




## Article

# Edible Coating from Enzymatically Reticulated Whey Protein-Pectin to Improve Shelf Life of Roasted Peanuts

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**Abstract:** Edible coatings are a viable alternative method to enhance food shelf life that can be designed using different biopolymers. This study evaluated the effect of a whey protein–pectin coating reticulated by microbial transglutaminase (*mTG*) on improving roasted peanuts’ shelf life. Peroxide value, water content, peanut color, and the solution’s contact angle were studied. The latter was improved by the presence of the enzyme. The results showed that the presence of the coating on the peanut surface reduces the peroxide value and water content, probably as a consequence of an improved barrier effect due to the presence of *mTG*, which protects the kernel. Enzymatically reticulated whey protein–pectin coatings are a promising alternative to enhance the shelf life of roasted peanut kernels using natural ingredients.

**Keywords:** coating; edible; peanut; transglutaminase



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## 1. Introduction

Peanuts (*Arachis hypogea*), from the family of legumes, are an important crop consumed all over the world. There are many different peanut cultivars available, however, four market types (runner, Virginia, Spanish, and Valencia) have been distinguished based on flavor, oil content, size, shape, and disease resistance. Of these, the Virginia type, is selected for its large size [1]. Due to their nutritional attributes, such as the richness in energy and proteins (approximately 22 to 30% crude protein, and about 47–50% of oil, of which 80% comes from unsaturated fatty acids) peanuts are a very important source of nutrients for developing countries. Recent studies have demonstrated that dietary inclusion of peanuts has been linked to a reduction of heart disease occurrence; for example, eating peanuts twice a week reduces the risk of death from heart disease by 24%, and studies on other diseases such as specific types of cancer, and improved weight management indicate that a regular peanut consumption helps to decrease blood pressure, which is important for hypertensive individuals [2].

Lipid oxidation in roasted peanuts is the major cause of decreased shelf life, due to the generation of undesirable aromas and adverse tastes, as well as a loss in nutritional value and formation of deleterious lipid radicals, such as carbon-centered, peroxy, alkoxy radicals, aliphatic aldehydes, ketones, and alcohols, all of which affect consumer’s consumption of roasted peanuts after prolonged storage [3–5].

Flavor, color, texture, and appearance are among the main quality factors in food [6]. Besides changes in flavor and aroma, spoilage causes undesirable changes in characteristics such as color and texture, these alterations occur mainly through chemical, microbiological, or physical changes in the food, including oxidation, drying, over-ripening, or through

microbial activity from native or contaminating bacteria, fungi, or viruses. It is known that there are organoleptic or nutritive attributes that can make food more or less attractive to the consumer [7].

In the food industry avoiding spoilage needs to be considered in the diverse stages of production, such as processing, packaging, distribution, retail display, transport, and storage, to minimize the effects of spoilage, and thus extend the shelf life of products [8]. Moreover, extending the shelf life of food helps industry to improve the utilization of raw materials, scheduling production, transportation, and logistics [6]. Factors such as light, water activity, lipid content and composition, temperature, relative humidity, and oxygen concentration of the environment, as well as packaging headspace, play a significant role in roasted-peanut lipid oxidation. Among these factors, oxygen concentration is one of the most important, making peanuts prone to oxidative and hydrolytic changes, thus affecting their flavor and reducing both the shelf life and nutritional quality [9].

Lipid oxidation can be reduced by the roasting process [10] or by using conventional packaging techniques, such as inert gas packaging, vacuum packaging, and modified atmosphere packaging [11], in which the oxygen is removed or reduced. Consumer interest in eco-friendly packaging promotes the use of modern packaging methods such as active packaging and biodegradable polymers like edible films or coatings [12,13]. Furthermore, in recent years consumers are becoming more interested in nutritional products prepared with less preservatives, minimally processed, and with high quality standards [14]. Edible coatings are thin layers of edible substances applied to the product's surface to help to prevent the exchange of water, oxygen, or any other component between the food and the surroundings, and which can be eaten as part of the product [15–17]. The main protective effects of edible coatings on food are the prevention of moisture loss, mechanic damage, decreased oxygen diffusion, and the facilitation of the transport of antimicrobial and antioxidant agents to extend the shelf life and pathogen growth on food surfaces [18–20]. It has been demonstrated that edible coatings applied to peanuts can act as a barrier, improving shelf life and keeping lipid stability, and reducing the oxidative process [21]. However, to obtain these results, the coating should act as a skin in reducing the kernel porosity, avoiding oxygen exchanges. Coatings made from polysaccharides and/or proteins have shown good barriers to oxygen, which make them good candidates for coating oxidizable products. Unfortunately, their hydrophilic nature has some drawbacks related to water permeability that favor the occurrence of water-related changes, the water absorption by the coating that reduces its barrier properties, and finally, the poor adhesion of hydrophilic coatings onto hydrophobic nut surfaces, which may result in coating dewetting, cracking upon drying, and flaking after drying [22].

It is well known that crosslinks improve both the oxygen and water permeability of whey protein–pectin films [23,24], and could improve the hydrophobicity [25]. Therefore, the aim of this work was to characterize the wettability of a coating solution based on whey protein and pectin crosslinked with microbial transglutaminase, and to evaluate the effect of dip coatings on the color, water loss, and lipid oxidation of roasted peanuts during 50 days of storage. Additionally, the ability to extend the shelf life of roasted peanuts without decreasing the consumer's acceptance was evaluated through a preliminary sensory evaluation.

## 2. Materials and Methods

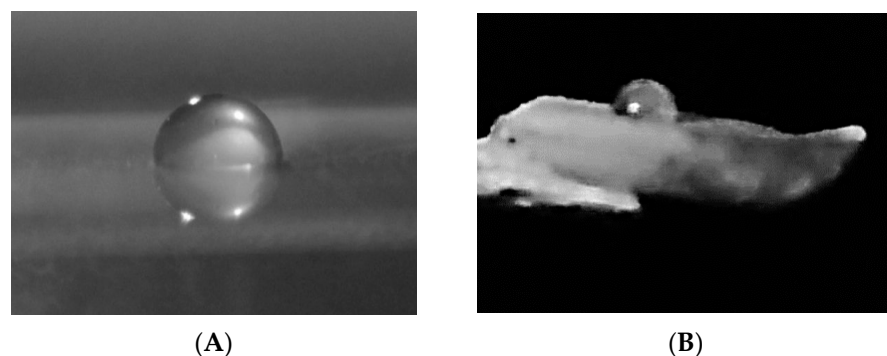
Commercial whey protein isolate (WPI) was obtained from Nature's Best (New York, NY, USA). Low methoxyl pectin (Pec) from citrus fruits, sorbitol, and all other reagents were purchased from Sigma (Steinheim, Germany). Microbial transglutaminase (mTG, Activa<sup>®</sup> WM), derived from the culture of *Streptoverticillium* sp., was supplied by Ajinomoto Co. (Tokyo, Japan). Roasted unsalted peanuts (variety, Virginia) were purchased from a local market. Reagents used for the coating solution were food grade products, other reagents were analytical grade commercial products.

### 2.1. Preparation of Coating Solutions

A WPI/Pec coating was prepared without (FFS) and with mTG (FFS + TG) as described by Davalos-Saucedo et al. [26]. The coating solutions produced were characterized for their wettability properties versus water, and tested as coatings on unsalted roasted peanuts.

### 2.2. Contact Angle

Drops of either solution (FFS and FFS + TG) were deposited on a glass microscope cover slip (Figure 1A) or on the internal surface of peanut cotyledons (Figure 1B), utilizing a microfluidic pump to keep the deposited volume constant at 8  $\mu$ L. Photos were taken using a Cannon Rebel T3 (12 MP color), with a lens EF-S 18–55 mm f/3.5–5.6. The camera was positioned in such a way that the drop and its reflection on the cover slip were observed. This view facilitated the identification of the solid–liquid–air interfaces.



**Figure 1.** Frontal view of a drop of water deposited on (A) a glass microscope cover slip, and (B) on a peanut cotyledon. The intersection of solid–liquid–air where the contact angle is measured is noticeable.

All images were converted to 8-bit gray scale and analyzed using the FIJI-ImageJ Drop Analysis LB-ADSA plugin to calculate the contact angle [27]. A cubic B-spline interpolation was performed to achieve sub-pixel resolution.

### 2.3. Peanut Coating

Approximately 1500 roasted unsalted peanuts seeds were separated into three groups and dipped for 10 sec in FFS, FFS+TG, and water as control. After dipping, all the samples were air-dried at room temperature for 10 min and finally, 20 randomly selected peanuts from each group were packed in thermally sealed low-density polyethylene bags (25 bags), and stored at room temperature. At each scheduled day of the study (0, 7, 14, and 50 days), two bags of peanuts were selected at random, opened and used for each test.

### 2.4. Color of Peanut Surfaces

A color reader (Model CR-20, Minolta, Tokyo, Japan) was used to determine the exterior color changes of the surface of the peanut samples. The color changes were quantified by the color values ( $L^*$ ,  $a^*$ ,  $b^*$ ), where  $L^*$  refers to the perceptual lightness of the peanuts, and ranges from black = 0 to white = 100. A negative value of  $a^*$  indicates green, while a positive one indicates red-purple color. Positive  $b^*$  indicates yellow, and negative, blue color [28,29]. The extent of color change of the peanut samples during storage was monitored as an indicator of storage stability of the coating materials. The color was measured on 10 peanuts randomly selected in each bag at each scheduled time, and the reported results ( $L^*$ ,  $a^*$ , and  $b^*$ ) are the mean of twenty determinations.

### 2.5. Water Content

Water content was determined as described by Rossi-Márquez et al. [30] by drying five peanuts down to a constant weight in a convection oven at 105 °C, according to the AOAC method [31]. Water content wet basis (w.b.) was calculated as follows:

$$\text{water content (\%)} = (\text{wet weight-dry weight})/\text{wet weight} \times 100 \quad (1)$$

The results were the mean of four determinations for each bag at each scheduled time.

### 2.6. Peroxide Value (PV)

Coated and uncoated peanuts (two bags for each scheduled time) were pressed using an Electric Automatic Oil Press Extractor (Fayelong, Hangzhou, China) to obtain oil. Peroxide value was measured according to the AOAC method [32] and was expressed as milliequivalents of oxygen per kilogram of oil.

### 2.7. Sensory Evaluation

A sensory evaluation of peanut samples was performed by presenting the different samples to a 10 member untrained sensory panel, as described by Rossi-Marquez et al. [30]. In particular, a triangular test (Table 1) was carried out to determine if consumers could distinguish between uncoated and coated samples (FFS, FFS + TG) randomly numbered and presented to the panel test at 0, 14, and 50 days of storage. Furthermore, a preliminary test to detect the overall acceptability of the coated and uncoated peanuts at different times of storage was performed by using a verbal three-point hedonic scale (dislike; neither like nor dislike; like) according to Meilgaard et al. [33]. At each scheduled day of the study, two bags of peanuts were opened, and four samples of peanuts were presented to each person.

**Table 1.** Triangle sensory evaluation design of uncoated and coated peanuts.

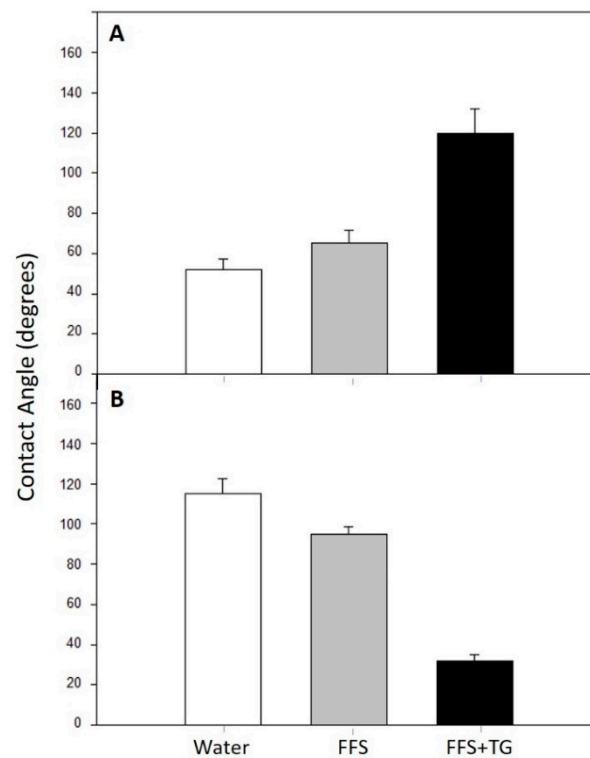
Test	Samples 1	Samples2	Sample3
A	Uncoated	FFS + TG	Uncoated
B	FFS	FFS	Uncoated
C	FFS + TG	FFS	FFS + TG

### 2.8. Statistical Analysis

All the experiments were carried out three times, and the results were analyzed using JMP version 8.0 software (SAS Institute, Cary, NC, USA). Statistical differences were obtained using the Tukey–Kramer test.

## 3. Results and Discussion

It has been reported that the hydrophobic nature of the surface of peanuts generates a dewetting of the hydrophilic coating solution after dipping, and moreover, shrinking and cracking of the coating may occur during drying, as well as flaking and peeling of the coating after drying [34]. To avoid these problems the wetting ability of FFS was tested both on glass and peanuts surfaces by measuring the contact angle of FFS crosslinked or not with mTG and compared with water. As seen on Figure 2, the FFS drops on the glass surface had a small but significant increase of contact angle ( $65^\circ \pm 3^\circ$ ) when compared to that of water ( $52^\circ \pm 3.5^\circ$ ), and which increased further in FFS + TG ( $120^\circ \pm 10^\circ$ ) corresponding to an increase on the hydrophobicity of the solutions. These effects were due to a decrease on the surface charges of the protein–pectin complexes occurring with the ionic interaction between the negative charges of pectin and the positive charges of proteins during the protein–pectin complexation. A further reduction of surface charges was found in FFS + TG due to the mTG mediated covalent binding among the polar aminoacidic residues, glutamine and lysine [25].



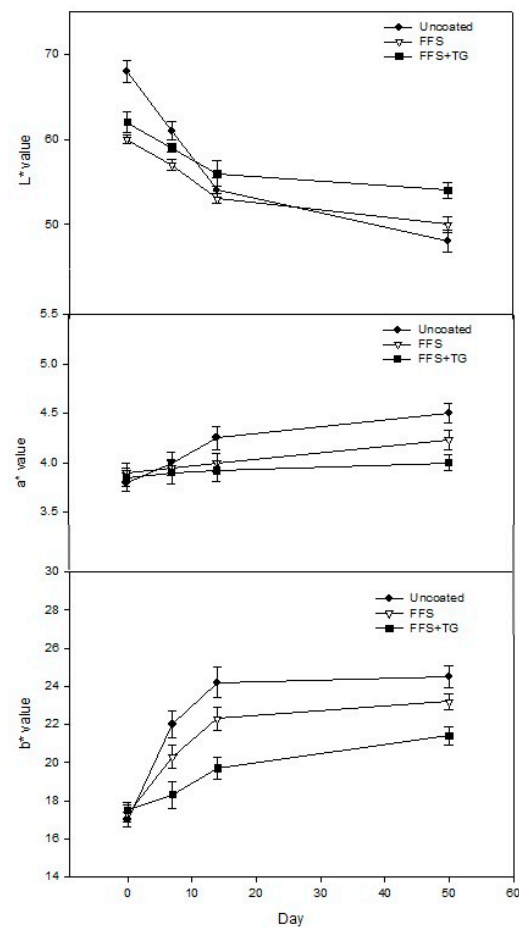
**Figure 2.** Contact angle of water (□), FFS (▒) and FFS + TG (■) on a glass microscope cover slip (A) and on a halved, trimmed roasted peanut (B).  $N = 3$ , values shown are mean  $\pm$  standard deviation.

The criterion of good adhesion of a coating (defined as wettability), is due to the attractive interactions across a coating–substrate interface that determine the adhesion between the two phases, and can be increased by improving intermolecular contact between the coating and substrate.

A good wettability of solid surfaces and consequently a homogeneous coating can be obtained when the contact angle of a coating solution applied on the surface to be coated is lowest. As seen in Figure 2B, the lowest contact angle on the peanut surface was obtained with FFS + TG. These results are in agreement with the increase of hydrophobic properties of FFS + TG, which increased its compatibility with the hydrophobic surface of peanuts, improving the adhesivity of the coating to the peanut surface and making a homogeneous coating surface.

### 3.1. Color of Peanut Surfaces

The color of foods is a fundamental parameter of the acceptability by the consumer. In some cases, it is associated with the oxidative effects that negatively affect the flavor and health effects of products. Figure 3 shows the  $L^* a^* b^*$  values of the uncoated and coated peanuts during 50 days of storage. In the uncoated samples, the  $L^*$  parameter decreased significantly, with a parallel increase in  $a^*$  and  $b^*$  values during 50 days of storage, confirming the oxidative process, with a change of peanut color to dark red.



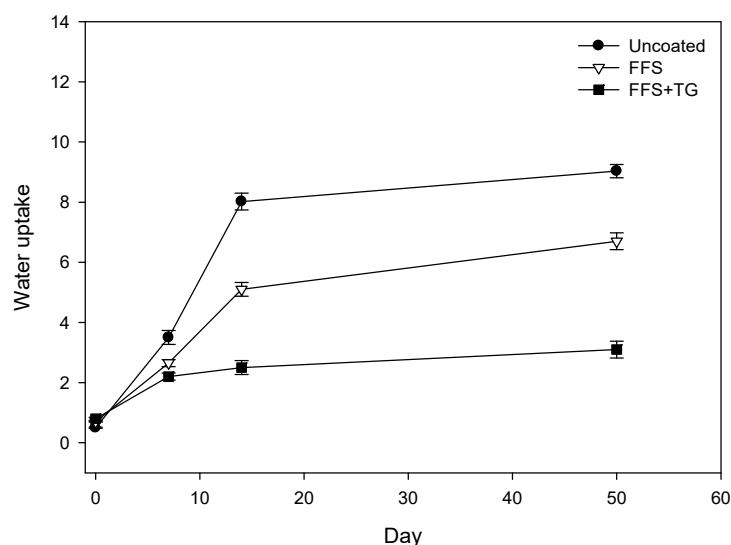
**Figure 3.** Color measurement of peanut surfaces for uncoated and coated peanuts with FFS or FFS+TG during 50 days of storage.  $N = 30$ , values are mean  $\pm$  standard deviation.

Both FFS or FFS+TG coatings, when applied on the peanuts surface, decreased the peanut's perceived lightness ( $L^*$ ) with respect to the control, due to the thin coating layer that reduces the light reflection, while no changes were observed in  $a^*$  and  $b^*$  values due to the transparency of the coatings. During storage, the results show that the coatings were able to counteract the decrease in lightness of the peanuts and the increase of red ( $a^*$ ) and yellow ( $b^*$ ), with a more significant effect from FFS+TG coatings, thus preserving the peanuts from developing red-dark color due to the oxidative process [35].

### 3.2. Water Content

There is a practical interest in the water content (WC) of the peanut kernel because the texture of the product is affected, as the desirable crispness is lost when the water content exceeds 2.9% (w. b.) [36]. Analyzing the WC in our samples (Figure 4) we observe that the coatings application did not affect it significantly ( $p < 0.05$ ), and the WC was within the range of consumer demand (0.4–1.6% w. b.). The WC increased linearly during the first 14 days of storing, with a slope that decreased from the uncoated (8.5% w. b.) to FFS-TG coated samples (2.4% w. b.).





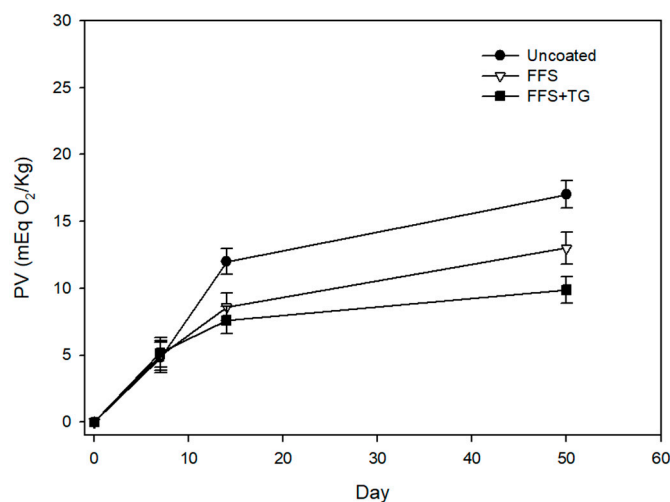
**Figure 4.** Water content (%) of uncoated and coated peanuts with FFS or FFS+TG during 50 days of storage.  $N = 24$ , values shown are mean  $\pm$  standard deviation.

Generally, when the water content value exceeds 8% (w. b.) conditions are very close to the danger zone for mold growth [37]. Figure 4 shows that uncoated samples reached 8% (w. b.) after two weeks. The FFS+TG coated peanuts preserved a qualitative value between 2.5–3.0% (w. b.) during 50 days of storage. It is known that packaged peanut kernels, if not packed under vacuum, seek their respective hygroscopic equilibrium moisture contents depending on the surrounding humidity. As the surrounding humidity in the packed samples was equivalent, the lowest increase in WC in the FFS+TG coated samples confirms the higher water vapor barrier properties of the FFS+TG coating [23,24,26,30]. Moreover, keeping a low water uptake over time can help to reduce the lipid oxidation, because water can solubilize prooxidants and can influence the formation of lipid oxidation secondary products [38]. Our results showed that the presence of the enzymatically reticulated edible coating could act as a barrier to water, as expected based on previous studies [23,24,30].

### 3.3. Peroxide Value (PV)

Peroxide value (PV) is the measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation, and can be defined as a reaction between fatty acids and oxygen, which results in oxidative degradation of lipids [39]. Thus, the presence of an edible coating on the surface of roasted peanuts can minimize the exposure to oxygen, consequently decreasing the oxidation of lipids [40].

The end of the shelf life was considered to be the time at which the PV reached 10 meq/kg of oil [41]. As reported in Figure 5, the peroxide value of all samples increased during 50 days of storage. For the coated samples, with and without enzyme, the increase on the peroxide value was significantly lower than the uncoated peanuts. These results are in agreement with Haq and Hasnain [42] and Riveros et al. [43] who applied a carboxymethyl cellulose (CMC) coating containing gum cordia and a whey protein coating, respectively, to peanuts and demonstrated the effect of the coating on the reduction of the PV. Similar results were obtained by Kazemian-Bazkiaee et al. [21] who applied an edible coating based on chitosan and  $\beta$ -glucan, and showed a significant decrease in the peroxide value in coated peanuts in the first 60 days. Boghori et al. [44] used a whey protein concentrate and different concentrations of Shiraz-thyme essential oil demonstrating that the coated samples had a lower peroxide concentration than the control sample.



**Figure 5.** Peroxide value (PV) of uncoated and coated peanuts with FFS or FFS+TG during 50 days of storage.  $N = 6$ , values shown are mean  $\pm$  standard deviation.

The peroxide value limit for the acceptability of roasted peanuts is from 20 to 30 mEqO<sub>2</sub>/Kg [39]. After 50 days, the sample FFS + TG remained under the limiting value, as well as below after coating without the enzyme; this effect can be explained due the formation of covalent bonds between proteins by the transglutaminase. Our results are in agreement with Wambura et al. [45], who applied different edible coatings to roasted peanuts and achieved a reduction in lipid oxidation compared to uncoated peanuts. Moreover, the presence of the edible coating (with or without TGase) had a barrier effect against vapor and water, which helped to reduce the oxidation effect on the peanuts. Similar results were obtained in previous studies using this coating formulation [23,24,30].

### 3.4. Sensory Evaluation

Finally, a preliminary sensory evaluation of uncoated, FFS and FFS+TG coated samples was performed. The results were analyzed using the cumulative binomial probabilities according to Rayner et al. [46], and by assuming that the panel was unable to detect significant differences at  $p < 0.05$  among the samples if the correct responses were less than the minimum of the correct responses expected [47].

The results in Table 2 show that the panelists were not able to detect differences among the three samples of peanuts tested immediately after coating drying, suggesting that the coating did not affect the sensory properties of the peanut samples. Conversely, after 14 days, nine out of 10 (Test A) and seven out of 10 (Test B) panelists seemed to significantly identify the differences among the uncoated samples and the FFS+TG or FFS samples, while no differences were appreciated between the FFS and FFS + TG samples (Test C). Similar effects were observed at 50 days of storage. These results suggest strong differences in the sensory properties among the uncoated and coated samples, while no differences were detected between FFS and FFS + TG samples. Researchers have mentioned that the presence of an edible coating based on proteins and polysaccharides does not alter either the natural taste or texture of food [40].

Finally, the preliminary results on the sensory evaluation of general preference (Table 3) show that the acceptability of the uncoated samples decreased from 80% to 0% during the 50 days of storage, similarly, the acceptability of the FFS coated samples decreased to 20% after 50 days. Furthermore, when the acceptability of the FFS-*m*TG coated samples was tested, a large number of consumers (>50%) reported liking the FFS-*m*TG coated samples from 0 to 50 days of storage.



**Table 2.** Triangle sensory evaluation of uncoated and coated peanuts.

Storing Time (Days)	Test	No. of Subjects	No. of Correct Responses	Minimum of Correct Responses at 5% Level [41]
0	A	10	3	7
	B	10	4	7
	C	10	2	7
14	A	10	9	7
	B	10	7	7
	C	10	3	7
50	A	10	10	7
	B	10	9	7
	C	10	4	7

**Table 3.** Hedonic evaluation of general preference of uncoated and coated foods.

Test	Storage Days		
	0	14	50
Preferences (%)			
Liked uncoated	82	22	0
Liked FFS	65	33	21
Liked FFS + <i>mTG</i>	72	65	53

#### 4. Conclusions

We demonstrated that coating with an edible whey protein–pectin film prepared in the presence of the enzyme *mTG* resulted in a useful method to improve the shelf life of roasted peanuts. The crosslink made by *mTG* decreases the surface charge of the whey protein–pectin complex, increasing the wettability of the hydrophobic surface and improving the adhesivity of the coating to the surface. Thus, reduction of water uptake and oxygen permeability preserves the crunchy texture, and the reduction of oxidation of peanuts, improving consumer acceptability.

The reported processes can preserve the quality of roasted peanuts after the opening of the primary packaging by protecting the onset of rancidity and avoiding the loss of crunchiness.

**Author Contributions:** Conceptualization, G.R.-M., P.D.P. and C.A.D.-S.; Methodology, P.D.P., G.R.-M. and M.H.; Validation, P.D.P., C.A.D.-S. and M.B.; Formal Analysis, C.A.D.S., P.D.P., G.R.M., M.H. and M.B.; Investigation, C.A.D.-S., G.R.-M. and P.D.P.; Writing-Original Draft Preparation, C.A.D.-S. and G.R.-M.; Writing-Review & Editing, P.D.P., G.R.-M., M.H. and C.A.D.-S.; Project Administration, P.D.P. All authors have read and agreed to the published version of the manuscript.

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