chips that resulted in high ACR reduction (up to 91.9%).

The objective of this study was to evaluate the effect of both TGase and PEC-based coating solution on the ACR formation and quality of the fried falafel. Oil and water content, texture analysis profile, and in vitro gastric digestion were investigated. Results demonstrated the effectiveness of our coatings providing healthier falafel.

2. Materials and Methods

2.1. Materials

ACR standard ≥99.8%, and methanol were supplied from the Sigma–Aldrich Chemical Company (St. Louis, MO, USA). Acetonitrile HPLC analytical grade, n-hexane, and formic acid were obtained from Carlo Erba reagents S.r.l. (Cornaredo, MI, Italy). Oasis HLB 200 mg, 6 mL solid phase extraction (SPE) cartridges were from Waters (Milford, MA, USA). Syringe filters (0.45 and 0.22 μm PVDF) were from Alltech Associates (Deerfield, Italy). PEC of *Citrus* peel low-methylated (7%) (Aglupectin USP) was purchased from Silva Extracts s.r.l. (Gorle, BG Italy) and ACTIVA WM *Streptoverticillium* TGase was supplied by Ajinomoto Co. (Tokyo, Japan). All the other reagents were of analytical grade. Corn oil and chickpea were purchased from a local super market.

2.2. Preparation of PEC Coating Solutions

PEC-based solutions (1% *w*/*v*) were prepared according to Al-Asmar et al. [22] and Esposito et al. [24], from a PEC stock solution (2% *w*/*v*), then diluted with water and the pH was adjusted to 7.5. Finally, the solution was stirred for 30 min at 25 °C.

2.3. Falafel Preparation and Dipping Process

2.3.1. Falafel Dough

The dough was made of a mixture of soaked chickpeas, onion, parsley, falafel spices, salt and sodium bicarbonate. The mixture was blended for 2 min by water. Then the falafel balls were formed by using a special scoop, and fried as described below.

2.3.2. Falafel Dough Treated with TGase

After prepared the falafel dough as described above, TGase (5 and 20 U/g protein) was added to the dough after incubation at 37 °C for 2h, the falafel balls were formed and fried. The control falafel samples were obtained without TGase but treated under the same conditions.

2.3.3. PEC Dipping

The falafel dough treated or not with TGase was frozen at −20 °C for 2 h, then dipped in 1% PEC. The control was dipped in water, then frozen again for 30 min then fried.

2.4. Frying Process

Falafel balls were fried at 180 ± 5 °C for 5 min, by using 2 L of corn oil, using a deep-fryer apparatus (GIRMI, Viterbo, Italy) [22,25]. The oil was replaced with fresh one for each group. Each fried group were flipping from side to side every 2 min. After frying, each sample was allowed to drain for 2 min to remove the excess oil [22].

2.5. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

For SDS-PAGE of falafel balls, an aliquot of 250 μL of sample buffer (15 mM of Tris–HCl, pH 6.8, containing 0.5% (*w*/*v*) of SDS, 2.5% (*v*/*v*) of glycerol, 200 mM of β-mercaptoethanol, and 0.003% (*w*/*v*) of bromophenol blue) was added to 25 mg of falafel dough and/or fried falafel (either untreated or treated with TGase) and analyzed by 12% SDS-PAGE. The samples were heated at 100 °C for 5 min, and then centrifuged for 10 min at 13,000× *g*. 10 μL of each supernatant were analyzed by SDS-PAGE (12%). SDS-PAGE was performed as described by Laemmli [26], at constant voltage (80 V for 2–3 h), and the proteins were stained with Coomassie Brilliant Blue R250 (Bio-Rad, Segrate, Milan, Italy). Bio-Rad Precision Protein Standards were used as molecular weight markers.

2.6. In Vitro Gastric Digestion

Fried falafel balls either treated or not by (20 U/g protein) TGase were subjected to *in vitro* digestion by using adult model [12,27,28], under gastric physiological conditions. For our analyses, 100 mg of each sample were incubated with 4 mL of Simulated Salivary Fluid (SSF, 150 mM of NaCl, 3 mM of urea, pH 6.9) containing 75 U of amylase enzyme/g protein for 5 min at 37 °C and 170 rpm. The amylase activity was blocked by adjusting the pH at 2.5. Afterwards the samples were subjected to gastric digestion as described by Giosafatto et al. [12] with some modifications. Briefly, 100 μL of Simulated Gastric Fluid (SGF, 0.15 M of NaCl, pH 2.5) were placed in 1.5 mL microcentrifuge tubes and added to 100 μL of oral phase then incubated at 37 °C. Therefor, 50 μL of pepsin (0.1 mg/mL dissolved in SGF) were added to start the digestion reaction. At intervals of 1, 2, 5, 10, 20, 40, 60 min, 40 μL of 0.5 M of ammonium bicarbonate (NH_4HCO_3) were added to each vial to stop the pepsin reaction. The control was set up by incubating the sample for 60 min without the protease. The samples were then analyzed using the SDS-PAGE (12%) procedure described above.

2.7. ACR Standard Preparation

The standard stock solution (1.0 mg/mL) was prepared as described by Al-Asmar et al. [22]. Then it was diluted at different concentrations (100, 250, 500, 1000, 2000, 3000, 4000, 5000 and 10000 μg/L), respectively. All series of standard solutions were stored in glass dark bottles (light-resistant) at 4 °C until used.

2.8. Extraction of ACR from the Falafel Balls

ACR extraction from fried falafel balls was carried out according to Al-Asmar et al. [22], with some modifications. About 160 g of fried falafel balls, after cooling, were immersed in hexane for 30 min to remove the oil from their surfaces [29]. The falafel balls were then grinded by using a rotary mill (Grindomix GM200, Retsch GmbH, Haan, Germany) at speed of 1300 rpm for 1 min. Each sample was allowed to dry by freeze-drying before being subjected to ACR extraction following the protocols reported by Wang et al. [30] and Krishna et al. [31]. Two different falcon tubes were set up for each sample, one for detecting ACR formed in the sample itself, and the second one to carry out the recovery test. In both tubes 1 g of grinded sample was put and only in the second one 100 μg/L of ACR standard were added. 50 μL of Carrez reagent potassium salt and 50 μL of Carrez reagent zinc salts were added to each sample. In each tube 10 mL of HPLC water were added. The samples were extracted in an incubated shaker for 30 min at 25 °C and 170 rpm, then followed by centrifugation at 8000 rpm for 10 min at 4 °C. The supernatant was filtered through a 0.45 μm syringe filter for Oasis HLB SPE cartridges clean-up. The SPE cartridge was conditioned with 2 mL of methanol followed by 2 mL of HPLC water before loading 3 mL of filtered supernatant, the first 0.5 mL were discarded and the remaining elute were collected (≈1.5 mL). The all different extracts were kept in dark glass vials at 4 °C before analysis. The clean sample extracts were further filtered through 0.2 μm nylon syringe filters, and analyzed by TOF LC-MS. Each analysis was performed in triplicate.

2.9. LC-MS Analysis for ACR Content of Fried Falafel

The determination of ACR concentration was performed using the Agilent 6230 TOF-LC/MS coupled to series HPLC system, a vacuum degasser, binary pumps, and a temperature-controlled column oven at 30 °C. The following MS parameters were used: positive ion mode, nebulizer pressure 35 psi, drying gas (N_2) 5 L/min and 325 °C, capillary voltage 3500 V and fragmentor 175 V.

The column used was a Synergi™ 4 μm Hydro-RP 80 Å HPLC Column 250 × 3 mm [22,32] (from Phenomenex, Torrance, CA, USA). The operating conditions were as follows: the mobile phase was a gradient elution: mixture water/acetonitrile (97/3, *v*/*v*) containing 0.10% (*v*/*v*) formic acid Solvent A and Solvent B was acetonitrile containing 0.10% (*v*/*v*) formic acid. The program elution was applied as follows: 100% A (0% B) for 8 min, increased to 80% B (20% A) from 8 to 15 min, and kept at 80% B (20% A) for 10 min, increased to 100% A (0% B) from 25 to 30 min, and kept at 100% A for 5 min, at flow rate 0.4 mL/min. The injection volume was 20 μL. The total chromatographic runtime was 35 min for each sample; ACR elutes at retention time 3 min, then the peak identification was based on the Extracted Ion Chromatogram (EIC); by selecting ion at *m*/*z* 72, calibration curves were obtained by plotting the peak area of ACR versus concentration of ACR (range of concentration: 0.05–10 mg/L). The equation was obtained by applying the linear regression was $y = 102.21x - 10.706$, with a R^2 equal to 0.9991; this equation was used to calculate the amount of ACR in all analyzed samples.

2.10. Oil Content

The oil content of each fried falafel balls were grinned into pieces of (3–5 g). The oil content was measured gravimetrically in triplicate, by using Soxhlet method [33], and reported as a percentage on dry matter weight.

2.11. Water Content Analysis

Water content of each fried falafel ball samples were measured gravimetrically in triplicate, according to AOAC [34].

2.12. Texture Profile Analysis (TPA)

Texture profile analysis of falafel balls for each sample was carried out as described by Rossi Marquez et al. [25] with some modifications. In particular, each falafel balls sample was analyzed using an Instron universal testing instrument model No. 5543A (Instron Engineering Corp., Norwood, MA, USA) equipped with a 2 kN load cell in compression mode with a cylindrical probe (55 mm in diameter). The instrumental TPA described by Bourne [35], was used. The test was configured so that the three TPA parameters, hardness, chewiness and gumminess, were calculated at the time of the test by determining the load and displacement at predetermined points on the TPA curve. Pre- and post-test speeds were 2.0 mm/s, while test speed was 1.0 mm/s. Samples, prepared as described above, were centered and compressed to 30% of deformation. Hardness (N) was derived from the positive peak obtained at the first compression of the product or a maximum exhibited compression force. Chewiness (N mm) was the mathematical product by the software Bluehill (version 2.21) from the hardness, cohesiveness, and springiness [36]. Gumminess (N) was calculated by the software Bluehill automatically by multiply the hardness with the cohesiveness that is a ratio of the positive force areas under first and second compressions. All the TPA analyses were carried out with at least eight balls per each treatment.

2.13. Statistical Analysis

All the experiments were performed three times, and the data were analyzed by using the JMP version 10.0 software (SAS Institute, Cary, NC, USA). Statistical differences were considered using the Tukey-Kramer HSD test to be significant at ($p < 0.05$).

3. Results and Discussion

3.1. Modification of the Protein Component of Falafel Balls by Means of TGase

Adding different concentrations (0, 5 and 20 U TGase/g chickpea protein) to the falafel dough and mixed very well, after incubation for (2 h at 37 °C) we analyzed the SDS-PAGE (12%), for the dough and also for the falafel after frying. The pH value of the falafel dough was 7, treatment that does not contain TGase was the control for this experiment. Figure 1, demonstrated that TGase (5 and 20 U/g protein) were able, under these experimental conditions, to modify chickpea proteins and this modification does not change during frying. The results indicated that chickpea proteins in a good substrate for the TGase. Moreover, the large polymers were clear demonstrated by increasing the TGase concentration to (20 U/g protein) and disappearance of protein bands with a lower molecular mass (Figure 1, Panels A and B).

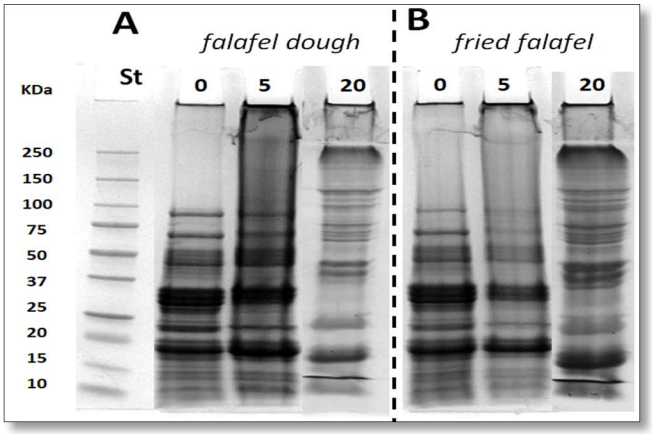

Figure 1. Panel A: SDS-PAGE of falafel dough without TGase (lane 1), with 5 U/g TGase-treated (lane 2) and 20 U/g TGase-treated (lane 3); Panel B: SDS-PAGE of fried falafel without TGase (lane 1), with 5 U/g TGase-treated (lane 2) and 20 U/g TGase-treated (lane 3); St, Molecular weight standards, Bio-Rad. (Panel A) falafel dough; (Panel B) fried falafel.

3.2. Effect of TGase and/or 1% PEC Coating Solution on the ACR Content of Falafel Balls

According to EFSA scientists report, ACR is an health concern, ACR is formed during frying, baking or roasting starchy rich food and also in the food contain higher protein concentration and sulfhydryl groups [37,38]. Traditional fried falafel balls were analyzed for their ACR content, which was equal to 7229 μg/kg. Whereas, the falafel prepared by adding 5 or 20 U TGase/g protein, demonstrated a significant reduction of the ACR concentration, 10.8% and 34.4% respectively (Figure 2, Panel A). This reduction could be explained by two different ways: (i) by the ability of TGase to crosslink the lysine and glutamine providing a network that could retain free amino acids involved into ACR formation; (ii) the higher water content of the falafel balls prepared in the presence of TGase compared to the water content in falafel prepared in the absence of the enzyme (Figure 3). In fact, in our previous paper Al-Asmar et al. [22] we have demonstrated that, in French fries, higher moisture content was lowering ACR content.

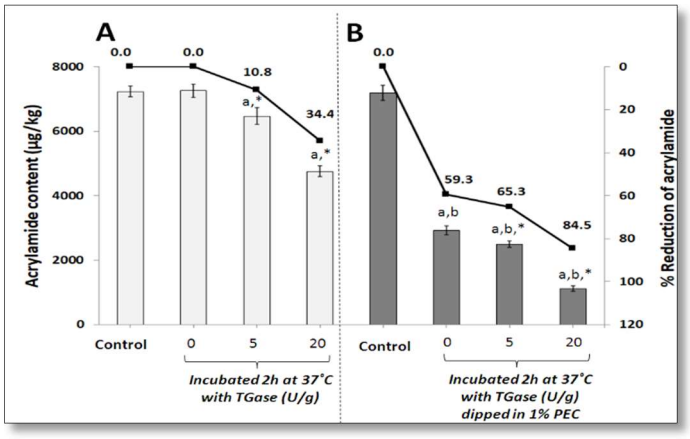

Figure 2. Effect of different concentrations of TGase on ACR content of fried falafel prepared without dipping (Panel A) or with dipping into 1% PEC-based coating solution (Panel B). The ACR content was determinated on fat-free dry matters and reported as percentage of ACR reduction of fried falafel balls. The columns significantly different from those obtained by analyzing the control are indicated by "*a*", the columns indicated by "*b*" were significantly different from those obtained without dipping, whereas the columns indicated by "*" were significantly different from those prepared with different TGase concentrations (Tukey means comparison, $p < 0.05$). Control of Panel A represents ACR content in fried traditional falafel. Control of Panel B represents ACR content in fried falafel dipped in water.

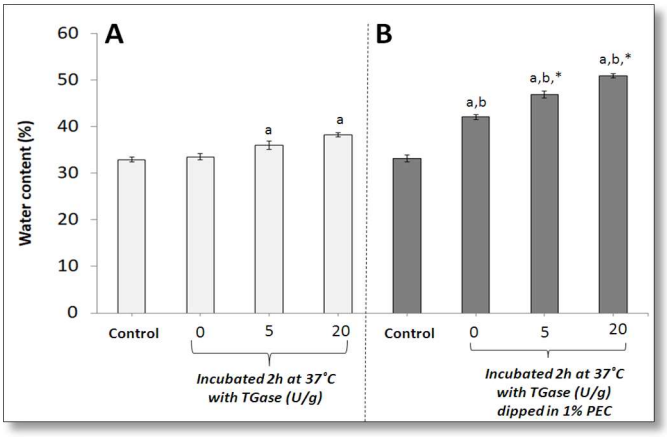

Figure 3. Effect of different concentrations of TGase on water content of fried falafel balls prepared without dipping (Panel A) or with dipping into 1% PEC coating solution (Panel B).The columns significantly different from those obtained by analyzing the untreated are indicated by "*a*", the columns indicated by "*b*" were significantly different from those obtained without dipping, whereas the columns indicated by "*" were significantly different from those prepared with different TGase concentrations (Tukey means comparison, $p < 0.05$). Control of Panel A represents ACR content in fried traditional falafel. Control of Panel B represents ACR content in fried falafel dipped in water.

To study the effect of coating, the ACR content was also determined in the falafel dipped in both water and 1% PEC-based solutions. The results, shown in Figure 2, Panel B, indicate that the ACR content was 40.7%, in comparison to the ACR content of falafel dipped in water or not dipped. As it is shown in both Panels A and B of Figure 2, falafel balls prepared by TGase followed by dipping into 1% PEC-based solution showed a significant reduction in ACR content in comparison to the uncoated falafel. The lowest amount of ACR (1118 μg/kg) was exhibited by falafel balls prepared by 20 U/g TGase and coated with 1% PEC-based solutions, where the percentage of the ACR reduction was 84.5%. In addition, the recovery test was performed to assess the extraction efficiency for each sample; to this aim the ACR content before and after the addition of 100 μg/L of ACR standard was determined. Percentage recovery was determined according to the following formula [22].

$$\text{Recovery}(\%) = \frac{\text{ACR (detected after standard addition)} - \text{ACR (sample)}}{\text{ACR (standard added)}} \times 100 \quad (1)$$

The results of recovery studies are showed in Table 1. The recovery values were in the range of 89%–102%, that are statistically in line with experimental deviations.

Table 1. Recovery test for ACR in all falafel types (in each sample 100 μg\L of ACR standard were added).

Falafel Type	ACR Content in Spiked Sample (μg/kg)	Recovery (%)
Traditional falafel	7329 ± 188	100
Incubated 2 h at 37 °C without TGase	7349 ± 140	94.2
Incubated 2 h at 37 °C with TGase 5 U/g	6560 ± 147	92.6
Incubated 2 h at 37 °C with TGase 20 U/g	4852 ± 217	96.8
Dipped in water	7294 ± 348	102
Incubated 2 h at 37 °C without TGase and dipped in 1% PEC	3017 ± 115	90.7
Incubated 2 h at 37 °C with TGase (5 U/g) and dipped in 1% PEC	2583 ± 65	89.3
Incubated 2 h at 37 °C with TGase (20 U/g) and dipped in 1% PEC	1219 ± 83	101

3.3. Effect of TGase and/or 1% PEC Coatings on Water and Oil Content of Falafel Balls

Analyzing the water content of fried falafel, indicated that the falafel prepared in the presence of TGase (5 or 20 U/g protein) contains more water in comparison to the falafel prepared without TGase or to the control (Figure 3, Panel A). Moreover, dipping the falafel into 1% PEC-based solution increasing the water content significantly compared to the control (Figure 3, Panel B).

Treating falafel with TGase and PEC-based solution increased the water content significantly compared to both controls. The TGase concentration (20 U/g protein) together with dipping into 1% PEC-based solution retained the highest water value. The obtained results could be explained by the TGase-mediated crosslinking that is responsible of water evaporation reduction during frying [22,25].

The oil content of the falafel balls is the main problem of such fast food products that contain about 25% oil, based on the dry weight. The oil is responsible for several health problems such as cardiovascular disease, overweight, and obesity [39]. Treated the falafels with (5 or 20 U/g protein) TGase does not show any reduction on the oil content (Figure 4, Panel A). However, the presence of PEC –based solution significantly reduces the oil content to about 23%. Many authors [25,40,41], have reported that PEC leads to a lower oil uptake. For example, in the previous paper Al-Asmar et al. [22] we have demonstrated that PEC-based coating reduces oil uptake in French fries. To our knowledge, few studies have been devoted to decrease oil absorption in fried falafel balls. Pinthus et al. [42] and Mansour. [43], have investigated the influence of added powdered cellulose and methyl cellulose or fibers or hydrocolloids, and have demonstrated their ability to reduce the oil uptake during falafel ball deep frying.

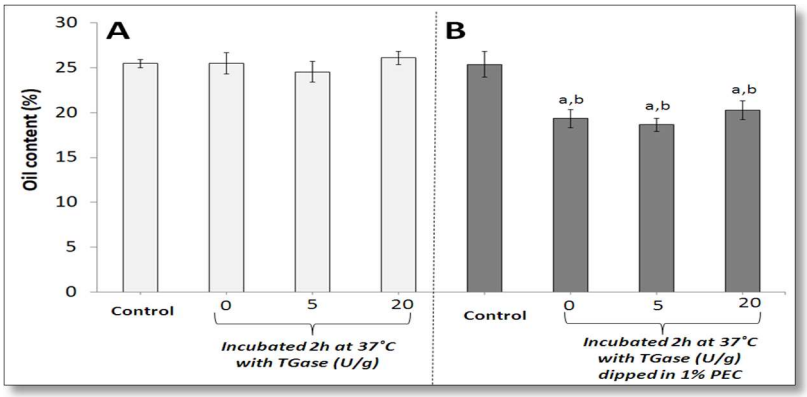

Figure 4. Effect of different concentrations of TGase without dipping (Panel A) or with dipping into 1% PEC coating solution (Panel B), on oil content prepared based on dry matters of fried falafel balls. The columns significantly different from those obtained by analyzing the untreated are indicated by "*a*", whereas the columns indicated by "*b*" were significantly different from those obtained without dipping (Tukey means comparison, $p < 0.05$). Control of Panel A represents ACR content in fried traditional falafel. Control of Panel B represents ACR content in fried falafel dipped in water.

3.4. Effect of TGase and/or 1% PEC Coatings on Texture Profile Analysis (TPA)

To verify whether or not enzyme treatment and coating could influence the quality of falafel balls, texture profile analysis (TPA) was performed. Thus, falafel hardness, chewiness, and gumminess were assessed. As shown in Table 2, falafel balls prepared with TGase showed an increasing on all three parameters. Moreover, TGase-treated falafel balls coated with 1% PEC-based solution showed as well significantly higher hardness, chewiness and gumminess values compared to the sample incubated in the absence of the enzyme and dipped into PEC-based solution.

Table 2. Texture Profile Analysis (TPA) of falafel balls prepared in the presence or absence of different concentrations of TGase dipped or not into 1% PEC solution prepared at pH 7.5.

Falafel Type	Hardness (N)	Chewiness (N mm)	Gumminess (N)
Traditional falafel	56.41 ± 5.50	184.28 ± 3.10	23.20 ± 1.20
Incubated 2 h at 37 °C without TGase	52.22 ± 5.30	180.97 ± 2.80	22.15 ± 1.20
Incubated 2 h at 37 °C with TGase 5 U/g	70.88 ± 3.25 [a]	238.44 ± 2.70 [a]	38.87 ± 1.50 [a]
Incubated 2 h at 37 °C with TGase 20 U/g	96.57 ± 4.80 [a]	280.25 ± 15.10 [a]	49.64 ± 3.80 [a]
Dipped in water	52.18 ± 3.40	178.13 ± 4.10	21.42 ± 3.01
Incubated 2 h at 37 °C without TGase and dipped in 1% PEC	58.13 ± 4.90	183.23 ± 11.69	23.29 ± 4.50
Incubated 2 h at 37 °C with TGase (5 U/g) and dipped in 1% PEC	114.31 ± 8.20 [a,b]	453.18 ± 11.30 [a,b]	67.60 ± 4.50 [a,b]
Incubated 2 h at 37 °C with TGase (20 U/g) and dipped in 1% PEC	136.07 ± 12.28 [a,b]	518.50 ± 18.05 [a,b]	78.24 ± 2.01 [a,b]

Notes: The results significantly different from those obtained by analyzing the untreated are indicated by "a", whereas the results indicated by "b" were significantly different from those obtained without dipping (Tukey means comparison, $p < 0.05$).

3.5. Effect of TGase on Digestibility of Falafel Balls

To test the effect of enzyme-treatment on digestibility of falafel, an in vitro digestion (IVD) model was used. The protocol come from FA1005 (INFOGEST) [44], IVD experiments were carried out using falafel balls prepared in the presence and absences of 20 U TGase/g. At the end of the simulated gastric digestion, samples were analyzed by SDS-PAGE. A it is possible to see in Figure 5, the sample "C" exhibits proteins fully precipitated, not matter if they were incubated or not with TGase (see Figure 5, lane "C", of both Panels A and B), such samples were treated with simulated gastric fluid (SGF) that did not contain pepsin. This result is likely due to the gelation and aggregation of the food sample during the heat treatment [45]. In the other hand, as expected, when samples were treated with SGF containing pepsin, the protein component present in falafel was gradually digested. TGase slightly decreased the protein digestibility rate of the falafel balls, even though, at the end of digestion in both systems, they were completely hydrolyzed by the gastric enzyme pepsin (Figure 5). These results were also supported by the densitometry analysis (Figure 6) of the protein bands having a molecular mass between 35 and 25 kDa, (Figure 5, Panel A, samples prepared without TGase), and the high molecular mass polymers of 250 kDa (Figure 5 panel B, falafel balls prepared by the means of TGase). As shown in Figure 6 the digestion rate is slower in the food incubated with the TGase enzyme , even though at the end of the digestion, the proteins analyzed are fully digested by pepsin in both untreated and TGase treated falafel.

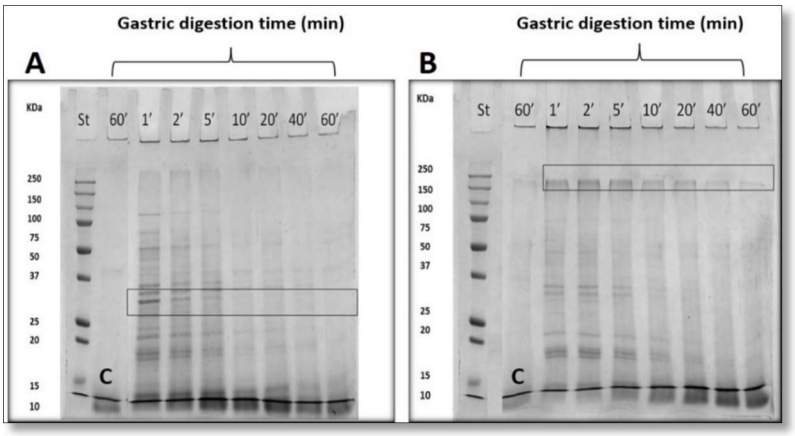

Figure 5. SDS-PAGE profile of falafel subjected to in vitro experiments. Panel A: traditional falafel prepared in the absence of TGase. Panel B: falafel prepared in the presence of TGase (20 U/g). The bands in the frame are those subjected to densitometry analysis. C is control sample incubated with simulated gastric solution not containing pepsin. St, Molecular weight standards, Bio-Rad.

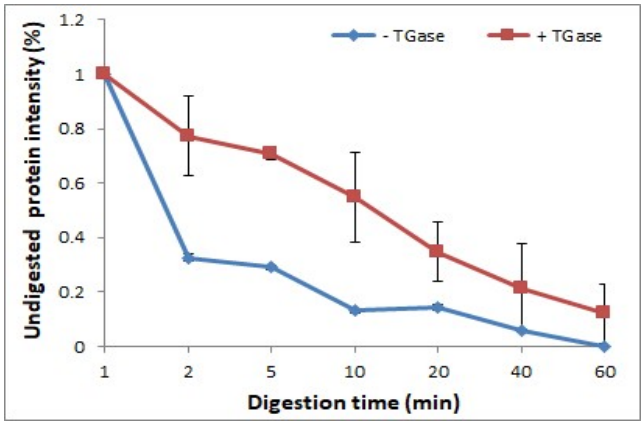

Figure 6. Densitometry analysis of the SDS-PACE bands relative to in vitro digestion shown in Figure 5. Both falafel types, traditional and (20 U/g) TGase-containing samples, were subjected to densitometry analysis.

4. Conclusions

In this paper for the first time falafel balls, a typical Middle Eastern food, were produced by using in their dough TGase. After the preparation of the balls, they were treated by dipping them in a PEC-based coating. We demonstrated that the use of the enzyme provoked the reduction of the ACR in falafel balls, which was even more evident when TGase-prepared balls were coated by PEC, that was able to decrease the ACR concentration also in the falafel prepared without TGase. However, TGase also had an effect on the texture profile parameters. On the other hand, the PEC coating protection allowed to reduce the oil content of this food product, either treated or not by means of TGase. In addition, protein gastric digestion, carried out under physiological conditions, showed that enzymatic treatment slightly decreased the digestion rate, although the proteins were fully digested at the end of the experiment in both unprocessed and TGase-processed systems.

Author contributions: L.M. supervised and conceived the project; L.M. and A.A. designed the experiments; A.A. performed the experiments; A.A., C.V.L.G., and L.P. analyzed the data; L.M. and A.A. co-wrote the paper; C.V.L.G. and L.M. reviewed and re-edited the paper; all authors discussed the results and commented on the manuscript.

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