coatings

MDPI

Article

Hydrocolloid-Based Coatings are Effective at Reducing Acrylamide and Oil Content of French Fries

Asmaa Al-Asmar [1,2], Daniele Naviglio [1], Concetta Valeria L. Giosafatto [1] and Loredana Mariniello [1,*]

[1] Department of Chemical Sciences, University of Naples "Federico II", 80126 Naples, Italy; asmaa.alasmar@unina.it (A.A.-A.); naviglio@unina.it (D.N.); giosafat@unina.it (C.V.L.G.)

[2] Analysis, Poison control and Calibration Center (APCC), An-Najah National University, P.O. Box 7, Nablus, Palestine

* Correspondence: loredana.mariniello@unina.it; Tel.: +39-081-253-9470

Received: 6 March 2018; Accepted: 16 April 2018; Published: 18 April 2018

Abstract: French fries are popular products worldwide. However, this product is a sufferable source of high acrylamide due to high temperature and low moisture. The main objective of this study was to evaluate the effect of grass pea flour (GPF), transglutaminase (TGase)-treated (GPF + TGase), chitosan (CH), and pectin (PEC) hydrocolloid coating solutions on the formation of acrylamide, water retention as well as on oil content. In addition, the Daily Intake (DI) and Margin of Exposure (MOE) were calculated to estimate variations in risk assessment by applying coating solutions before frying. Our results showed that the highest acrylamide content was detected in the control sample, reaching a value of 2089 μg kg^{-1}. Hydrocolloid coating solutions were demonstrated to be an effective way to reduce acrylamide formation, with the percentage of acrylamide reduction equal to 48% for PEC, >38% for CH, ≥37% for GPF + TGase, and >31% for GPF, respectively. We hypothesized that the coatings were able to increase the water retention and, thus reduce the Maillard reaction, which is responsible for acrylamide formation. In fact, the MOE value for coated French fries was increase, resulting in being closer to the safety level to avoid carcinogenic risk. Moreover, our coatings were effective in reducing oil uptake.

Keywords: French fries; acrylamide; hydrocolloid coating; transglutaminase; margin of exposure

1. Introduction

Acrylamide (H_2C=CH–CO–NH_2) is a compound that is highly soluble in water, about which heightened concerns regarding its exposure arose in 2002 when Swedish researchers discovered its formation when certain foods were prepared at temperatures above 120 °C and in the presence of low moisture [1,2]. Its formation, at least in part, is due to the Maillard reaction between free asparagines and reducing sugars. According to the European Food Safety Authority (EFSA), acrylamide forms in numerous baked and/or free asparagine rich fried foods including French fries, potato crisps, breads, biscuits, and coffee (roasted beans). Acrylamide is also known to be present in cigarette smoke. The EFSA report in 2015 mentioned that rats and mice exposed to acrylamide have shown some signs of developmental toxicity, increased incidence of skeletal variations, slightly impaired body weight gain, histological changes in the central nervous system, and some neurobehavioral effects. Acrylamide and its metabolite glycidamide (a reactive epoxide with the formula $C_3H_5NO_2$) are genotoxic and carcinogenic. Since any level of exposure to a genotoxic substance could potentially damage DNA and lead to cancer—as also stated by the International Agency for Research on Cancer (IARC, 1994) [3]—EFSA scientists conclude that acrylamide is a health concern [4].

The presence of acrylamide in food products has been studied in different countries and many food organizations have affirmed that deep-fried potato chips contain high amounts of acrylamide [5]. In addition, it was reported by Mestdagh et al. [6] that the ratio of fructose to glucose impacted both the color and acrylamide levels of fried potato strips, with higher fructose concentrations favoring acrylamide formation. Any factor, such as food formulation, pH, water content, temperature, and frying time can influence the Maillard reaction, which is also responsible for acrylamide formation [2]. French fries can contain more than 2000 μg kg^{-1} of acrylamide [7,8]. Recently, it has been found that the acrylamide content of 40 potato crisp brands from the Spanish market ranged from 108 to 2180 μg kg^{-1} [9]. Furthermore, the acrylamide level has recently been determined [10] in potato crisps, corn-based extruded snacks, and other savory snacks that are very popular in Italy together with the exposure risk assessment through the Margin of Exposure (MOE), showing that the acrylamide ranged from 21 to 3444 μg kg^{-1}, with the highest level in potato samples; the MOE assessment revealed that five out of six consumer groups showed exposure values associated with an augmented carcinogenic risk [10].

To prevent acrylamide formation during the frying process, some precautions have been reported [11]. The simplest precautions refer to the use of potato blanching either with water or acidic solutions, containing, for example, ascorbic acid or citric acid [12,13]. These strategies could also be applied together with a way of choosing specific potato cultivars described as containing lower amounts of both free asparagines and reducing sugars or controlling the storage conditions (potatoes stored at a temperature of 6 °C or higher have shown a lower amount of reducing sugars) or the recurring addition of salts in the soaking water [14]. Another more complex but expensive alternative process could be the recurring use of the asparaginase enzyme [15].

Some authors have proven the effectiveness of hydrocolloid solutions to control the acrylamide formation of different dried foods [16–18]. Hydrocolloids (proteins and polysaccharides) are hydrophilic polymers that can modify the functional properties of aqueous food systems such as thickening, gelling, and emulsifying properties [19,20]. Other studies have shown that the addition of some hydrocolloids to food preparations could enhance water retention [21–23], thus, becoming responsible for acrylamide reduction. In previous studies, the effect of enzyme transglutaminase (TGase, E.C. 2.3.2.13) on different hydrocolloid film properties was investigated [24–28]. This enzyme catalyzes the formation of isopeptide bonds among glutamine and lysine residues, thus providing a certain degree of reticulation in the hydrocolloid-based films [26]. Rossi Marquez et al. [29] demonstrated that a coating with whey protein/pectin film prepared in the presence of TGase resulted in a useful way to reduce oil content in fried potatoes. Moreover, a blanching pre-treatment and pectin coating were able to reduce the acrylamide formation in fried banana chips [16]. Additionally, alginic acid and PEC are promising inhibitors of acrylamide formation in fried potatoes [17]. Hydrocolloid-based coatings have also been used as effective protection to reduce oil uptake [29,30]. In fact, some studies have demonstrated that pectin [31,32], methylcellulose [32–34], whey protein, and egg white [35] were able to reduce the oil uptake in potato chips.

Hydrocolloids are effective coatings to enhance the quality of food products as reported in several reports [26,36]. Despite their effectiveness in regulating the transmission of gases such as oxygen and carbon dioxide besides water vapor, an increasing interest in studying their role in acrylamide reduction in processed foods has been registered [16,17,37].

The aim of the present study was to verify whether some hydrocolloid-based coatings, used previously in our laboratories [25,38] as effective film forming solutions, were also able to influence the formation of acrylamide in fried potatoes, the most widespread consumed fried food worldwide. In this study, we evaluated the effect of four different hydrocolloid coating solutions made of: (1) grass pea flour (GPF); (2) TGase-treated (GPF + TGase); (3) chitosan (CH); and (4) pectin (PEC) in reducing the formation of acrylamide during the frying process of potatoes, and to estimate the influence of the coating on the MOE in fried potatoes. Finally, the effect of coatings on reducing oil uptake was also investigated.

2. Materials and Methods

2.1. Materials

Acrylamide standard ≥ 99.8%, catalogue No. 23701 and methanol were obtained from the Sigma–Aldrich Chemical Company (St. Louis, MO, USA). Acetonitrile HPLC (high pressure liquid chromatography) analytical grade, n-hexane, and formic acid were supplied from Carlo Erba reagents srl (Milan, Italy). Water purified by a Milli-Q-RO system (Millipore, Bedford, MA, USA) was used. Glycerol was purchased from the Merck Chemical Company (Darmstadt, Germany) whereas Oasis HLB (Hydrophilic-Lipophilic-Balanced) 200 mg, 6 mL solid phase extraction (SPE) cartridges were from Waters (Milford, MA, USA). The syringe filters (0.45 µm, 0.22 µm PVDF (polyvinylidene difluoride) were from Alltech Associates (Deerfield, IL, USA). Pectin (PEC) of *Citrus* peel low-methylated (7%) (Aglupectin USP (United States Pharmacopoeia)) was purchased from Silva Extracts srl (Gorle, Italy). Chitosan (CH) was obtained from Professor R. Muzzarelli (University of Ancona, Ancona, Italy), prepared as described by [39] with a degree of 9.0% *N*-acetylation. Grass pea seeds (GP) and corn oil were from a local market (Naples, Italy) and ACTIVA WM containing *Streptoverticillium* Ca^{2+} independent TGase was supplied by Ajinomoto Co. (Tokyo, Japan; Japan product No. AJ301402, lot No. 00.02.03).

2.2. Preparation of Coating Solutions

To obtain GP flour (GPF), seeds were ground using a variable speed laboratory blender LB 20ES (Waring Commercial, Torrington, CT, USA), so that the GPF could pass through a 425 µm stainless steel sieve (Octagon Digital Endecotts Limited, London, UK). GPF-based solutions were prepared by dissolving 8.3 g of GPF (containing 24% *w*/*w* proteins) in 100 mL Milli-Q water. The solution was shacked for 1 h and its pH was adjusted to 9.0, followed by centrifugation at 12,096× *g* for 10 min. After centrifugation, 60 mL of the supernatant were taken and the pH was adjusted to 7.0, and the final volume of 100 mL was reached with water after adding 16 µL of glycerol (8% *w*/*w* with GPF proteins). The solution was then divided in two 50 mL falcon tubes and TGase (33 U/g of GPF proteins) was added in only one tube; then both samples (GPF with and without TGase) were incubated for 2 h at 37 °C. The enzymatic reaction was stopped by adjusting the pH to 9.0. The ratio between the enzyme and its substrate in order to have TG-mediated crosslinking of GPF the proteins, was established in our laboratories (unpublished data). CH-based solutions (0.6% *w*/*v*) were prepared from a CH stock solution (2% *w*/*v* of hydrochloric acid 0.1 N stirred overnight) [25] then diluted with water adjusting the pH to 4.0; finally the solution was stirred for 30 min at 25 °C. PEC-based solutions (1% *w*/*v*) were prepared according to Esposito et al. [40] from a PEC stock solution (2% *w*/*v*), then diluted with water; the pH was adjusted to 7.5; finally the solution was stirred for 30 min at 25 °C. PEC and CH concentrations were chosen according to studies reported in the literature [38].

2.3. French Fry Preparation and Frying Process

Potatoes (cultivar Musica) were obtained from the Department of Agriculture, University of Naples "Federico II" (Naples, Italy) and stored at 4 °C until use. Before performing the experiments, potatoes were cut into 1 cm × 1 cm× 6 cm sticks as described by Rossi Marquez et al. [29] and treated as follows: 100 g of potatoes (18 sticks) were dipped for 30 s into either distilled water (sample used as "control") or one of the following coating solutions: (1) grass pea flour (GPF); (2) TGase-treated (GPF + TGase); (3) chitosan (CH); and (4) pectin (PEC). Then, each sample was allowed to drip for 2 min before frying. The frying conditions were the following: 1.5 L corn oil was preheated (using a controlled temperature deep-fryer apparatus (GIRMI, Viterbo, Italy) to the processing temperature (170 °C), then the potato sticks were fried for exactly 6 min [7]. The oil was replaced with fresh oil for each differently coated sample. Each potato stick was fried by flipping from side to side every 2 min. After frying, each sample was allowed drain for 2 min to remove the excess oil. The described procedure was repeated three times and the results were averaged.

2.4. Acrylamide Standard Preparation

A standard stock solution (1.0 mg/mL) was prepared by dissolving 10.0 mg of the acrylamide standard in 10 mL of Milli-Q water by using a volumetric flask. From the stock solution, calibration standards at different concentrations (100, 250, 500, 1000, 2000, 3000, 4000, and 5000 µg/L), were prepared, respectively. The limit of detection (LOD) was 29.6 µg/L, and the limit of quantitation (LOQ) was 89.1 µg/L. All series of standard solutions were stored in glass dark bottles (light-resistant) at 4 °C until used (Figures S1 and S2).

2.5. Extraction of Acrylamide from the Fried Potato Strips

About 100 g of fried potato sticks, accurately weighed after cooling, were immersed in n-hexane for 30 min to remove the oil from their surfaces [17]. The sticks were then ground with a rotary mill Grindomix GM200, (Retsch GmbH, Haan, Germany) at a speed of 1300 rpm for 1 min. Each sample was allowed to dry by freeze-drying before being subjected to acrylamide extraction following the protocols reported by Wang et al. [41], and Krishna et al. [42] with some modifications: two different Falcon tubes were set up for each sample, one for detecting acrylamide formed in the sample itself ("basic" acrylamide), and the second one to carry out the "Recovery test" (Table S1). In both tubes, 1.0 g (dry weight) of sample, accurately weighed, was put in both tubes and only in the second one was the 500 µg kg^{-1} of acrylamide standard added. In each tube, 10.0 mL of Milli-Q 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 7741 × g for 10 min at 4 °C. The supernatant was filtered through a 0.45 µm syringe filter for the clean-up of the Oasis HLB SPE cartridges. The SPE cartridge was preventively conditioned with 2.0 mL of methanol followed by washing with 2 mL of water before loading 2.0 mL of the filtered supernatant, the first 0.5 mL was discarded and the remaining elute collected (≈1.5 mL; exact volume was measured by weight and converted by means of density). All 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 before HPLC-UV (ultra violet) analysis (Figure S3). Each analysis was performed in triplicate. The acrylamide recovery test was between 106% and 86% (Table S1) in accordance with the data reported in the literature.

2.6. HPLC-UV Analysis

HPLC-UV analysis was performed by using the RP-HPLC (RP: reverse phase) method on an Agilent 1100 series HPLC instrument equipped with an on-line degasser, a dual pump, and a diode array detector (Hewlett Packard, Wilmington, DE, USA). The column used was a Synergi™ 4 µm Hydro-RP 80 Å HPLC Column 250 × 4.6 mm [43,44] (Phenomenex, Torrance, CA, USA). The operating conditions were as follows: the wavelength detection was 210 nm, a gradient elution of 0.1% formic acid (v/v) in water: acetonitrile (97:3, v/v) was applied. Solvent A was water and Solvent B was acetonitrile, both solvents containing 0.10% (v/v) formic acid; flow rate, 1.0 mL/min. The gradient elution program was applied as follows: 97% A (3% B) for 10 min, increased to 20% A (80% B) from 10 to 12 min, and kept at 20% A (80% B) for 5.0 min, increased to 95% B (5% A) from 17 to 19 min, and kept at 95% B for 5 min, increased to 97% A (3% B) from 24 to 26 min, and kept for 4 min. The injection volume was 20 µL. The total chromatographic runtime was 30 min for each sample and the temperature was kept at 30 °C (GECKO 2000 "HPLC column heater", SpectraLab Scientific Inc., Markham, ON, Canada) to ensure optimal separation. In all samples (acrylamide standard and fried potato-derived), the acrylamide retention time was 4.9 min. The method presented a relative standard deviation lower than 5% with three repetitions; this result was in accordance with the data reported in literature.

2.7. Daily Intake (DI) and Margin of Exposure (MOE) of Acrylamide Risk Assessment

The daily intake (DI) and consequently, the Margin of Exposure (MOE) was calculated by taking into account the six following age groups (as stated from EFSA): Toddlers (1–3 years old); Children (3–10 years old); Adolescents (10–18 years old); Adults (18–65 years old); Elderly (65–75 years old); Very Elderly (more than 75 years old). DI (ng (kg body weight)$^{-1}$ day^{-1}) was calculated according to the equation [10]:

$$\mathrm{DI} = \frac{C \times Q}{\mathrm{BW}} \quad (1)$$

where C is the average concentration of acrylamide detected in each fried potato samples (ng g^{-1}), Q is the average daily consumption of fried potato samples of each age groups (g day^{-1}), and BW is the body weight of each age group (kg).

The average daily consumption Q was taken from the EFSA report [4], and the BW values were taken by Leclercq et al. [45] that considered the BW of Italian consumers belonging to the described six age groups. The DI results were used to estimate the MOE, a parameter that indicates the level of health concern for toxic and/or carcinogenic molecules. Here, we calculated the MOE for acrylamide, taking into account that it is classified as both a neurotoxic and carcinogenic agent. To calculate the MOE, it is necessary to refer to the BMDL_{10} = Benchmark Dose Lower confidence limit (mg (kg BW)$^{-1}$ day^{-1}), which represents the minimum dose range of a substance that produces a clear, low level health risk, usually in the range of 1%–10% [4]. The BMDL_{10} based on neurological changes (acrylamide as neurotoxic agent) is 0.43 mg (kg BW)$^{-1}$ day^{-1}, while the BMDL_{10} considering acrylamide as a carcinogenic agent is 0.17 mg (kg BW)$^{-1}$ day^{-1}, according to the EFSA report 2015 [4].

The MOE assessment was calculated according the following equation [10]:

$$\mathrm{MOE} = \frac{\mathrm{BMDL}_{10}}{\mathrm{DI}} \quad (2)$$

2.8. Oil Content

After frying and cooling, the oil content of each ground sample (3–5 g) was determined in triplicate and reported as a percentage on dry matter weight by n-hexane solvent extraction using the Soxhlet method [46], and the oil reduction due to coating was calculated as per the following equation.

$$\text{Oil reducing due to coating } (\%) = \frac{\text{oil content (control)} - \text{oil content (coated)}}{\text{oil content (control)}} \times 100 \quad (3)$$

2.9. Water Content Analysis

Water content of fried potato samples was determined according to AOAC (Association of Official Analytical Chemists) [47]. After frying, the potato sticks that had been coated with a different hydrocolloid coating solution and the control (coated with water) were dried in an oven at 105 °C until constant weight was achieved. Water content, water loss during frying and water retention in all of the samples were calculated as following:

$$\text{Water content } (\%) = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100 \quad (4)$$

$$\text{Water loss during frying } (\%) = \frac{(\text{initial water } - \text{water after frying})}{\text{initial water}} \times 100 \quad (5)$$

$$\text{Water retention } (\%) = \left(\frac{\text{water contant of coated}}{\text{water contant of control sample}} - 1\right) \times 100 \quad (6)$$

2.10. Statistical Analysis

All the experiments were carried out in triplicate, 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. Effect of Hydrocolloid Coating Solutions on the Acrylamide Content

The aim of the present work was to evaluate the effectiveness of hydrocolloid-based coatings in reducing acrylamide formation in potato French fries. Acrylamide analyses, based on fat-free dry matters (FFDM), were carried out by RP-HPLC and reported in Figure 1.

As reported in the Materials & Methods (Section 2.3), potato chips were coated by dipping with four different hydrocolloid solutions. The first solution was made of GPF proteins, the second was made of the same proteins enzymatically modified by means of TGase, and the third was a solution made of 0.6% w/v CH, while the fourth one was made of 1% w/v PEC. A potato sample dipped in distilled water was used as the control.

The highest acrylamide content was detected in the control sample, reaching a value of 2089 ± 36 μg kg^{-1}. The results of Figure 1 show that the most effective coating solution was the one made of PEC, since the potato samples protected by this edible film, contained only 52% w/w of acrylamide when compared to the uncoated ones. Zeng et al. [17] tested PEC-based hydrocolloid solutions and demonstrated their effectiveness in reducing acrylamide in both the model systems and fried potatoes. Even though these authors obtained a 50% reduction in acrylamide content, they used a 2% pectin solution into which the samples were immersed for 5 h. These conditions are less sustainable and, thus, more expensive than the conditions we studied in the present paper. No comparison with previous results could be made with the GPF coating solutions since the present paper is the first study to have used this GPF and GPF + TGase as coatings. GPF was able to reduce the acrylamide content of 31% w/w, while in the presence of TGase, the acrylamide content was reduced to 37% w/w, thus suggesting that the presence of peptide bonds, catalyzed by the enzyme, increased the coating compactness and somehow influenced the acrylamide formation, probably due to the edible film possessing a more straightened protein network with reduced pores [24–28].

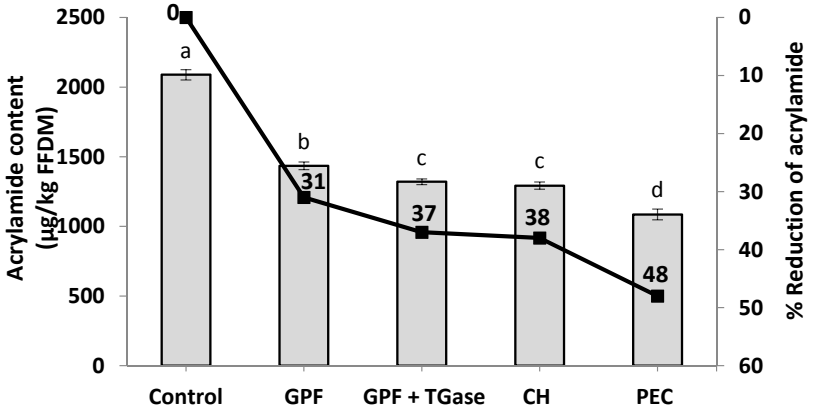

Figure 1. Effect of different hydrocolloid coatings on acrylamide content for French fries (y-axis on the left based on fat-free dry matters (FFDM)) and % reduction of acrylamide (y-axis on the right). Samples were coated with hydrocolloid-based coatings made of GPF, grass pea flour; GPF + TGase, grass pea flour treated by transglutaminase; PEC, pectin; CH, chitosan. "Uncoated" represents the control sample dipped in distilled water. Means with different letters (a–d) are significantly different (Tukey means comparison, $p < 0.05$). Data reported are the average values of three repetitions. Further experimental details are given in the text.

Our results also showed that the ability of reducing the acrylamide content of 38% (*w*/*w*) was exhibited by a CH coating solution, a reduction very similar to the one obtained using the coating made of GPF treated with TGase. While the latter reduction may be related to the pore dimension, the acrylamide reduction due to CH could be related to the high number of amino groups in this polysaccharide. In fact, Sansano et al. [18], while investigating the effect of CH on the reduction of acrylamide in fried batter, demonstrated that the polysaccharide influence was higher than the one performed by reducing sugars such as fructose added to the model system. Hence, the authors suggested that the CH amino groups might compete with free asparagine amino groups in binding the reducing sugars, which is the first step of the reactions that ends with acrylamide formation.

3.2. Effect of Coating to Acrylamide Risk Assessment

Risk assessment is usually evaluated by the MOE, which is calculated as the ratio between the dose at which a small but measurable adverse effect is first observed, and the daily level of intake that accounts for both the BW and different population groups. EFSA published in its report the MOE for acrylamide, which is classified as a substance that can be both genotoxic (neurotoxic) and carcinogenic. Acrylamide becomes a health concern for consumers that were divided in groups according to their ages, since the effect of exposure to a toxic/carcinogenic agent depends on the BW that is correlated, mostly, to age. In the present study, we verified that some hydrocolloid-based coatings were effective in reducing the acrylamide content and, thus, we also wanted to verify how the DI would change if the potato sticks were coated before being fried. Table 1 shows the DI calculated by Equation (1) in six different age groups. Indeed, we only excluded the Infants out of all seven groups that EFSA considered in its reports. In fact, Infants are classified as population members that are less than one year old and, thus, are not supposed to eat fried foods or potatoes. DI value relatives were calculated by multiplying the concentration of acrylamide detected in the samples by the average consumption (g day^{-1}) in each age group. The minimum, median, and maximum of the mean values were obtained by the EFSA through surveys across 20 European countries [4]. For the sake of brevity, Table 1 only reports the median values.

Table 1. Dietary intake of acrylamide consumption based on the median of the estimated consumption of fried potatoes treated with the coating solutions. Samples were coated with different hydrocolloid-based coatings made of GPF, grass pea flour; GPF + TGase, grass pea flour treated by transglutaminase; PEC, pectin; and CH, chitosan. "Uncoated" represents the control sample dipped in distilled water.

Fried Potato Samples	Age Groups	Acrylamide Intake (ng (kg body weight)$^{-1}$ day^{-1})
Control	Toddlers	1387
	Other children	1521
	Adolescents	1072
	Adults	719
	Elderly	536
	Very elderly	417
GPF	Toddlers	952
	Other children	1045
	Adolescents	737
	Adults	494
	Elderly	368
	Very elderly	287
GPF + TGase	Toddlers	877
	Other children	962
	Adolescents	678
	Adults	455
	Elderly	339
	Very elderly	264

Table 1. *Cont.*

Fried Potato Samples	Age Groups	Acrylamide Intake (ng (kg body weight)$^{-1}$ day^{-1})
CH	Toddlers	858
	Other children	941
	Adolescents	663
	Adults	445
	Elderly	332
	Very elderly	258
PEC	Toddlers	720
	Other children	790
	Adolescents	557
	Adults	374
	Elderly	279
	Very elderly	217

It was clear that the presence of the coating would allow all population groups to have a lower DI, so being more protected from the damage that acrylamide from fried potatoes could give them. The most effective was the PEC coating, since, taking into account the median DI value, we observed that all of them were roughly 50% less that the DI values exhibited in the control samples. We also verified that the GPF + TGase coating was as effective as the CH-based coatings even though they gave rise to a DI that was about 40% lower than the DI of the control. DI was useful to calculate the MOE according to Equation (2), and considered both the neurotoxic and carcinogenic risks of acrylamide. Thus, a $BMDL_{10}$ equal to 0.17 mg (kg BW)$^{-1}$ day^{-1} for carcinogenic risk (Figure 2, left panels) and a $BMDL_{10}$ equal to 0.43 mg (kg BW)$^{-1}$ day^{-1} for neurotoxic risk (Figure 2, right panels) were used to calculate the MOE in the six different age groups. Figure 2 also reports the MOE for the minimum (Figure 2A), medium (Figure 2B), and the maximum (Figure 2C) of acrylamide consumption levels as estimated from the EFSA throughout surveys across 20 European countries [4].

In each panel, the values of the MOE that were considered of no concern to the consumers according to EFSA regulation are reported: 10,000 and 125 are the minimum safety levels of the MOE for carcinogenic and neurotoxic risks, respectively. For the sake of clarity, it is worth reminding that, since MOE is inversely proportioned to the DI of acrylamide, a lower MOE level indicates a higher risk. Thus, as reported in Figure 2, for each age group, the MOE values were below the minimum safety level carcinogenic risk, even though it was clear that the use of hydrocolloid-based coatings increased the MOE values even though by only about 50%–60%. In any case, this result proved that recurring coatings could provide advantages to consumers, especially for the ones from 1 to 65 years old. In fact, this population consumes a higher amount of fried potato than the elderly or very old people. We want to underline that, as expected, the evaluation of the MOE indicated that the PEC-based hydrocolloid coating was the most effective among the others tested. Regarding the MOE values that assess safety for neurotoxic risk, we can see from the Figure 2, right panels, that a healthy concern due to a MOE value below the 125 safety level, was for the control samples of Toddlers and Other children groups, thus coating fried potatoes is effective in regard to maximum consumption levels (Figure 2C on the right). In fact, for minimum and median levels (Figure 2A,B on the right), acrylamide is not a health concern regarding neurotoxicity since the MOE values were much higher than the minimum safety level.

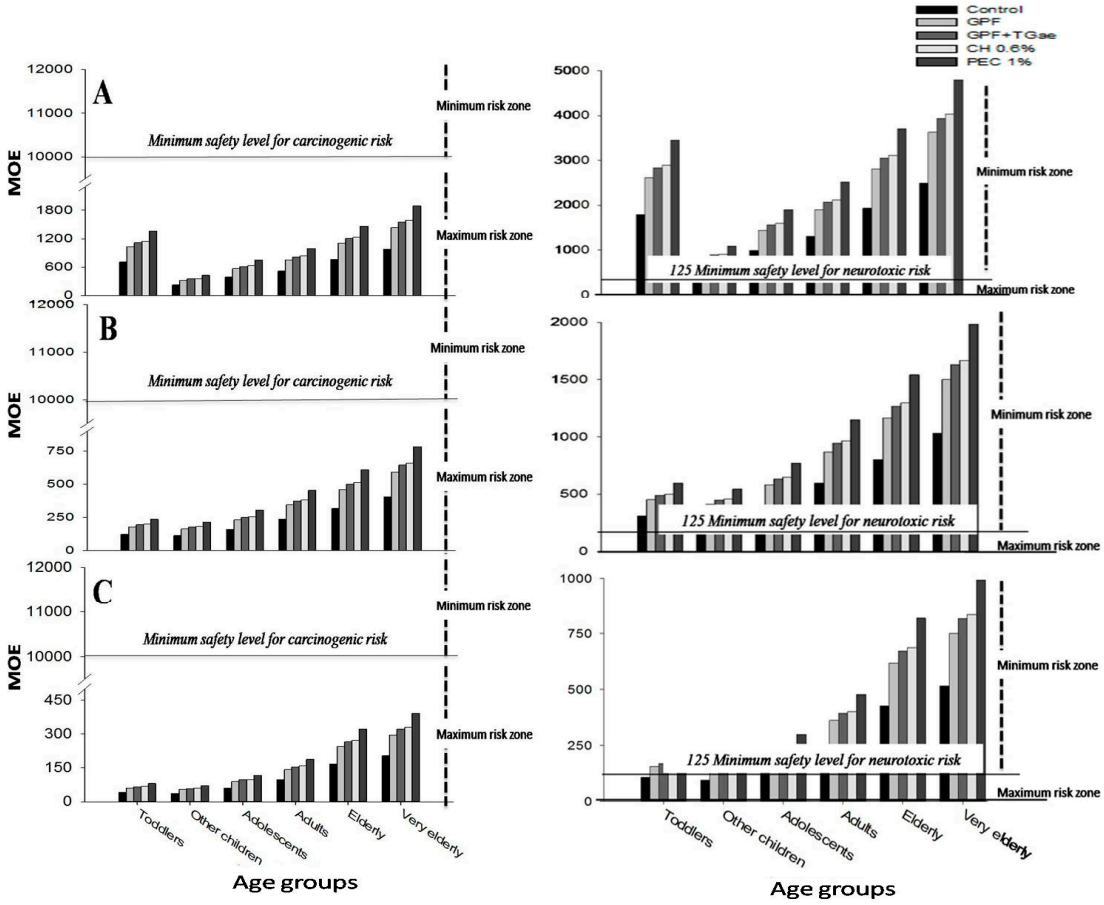

Figure 2. Margin of exposure (MOE) values for carcinogenicity (left panel) and neurotoxic (right panel) of acrylamide through the consumption of French fries that were both uncoated and coated with hydrocolloid coating solutions. Samples were coated with different hydrocolloid-based coatings made of GPF, grass pea flour; GPF + TGase, grass pea flour treated by transglutaminase; PEC, pectin; and CH, chitosan. "Uncoated" represents the control sample dipped in distilled water, across different consumer age groups: (**A**) minimum, (**B**) median, and (**C**) maximum of consumption levels estimated from the 2015 EFSA report. Further experimental details are given in the text.

3.3. Effect of Coating Materials on the Water and Oil Content

It is well known that during frying, the food water content decreases, while the oil replaces water. In a previous work, Rossi Marquez et al. [29] demonstrated the effectiveness of hydrocolloid-based coatings in reducing oil uptake in fried potato strips by using a coating made of PEC and whey proteins, modified by the means of TGase. In the present paper, the effectiveness of the four different hydrocolloid-based coatings in the oil uptake was also verified. As shown in Table 2, all four types of hydrocolloid-based coatings were effective in reducing oil content, even if the most significant reduction was obtained with the PEC-based coatings. Regarding water, as expected according to our hypothesis, the water content increased, reaching 56.1% (w/w) in PEC-coated samples, while the controls exhibited 41.9% (w/w) of water. During frying, the water evaporates through the dry crust and is substituted by oil. Oil uptake in fried foods can be considered a health concern since high consumption of fatty acids has been related to obesity and other health problems such as heart disease [48].

Table 2. Effect of hydrocolloid coatings on the quality of potato French fries. Samples were coated with different hydrocolloid-based coatings made of GPF, grass pea flour; GPF + TGase, grass pea flour treated by transglutaminase; CH, chitosan; and PEC, pectin. "Uncoated" represents the control sample dipped in distilled water *.

Coating Solutions	Oil Content (%)	Oil Reducing Due to Coating (%)	Water Content (%)	Water Loss During Frying (%)	Water Retention Due to Coating (%)
Control	20.1 ± 0.7^{a}	–	41.9 ± 1.1^{a}	36.9 ± 1.1^{a}	–
GPF	18.2 ± 0.4^{b}	9.3 ± 1.9^{a}	44.9 ± 1.1^{b}	33.9 ± 1.1^{b}	7.1 ± 0.7^{a}
GPF + TGase	$16.5 \pm 0.9^{b,c}$	15.9 ± 1.6^{b}	49.3 ± 0.7^{c}	29.5 ± 0.7^{c}	17.5 ± 1.7^{b}
CH	$15.7 \pm 0.5^{c,d}$	21.5 ± 2.7^{c}	51.3 ± 1.0^{c}	27.4 ± 1.3^{c}	22.3 ± 2.4^{c}
PEC	14.1 ± 0.4^{d}	29.4 ± 2.4^{d}	56.1 ± 0.6^{d}	22.7 ± 0.6^{d}	33.8 ± 1.4^{d}

*: Means with different letters ($^{a–d}$) are significantly different per column (Tukey means comparison, $p < 0.05$).

As shown in Table 2, all four hydrocolloid-based coatings used in this study were able to reduce the oil content when compared to the control, potato sticks coated with water. The most effective coating was PEC, which was able to reduce the oil content of the fried sample by about 29.4% (w/w) and retain 33.8% (w/w) of the water content of the sample. These results were in agreement with Hua et al. [32], since these authors indicated that the coating prepared with 1.0% (w/v) low-methoxyl sunflower head pectin and 0.05 mol/L $CaCl_2$ reduced the oil uptake by about 30%. Other authors [31,49] have reported that pectin leads to a lower oil uptake. In the present paper, the effectiveness of both protein-based hydrocolloid solutions (GPF and GPF + TGase) in reducing oil content uptake was also proven since the oil content was reduced by 9% and 15.9%, respectively. Thus, the TGase-mediated crosslinking was able to reduce the oil content twice when compared to the GPF sample. These results were better than the ones obtained by Aminlari et al. [35] where, using protein-based hydrocolloids such as whey and egg proteins, they only achieved 5% and 12% oil uptake reduction, respectively. The effectiveness of the hydrocolloid-based coatings in reducing the excessive oil uptake can be referred to their ability of reducing the heat transfer coefficient, as extensively discussed in the review by Kurek et al. [30].

4. Conclusions

In summary, it was demonstrated that a hydrocolloid-based coating was responsible for the reduction in acrylamide formation due to its capability to increase water retention. For the same reason, our hydrocolloid-based coatings were also effective in reducing oil uptake as they provided a reduction in the heat transfer coefficient during frying. It is worth noting that, of the four types of coatings studied, the PEC-based coating gave the best performances. We are confident that, due to their low cost and their colorless and tasteless properties, hydrocolloids could be hopefully adopted in the future as strategies for consumers, but also enterprises that produce commercial fried foods to maintain a lower acrylamide content.

Supplementary Materials: The following are available online at http://www.mdpi.com/2079-6412/8/4/147/s1, Figure S1: HPLC chromatograms of water as blank obtained at 210 nm. The mobile phase was (97/3 v/v) water/acetonitrile containing 0.1% v/v formic acid at 1.00 mL/min, Figure S2: HPLC chromatograms of acrylamide standards obtained at 210 nm; acrylamide concentrations were 0.1, 0.25, 0.5, 1, 2, 3, 4, and 5 mg/L of acrylamide. The mobile phase was (97/3 v/v) water/acetonitrile containing 0.1% v/v formic acid at 1.00 mL/min, Figure S3: HPLC chromatograms were: A (green line) 1000 µg/L acrylamide standard; B (violet line) acrylamide extracted from the French fry potato sample; and C (pink line) acrylamide from the French fry potato sample into which 500 µg/kg acrylamide standard was added and in the sample were eluted in the best chromatographic conditions by using mobile phasewater/acetonitrile (97/3 v/v) containing 0.1% v/v formic acid at 1.00 mL/min, and UV detection at 210 nm, Table S1: Recovery test for acrylamide in all samples (in each sample 500 µg/kg of standard were added) *.

Acknowledgments: The authors acknowledge the financial support from Italian Ministery of University and Reaserch (MIUR) to D.N. through the program FFABR 2017 (CUP E65D17000430001), and Biofarmex srl (Salerno, Italy). In addition, the authors are grateful to Mrs. Maria Fenderico for technical support and to Prof. Domenico Carputo (Department of Agriculture, University of Naples "Federico II", Naples, Italy) for providing potato samples.

Author Contributions: Loredana Mariniello conceived and supervised the project. Loredana Mariniello, Asmaa Al-Asmar, and Daniele Naviglio designed the experiments. Asmaa Al-Asmar performed the experiments. Concetta Valeria L. Giosafatto and Daniele Naviglio analyzed the data. Asmaa Al-Asmar and Loredana Mariniello co-wrote the paper. All authors discussed the results and commented on the manuscript.

Conflicts of Interest: The authors declare that they do not have any conflict of interests.

References

1. Tareke, E.; Rydberg, P.; Carlson, P.; Eriksson, S.; Törnqvist, M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J. Agric. Food Chem.* **2002**, *50*, 4998–5006. [CrossRef] [PubMed]
2. Mottram, D.S.; Wedzicha, B.L.; Dodson, A.T. Food chemistry: Acrylamide is formed in the Maillard reaction. *Nature* **2002**, *419*, 448–449. [CrossRef] [PubMed]
3. IARC. Acrylamide. In *Monographs on the Evaluation of Carcinogenic Risks to Humans Some Industrial Chemicals*; IARC: Lyon, France, 1994; pp. 389–433.
4. EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain). Scientific opinion on acrylamide in food. *EFSA J.* **2015**, *13*, 4104. [CrossRef]
5. Medeiros, R.; Mestdagh, F.; De Meulenaer, B. Acrylamide formation in fried potato product present and future a critical review on mitigation strategies. *J. Food Chem.* **2011**, *50*, 8–11. [CrossRef]
6. Mestdagh, F.; De Wilde, T.; Castelein, P.; Ne'meth, O.; Van Peteghem, C.; De Meulenaer, B. Impact of the reducing sugars on the relationship between acrylamide and Maillard browning in French fries. *Eur. Food Res. Technol.* **2008**, *227*, 69–76. [CrossRef]
7. Gökmen, V.; Palazoğlu, T.K.; Şenyuva, H.Z. Relation between the acrylamide formation and time history of surface and core regions of French fries. *J. Food Eng.* **2006**, *77*, 972–976. [CrossRef]
8. Pedreschi, F.; Kaack, K.; Granby, K. Acrylamide content and color development in fried potato strips. *Food Res. Int.* **2006**, *39*, 40–46. [CrossRef]
9. Mesías, M.; Morales, F.J. Acrylamide in commercial potato crisps from Spanish market: Trends from 2004 to 2014 and assessment of the dietary exposure. *Food Chem. Toxicol.* **2015**, *81*, 104–110. [CrossRef] [PubMed]
10. Esposito, F.; Nardone, A.; Fasano, E.; Triassi, M.; Cirillo, T. Determination of acrylamide levels in potato crisps and other snacks and exposure risk assessment through a Margin of Exposure approach. *Food Chem. Toxicol.* **2017**, *108*, 249–256. [CrossRef] [PubMed]
11. Krishnakumar, T.; Visvanathan, R. Acrylamide in food products: A Review. *J. Food Process. Technol.* **2014**, *5*, 344. [CrossRef]
12. Rydberg, P.; Eriksson, S.; Tareke, E.; Karlsson, P.; Ehrenberg, L.; Tornqvist, M. Investigations of factors that influence the acrylamide content of heated foodstuffs. *J. Agric. Food Chem.* **2003**, *51*, 7012–7018. [CrossRef] [PubMed]
13. Mestdagh, F.; Maertens, J.; Cucu, T.; Delporte, K.; Van Peteghem, C.; De Meulenaer, B. Impact of additives to lower the formation of acrylamide in a potato model system through pH reduction and other mechanisms. *Food Chem.* **2008**, *107*, 26–31. [CrossRef]
14. Pedreschi, F.; Granby, K.; Risum, J. Acrylamide mitigation in potato chips by using NaCl. *Food Bioprocess Technol.* **2010**, *3*, 917–921. [CrossRef]
15. Xu, F.; Oruna-Concha, M.-J.; Elmore, J.S. The use of asparaginase to reduce acrylamide levels in cooked food. *Food Chem.* **2016**, *210*, 163–171. [CrossRef] [PubMed]
16. Suyatma, N.E.; Ulfah, K.; Prangdimurti, E.; Ishikawa, Y. Effect of blanching and pectin coating as pre-frying treatments to reduce acrylamide formation in banana chips. *Int. Food. Res. J.* **2015**, *22*, 936–942.
17. Zeng, X.; Cheng, K.-W.; Du, Y.; Kong, R.; Lo, C.; Chu, I.K.; Chen, F.; Wang, M. Activities of hydrocolloids as inhibitors of acrylamide formation in model systems and fried potato strips. *Food Chem.* **2010**, *121*, 424–428. [CrossRef]
18. Sansano, M.; Castello, M.L.; Heredia, A.; Andres, A. Protective effect of chitosan on acrylamide formation in model and batter systems. *Food Hydrocoll.* **2016**, *60*, 1–6. [CrossRef]
19. BeMiller, J.N. Hydrocolloids. In *Gluten-Free Cereal Products and Beverage*, 1st ed.; Arendt, E.K., Dal Bello, F., Eds.; Elsevier Academic Press: Burlington, NJ, USA, 2008; pp. 203–215.
20. Kohajdova, Z.; Karovicova, J. Application of hydrocolloids as baking improvers. *Chem. Pap.* **2009**, *63*, 26–38. [CrossRef]

21. Albert, S.; Mittal, G.S. Comparative evaluation of edible coatings to reduce fat uptake in a deep-fried cereal product. *Food Res. Int.* **2002**, *35*, 445–458. [CrossRef]
22. Pedroso, R.A.; Demiate, I.M. Evaluation of the influence of starch and carrageenan on physicochemical and sensory characteristics of cooked turkey ham. *Cienc. Tecnol. Aliment.* **2008**, *28*, 24–31. [CrossRef]
23. Ramos, N.A.G.; Farias, M.E.; Almada, C.; Crivaro, N. Stability of sausages with emulsifiers and hydrocolloids. *Inf. Tecnol.* **2004**, *15*, 91–94.
24. Mariniello, L.; Di Pierro, P.; Esposito, C.; Sorrentino, A.; Masi, P.; Porta, R. Preparation and mechanical properties of edible pectin-soy flour films obtained in the absence or presence of transglutaminase. *J. Biotechnol.* **2003**, *102*, 191–198. [CrossRef]
25. Di Pierro, P.; Chico, B.; Villalonga, R.; Mariniello, L.; Damiao, A.E.; Masi, P.; Porta, R. Chitosan-whey protein edible films produced in the absence or presence of transglutaminase: Analysis of their mechanical and barrier properties. *Biomacromolecules* **2006**, *7*, 744–749. [CrossRef] [PubMed]
26. Porta, R.; Mariniello, L.; Di Pierro, P.; Sorrentino, A.; Giosafatto, C.V.L. Transglutaminase crosslinked pectin-and chitosan-based edible films: A review. *Crit. Rev. Food Sci. Nutr.* **2011**, *51*, 223–238. [CrossRef] [PubMed]
27. Giosafatto, C.V.L.; Di Pierro, P.; Gunning, P.; Mackie, A.; Porta, R.; Mariniello, L. Characterization of citrus pectin edible films containing transglutaminase-modified phaseolin. *Carbohydr. Polym.* **2014**, *106*, 200–208. [CrossRef] [PubMed]
28. Porta, R.; DiPierro, P.; Sabbah, M.; Regalado-Gonzales, C.; Mariniello, L.; Kadivar, M.; Arabestani, A. Blend films of pectin and bitter vetch (*Vicia ervilia*) proteins: Properties and effect of transglutaminase. *Innov. Food Sci. Emerg. Technol.* **2016**, *36*, 245–251. [CrossRef]
29. Rossi Marquez, G.; Di Pierro, P.; Esposito, M.; Mariniello, L.; Porta, R. Application of transglutaminase-crosslinked whey protein/pectin films as water barrier coatings in fried and baked foods. *Food Bioprocess Technol.* **2013**, *7*, 447–455. [CrossRef]
30. Kurek, M.; Scetar, M.; Gali, K. Edible coatings minimize fat uptake in deep fat fried products: A Review. *Food Hydrocoll.* **2017**, *71*, 225–235. [CrossRef]
31. Daraei Garmakhany, A.; Mirzaei, H.O.; Maghsudlo, Y.; Kashaninejad, M.; Jafari, S.M. Production of low fat French-fries with single and multi-layer hydrocolloid coatings. *J. Food Sci. Technol.* **2014**, *51*, 1334–1341. [CrossRef] [PubMed]
32. Hua, X.; Wang, K.; Yang, R.; Kang, J.; Yang, H. Edible coatings from sunflower head pectin to reduce lipid uptake in fried potato chips. *LWT Food Sci. Technol.* **2015**, *62*, 1220–1225. [CrossRef]
33. Tavera-Quiroz, M.J.; Urriza, M.; Pinotti, A.; Bertola, N. Plasticized methylcellulose coating for reducing oil uptake in potato chips. *J. Sci. Food Agric.* **2012**, *92*, 1346–1353. [CrossRef] [PubMed]
34. Luvielmo, M.M.; De Armas, D.S.; Paiva, F.F.; Krolow, A.C.R.; Ferri, N.M.L. Physicochemical and sensory characteristics of potato chips made from blanched potatoes of the cultivar BRS Ana and coated with methylcellulose. *Braz. J. Food Technol.* **2015**, *18*, 211–219. [CrossRef]
35. Aminlari, M.; Ramezani, R.; Khalili, M.H. Production of protein-coated low-fat potato chips. *Food Sci. Technol. Int.* **2005**, *11*, 177–181. [CrossRef]
36. Coltelli, M.-B.; Wild, F.; Bugnicourt, E.; Cinelli, P.; Lindner, M.; Schmid, M.; Weckel, V.; Müller, K.; Rodriguez, P.; Staebler, A.; et al. State of the art in the development and properties of protein-based films and coatings and their applicability to cellulose based products: An extensive review. *Coatings* **2016**, *6*, 1. [CrossRef]
37. Rannou, C.; Laroque, D.; Renault, E.; Prost, C.; Sérot, T. Mitigation strategies of acrylamide, furans, heterocyclic amines and browning during the Maillard reaction in foods. *Food Res. Int.* **2016**, *90*, 154–176. [CrossRef] [PubMed]
38. Sabbah, M.; Di Pierro, P.; Esposito, M.; Giosafatto, C.V.L.; Mariniello, L.; Porta, R. Stabilization of charged polysaccharide film forming solution by sodium chloride: Nanoparticle *Z*-average and Zeta-potential monitoring. *J. Biotechnol. Biomater.* **2016**, *6*, e128. [CrossRef]
39. Baxter, A.; Dillon, M.; Taylor, K.D.A.; Roberts, G.A.F. Improved method for i.r. determination of the degree of *N*-acetylation of chitosan. *Int. J. Biol. Macromol.* **1992**, *14*, 166–169. [CrossRef]
40. Esposito, M.; Di Pierro, P.; Regalado-Gonzales, C.; Mariniello, L.; Giosafatto, C.V.L.; Porta, R. Polyamines as new cationic plasticizers for pectin-based edible films. *Carbohydr. Polym.* **2016**, *153*, 222–228. [CrossRef] [PubMed]

41. Wang, H.; Feng, F.; Guo, Y.; Shuang, S.; Choi, M.M.F. HPLC-UV quantitative analysis of acrylamide in baked and deep-fried Chinese foods. *J. Food Compos. Anal.* **2013**, *31*, 7–11. [CrossRef]
42. Krishna, V.N.; Meyyanathan, S.N.; Karthik, Y.; Hemnath, E.; Satiesh, K.R.; Usha, K. A simple and validated RP HPLC method for the estimation of acrylamide in potato chips. *World J. Pharm. Pharm. Sci.* **2014**, *3*, 1468–1476.
43. Michalak, J.; Gujska, E.; Kuncewicz, A. RP-HPLC-DAD studies on acrylamide in cereal-based baby foods. *J. Food Compos. Anal.* **2013**, *32*, 68–73. [CrossRef]
44. Michalak, J.; Gujska, E.; Klepacka, J. The effect of domestic preparation of some potato products on acrylamide content. *Plant Foods Hum. Nutr.* **2011**, *66*, 307–312. [CrossRef] [PubMed]
45. Leclercq, C.; Arcella, D.; Piccinelli, R.; Sette, S.; Donne, C.L.; Turrini, A. The Italian National Food Consumption Survey INRAN-SCAI 2005-06: Main results in terms of food consumption. *Public Health Nutr.* **2009**, *12*, 2504–2532. [CrossRef] [PubMed]
46. Association of Official Analytical Chemists. Method 960.39. In *Official Methods of Analysis*, 15th ed.; Helrich, K., Ed.; AOAC International: Arlington, VA, USA, 1990.
47. Association of Official Analytical Chemists. Method 950.46. In *Official Methods of Analysis*, 15th ed.; Helrich, K., Ed.; AOAC International: Arlington, VA, USA, 1990.
48. Sayon-Orea, C.; Bes-Rastrollo, M.; Basterra-Gortari, F.J.; Beunza, J.J.; Guallar-Castillon, P.; de la Fuente-Arrillaga, C.; Martinez-Gonzalez, M.A. Consumption of fried foods and weight gain in a mediterranean cohort: The sun project. *Nutr. Metab. Cardiovasc. Dis.* **2013**, *23*, 144–150. [CrossRef] [PubMed]
49. Mahajan, I.; Sonka, C.; Surendar, J. Study on the effective reduction of oil up-take by the application of edible hydrocolloid coatings on French fries. *Int. J. Res. Eng. Adv. Technol.* **2014**, *2*. Available online: http://www.ijreat.org/Papers%202014/Issue9/IJREATV2I3056.pdf (accessed on 6 March 2018).