

Chocolate Coating Effect on Whey Protein Isolate-Pullulan-Coated Freeze-Dried Chestnut

Mahamadou Elhadji Gounga^{1,*}, Khamis Ali Omar², Issoufou Amadou¹, Shi-Ying Xu²

¹Département des Sciences et Techniques de Productions Végétales, Faculté d'Agronomie et des Sciences de l'Environnement, Université Dandicko Dankoulodo de Maradi, BP 465, Maradi, Niger

²State Key Laboratory of Food Science and Technology, Synergetic Innovation Center of Food Safety and Nutrition, School of Food Science and Technology, Jiangnan University, Wuxi 214122, Jiangsu, China

*Corresponding author: bgounga@yahoo.fr; gounga.mahamadou@uddm.edu.ne

Abstract Chestnuts are characterized by a limited shelf-life because of their high water content and sugar content hence producers around the world are confronted with a storage problem as product losses are very high. The strategy of the conception, stemming from the current adoption of new food technologies combined with the consumer desirability to put to shelf new products on the market has made researchers and industrialists to upgrade and/or add value to the existing products in order to provide a wider choice of new products to the consumer disposal. The objective of this work was to come up with a suitable technology of extending the shelf-life of harvested chestnut fruits and to develop a new food product for commercial consumption. Whey protein isolate-Pullulan-coated roasted and freeze-dried chestnut (WPI-Pul-RFDC) was coated separately with dark chocolate (DCC) and milk chocolate (MCC). Color change of surface coating and decay incidence were studied at [7°C, 82 ± 5% RH] and [25 ± 2°C, 30 ± 2% RH] for 6 months storage. Further investigation on sensory evaluation were carried out using a taste panel of 120 consumers. 7°C and 25°C provided the best storage conditions for dark chocolate and milky chocolate coating respectively. The results obtained were effective in the control of overgrowth of spoilage organisms and surface discoloration, which is satisfactory in improving the quality and increasing the shelf-life of chestnut. The consumer acceptance testing revealed that chocolate-coating greatly improved the sensory attributes of chestnut as compared to the WPI-Pul coated sample acceptance. This is an alternative strategy to add value to chestnut thus minimizing the significant losses in harvested fruits hence providing a wider choice of new products to the consumer disposal.

Keywords: chestnut, chocolate coating, color change, shelf life, sensory evaluation

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1. Introduction

Nowadays, the social view of chestnuts has undergone a curious transformation, losing the traditional image of food for the poorest and becoming an ingredient of dishes and culinary preparations characterized by a high degree of sophistication.

In order to store chestnuts, different methods are used depending on the technical opportunities, food consumption and food processing methods. There are traditional methods, cold storage, frozen storage and drying [1,2,3].

More consumers are looking for convenience in the form of new product processed from fruits and vegetables. The market demand for high quality, longer shelf-life and ready-to-eat foods has undergone rapid expansion in recent years due to life styles, increasing purchase power and health conscious consumers. More so, the strategy of the conception, stemming from the current adoption of new food technologies combined with the consumer

desirability to put to shelf new products on the market has made researchers and industrialists to upgrade and/or add value to the existing products in order to provide a wider choice of new products to the consumer disposal.

Edible coatings in general and chocolate coating in particular may have that potential for application in the food industry to serve this purpose [4-13]. Whey protein isolate-based edible films and coatings have been utilized in coating peanuts [14,15,16,17,18] and walnuts [18] in order to improve appearance and increase shelf-life.

In our previous studies, roasted chestnuts were freeze-dried and coated with whey protein isolate-pullulan (WPI-Pul) edible coatings. The results were effective in the control of overgrowth of spoilage organisms and surface discoloration [19]. More so, WPI-Pul coatings improved greatly the sensory attributes of fresh and dried chestnuts [20].

Chocolate has long been used industrially to coat several nut products such as peanut, hazelnuts, cashews, raisins and almonds [6,21,22]. It is a highly nutritious energy source, with a fast metabolism and good digestibility [23]. Cocoa butter is the important ingredient

as it dictates the main properties (gloss, texture and mouth feel) of the chocolate [23,24]. The mouth feel and release of flavor are due to the sharp melting range of cocoa butter just below body temperature. Today, many applications of chocolate coatings to heterogeneous foods are applied, developed, or tested by industrial firms, but little data are available in the scientific and patent literatures.

However, chocolate coating has not been applied on WPI-Pul coated chestnut to develop a commercial and/or industrial product. The objective of this study was to further coat the WPI-Pul-coated chestnut with chocolate in order to extending the shelf-life of harvested fruits and to develop a novel food product for commercial consumption. The color changes that occur during storage and decay incidence were investigated.

Consumer perception is crucial for effective design and the marketing of food products hence it often determines their acceptability, especially for new products. If the chocolate-coated-chestnut is going to be used commercially in the near future, and given that new food products are meant to respond to consumer's acquired expectations, information on sensory attributes of chocolate-coated chestnuts is of utmost importance. Therefore, the 2nd objective of this work was to identify and compare the descriptive sensory properties of both dark chocolate- and milk chocolate-coated chestnuts in order to commercialize them as new products.

2. Materials and Methods

2.1. Chestnut Samples

Freshly roasted chestnuts (*Castanea molissima*) were purchased from a local chestnut shop (Jin Li Wang) in Wuxi, China. The fruits were peeled and frozen at -20°C for 72 h. Samples were then dried for 72h using a freeze-drier (Labconco Corporation, Kansas, USA). After freeze drying, the samples were transferred into a tightly closed plastic bag, and kept in a desiccator containing silica gel (0%RH) until required for use.

2.2. WPI-Pul Coatings

The freeze-dried chestnuts were coated with WPI-Pul coatings as described in Gounga, Xu and Wang [20]. Samples were dried for 30 min before further coating with chocolate.

2.3. Chocolate Coatings

The WPI-Pul-coated chestnuts were doubly coated separately with dark chocolate and milk chocolate. Dark chocolate was prepared using 45% cocoa mass, 13.5% cocoa butter, and 41.5% sucrose, while 10% cocoa mass, 29.55% cocoa butter, 8% cocoa powder, 33.5 sucrose, 14% milk powder and 4.5 whey powder were used in milk chocolate formulation. 0.4% soy lecithin and 0.05% vanillin were used in both formulations. The coating was performed with a pan coater (Suzhong Pharmaceutical Machinery Co. Ltd, Taixing, China) with a diameter of 1m. It is a conventional coater used in the confectionery

industry. The coating formulation was ladled onto the WPI-Pul-coated chestnuts and evenly distributed by rotating the pan at 28 rpm. The rotation was stopped once the chocolate was completely applied, then the drying process was started. The batches were dried in the pan using cool air (19°C). The drying process consisted of drying 1 side of the batch, then 180° rotation of the pan to flip the sample batch to the other side, then drying that side. The drying cycle was about 10 to 20 min each side, with about 4 to 6 flips. Glazing was done using gum solution. Figure 1 summarizes the schematic flow diagram of the formulation preparation of the chocolate-coated chestnut.

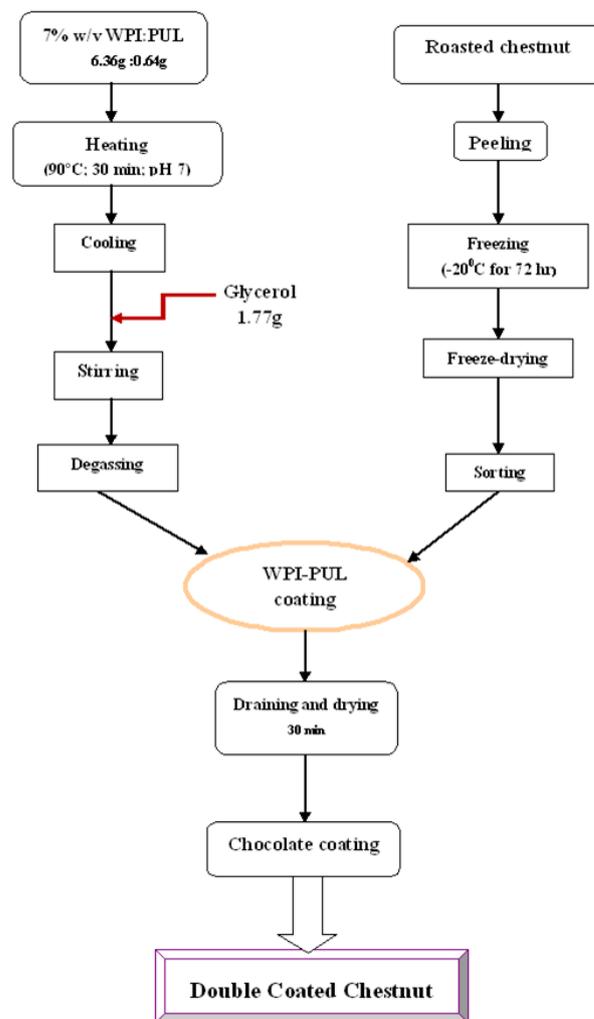


Figure 1. Flow chart for the production of chocolate coated chestnut

Dark chocolate-coated chestnuts (DCC) and milk chocolate-coated chestnuts (MCC) were examined for surface color change and decay incidence. A commercialized product of chocolate-coated peanut was provided by Liangfeng Food Group Import and Export Co., Ltd (Zhangjiagang, Jiangsu, China) for use as a reference sample (REF).

2.4. Colorimetric Measurements

A Minolta (Model CR-400, Minolta Co. Ltd., Osaka, Japan) was used periodically to measure the CIELAB color parameters, L^* , a^* and b^* in order to evaluate the external color evolution of chocolate coated chestnuts

during 0, 7, 14, 30, 60, 90, 120, 150 and 180 d storage at [7°C, 82 ± 5% RH] and [25 ± 2°C, 30 ± 2% RH]. Each measurement was taken at three locations of three replicates for each sample. A standard white calibration plate ($L_0 = 96.94$; $a_0 = 0.12$ and $b_0 = 1.68$) was employed to calibrate the equipment. Results were also reported as Hue angle and Chroma which are usually used to describe color change during storage and expressed respectively by the following equations [25]:

$$\text{Chroma} = (a^2 + b^2)^{1/2} \quad (1)$$

$$\text{Hue angle} = \tan^{-1}\left(\frac{b}{a}\right) \quad (2)$$

2.5. Decay Incidence (DI)

A sample was considered decayed when a visible surface lesion or mark or mycelial development was observed. The decay incidence was evaluated in triplicates on one thousand and five hundred fruits each and expressed as percentage of fruit infected. Chocolate-coated chestnuts were examined for visible decay after 0 30 60 120 180 days of storage at [(7°C, 82 ± 5% RH) and (25 ± 2°C, 30 ± 2% RH)]. To minimize contamination among fruits, ten coated nuts were put in a PVC bag for each sample (DCC, MCC, and REF). The product samples are different but the method is similar and adapted from previous work of Gounga et al. (2008) [19].

2.6. Consumer Acceptance Testing

Consumers were solicited among students of different study class categories of the School of Food Science and Technology of Jiangnan University, Wuxi, China. Tasting was carried out under white light in a large sensory analysis laboratory equipped with individual testing booths. Prior to tasting, panelists were asked a series of questions used to gather demographic data, including gender, degree of study (Bachelor, Master or PhD) and their origin. One hundred and twenty panelists (66 male and 54 female of which 42 Bachelor, 46 Masters and 32 PhD candidates) participated voluntarily in the study without remuneration.

Panelists seated at partitioned booths were asked to evaluate two samples for acceptability of attributes including color, sweetness, texture/appearance, crunchiness/mouth feel, flavor and overall acceptance using a structured 10 point intensity scale where 10 indicated the highest score and 1 the lowest score of the attribute being assessed with the possibility to indicate half point (Figure 2). The samples were presented in coded white plastic bowls: A (DCC) and B (MCC), each one containing 3 to 4 pieces. Consumers were asked to evaluate sample A, then B. An extra cup of pure water was provided for rinsing between samples. Evaluations were performed on whole product. Following the sensory testing, participants were asked to complete the additional demographic questions (Table 1) focused on buying intent and preference.

Cross the appropriate field on the scale X

1. Color	<div style="border: 1px solid black; display: flex; justify-content: space-between; width: 100%; height: 20px; margin-bottom: 5px;"></div> <div style="display: flex; justify-content: space-between; width: 100%; font-size: small;"> Dislike extremely neither like nor dislike like extremely </div>
2. Sweetness	<div style="border: 1px solid black; display: flex; justify-content: space-between; width: 100%; height: 20px; margin-bottom: 5px;"></div> <div style="display: flex; justify-content: space-between; width: 100%; font-size: small;"> Flat fairly sweet sweet </div>
3. Texture/ Appearance	<div style="border: 1px solid black; display: flex; justify-content: space-between; width: 100%; height: 20px; margin-bottom: 5px;"></div> <div style="display: flex; justify-content: space-between; width: 100%; font-size: small;"> Dislike extremely neither like nor dislike like extremely </div>
4. Crunchiness/ Mouth feel	<div style="border: 1px solid black; display: flex; justify-content: space-between; width: 100%; height: 20px; margin-bottom: 5px;"></div> <div style="display: flex; justify-content: space-between; width: 100%; font-size: small;"> Coarse medium fine, melts in the mouth </div>
5. Flavor	<div style="border: 1px solid black; display: flex; justify-content: space-between; width: 100%; height: 20px; margin-bottom: 5px;"></div> <div style="display: flex; justify-content: space-between; width: 100%; font-size: small;"> Non typical neutral typical </div>
6. Acceptance	<div style="border: 1px solid black; display: flex; justify-content: space-between; width: 100%; height: 20px; margin-bottom: 5px;"></div> <div style="display: flex; justify-content: space-between; width: 100%; font-size: small;"> Dislike extremely neither like nor dislike like extremely </div>

Figure 2. Questionnaire for sensory evaluation

Table 1. Change in chroma and Hue angle values of chocolate-coated chestnut during storage

Sample	Temp (°C)	Storage time (d)									
		0	7	14	30	60	90	120	150	180	
Chroma	DCC	7	5.36b	5.09c	5.13bc	5.24bc	5.17bc	5.07c	5.20bc	5.50ab	5.72a
		25	4.93a	4.87a	4.69cd	4.66cd	4.72bc	4.56de	4.46e	4.82ab	6.75f
	MCC	7	11.95a	11.84ab	11.53bc	11.26dc	11.19dc	10.73e	10.91de	11.13cd	11.15dc
		25	11.98b	11.80bcd	11.70de	11.78bcd	11.83bcd	11.72cde	11.53e	11.96bc	12.61a
	REF	7	12.97a	12.80a	12.42b	12.23c	12.06d	11.71ef	11.59f	11.86e	12.12d
		25	12.49a	12.39a	12.43a	12.31a	12.44a	12.01b	12.03b	12.44a	12.86a
Hue	DCC	7	40.00ab	39.16b	40.40ab	42.24ab	41.48ab	41.42ab	43.12a	41.84ab	41.0ab
		25	39.96cd	40.79bc	38.55d	40.17bc	41.53bc	40.88bc	40.08c	41.71b	43.41a
	MCC	7	46.32a	43.93b	42.42c	41.94c	42.03c	40.83d	41.16d	42.28c	42.73b
		25	46.71a	46.01a	45.82a	46.33a	46.67a	46.12a	45.93a	46.44a	47.06a
	REF	7	45.95a	45.70a	44.03b	43.45bc	43.68bc	43.04c	43.02c	44.11b	44.85b
		25	47.23b	47.07b	47.10b	47.25b	47.90c	46.90b	47.33c	47.20b	48.39a

DCC: Dark chocolate-coated chestnut; MCC: Milk chocolate-coated chestnut; REF: Reference sample consisted on peanut-coated milk chocolate used as control.

Any two means in the same row followed by the same letter are not significantly different ($p > 0.05$).

2.7. Statistical Analysis

All measurements were conducted in triplicate. The statistical analysis was performed using SAS Software (SAS 8.1 for window, SAS Inc., Cary, NC, USA). A one-way analysis of variance and Duncan's multiple range tests were conducted to determine the specific differences between means.

In order to evaluate consumer tests, difference among the two samples were explored through a one-way analysis of variance, ANOVA followed by a separation of means test using Fisher's Least Significance Difference LSD. A $p < 0.05$ was considered statistically significant in all tests.

3. Results and Discussion

3.1. Color Change in the Chocolate-coated Chestnut Surface

Color is one of the most important attribute of foods, being perceived as a quality and acceptance indicator. It determines the assessment of external quality in food industries and in food engineering research [26].

3.1.1. Lightness

L^* is the luminance or lightness component which ranges from 0 (black) to 100 (white). The changes of lightness values in chocolate coated chestnuts during storage period are given in Figure 3. The results of the ANOVA and DMRT showed that all L^* values increased significantly ($p < 0.05$) between 0 d and 7 d of storage, after then L^* remained almost constant ($p > 0.05$) for all tested samples in accordance with the visual impression of

surfaces. This could be attributed to the drying of the surface glazing [27,28] after which the coated chocolate found its brightness. A similar trend was observed with the reference sample in both storage conditions [7°C , $82 \pm 5\%$ RH] and [$25 \pm 2^\circ\text{C}$, $30 \pm 2\%$ RH]. The changes in L^* value in tested and reference samples as affected by time (x) are described by the following equations:

$$\text{DCC } 7^\circ\text{C}: y = -0.0589x^2 + 0.8x + 21.59 \quad (R^2 = 0.952) \quad (3)$$

$$\text{DCC } 25^\circ\text{C}: y = -0.048x^2 + 0.58x + 22.83 \quad (R^2 = 0.939) \quad (4)$$

$$\text{MCC } 7^\circ\text{C}: y = 0.032x^2 - 0.01x + 28.68 \quad (R^2 = 0.986) \quad (5)$$

$$\text{MCC } 25^\circ\text{C}: y = 0.1531x + 30.23 \quad (R^2 = 0.934) \quad (6)$$

$$\text{REF } 7^\circ\text{C}: y = 0.3436x + 28.36 \quad (R^2 = 0.950) \quad (7)$$

$$\text{REF } 25^\circ\text{C}: y = 0.0076x^2 + 0.059x + 30.65 \quad (R^2 = 0.898) \quad (8)$$

After 150 d, the sample stored at 7 and 25°C still had a glossy appearance, but L^* tended to decrease. Since it is a measure of the color in the light-dark axis, this falling value indicates that samples were turning darker, which did not affect the visual appearance. The result agrees with the findings of Simonot & Elias [29] who reported that the apparent color of the object changes when the surface of a smooth-colored object becomes rough. In their investigations on chocolate, Briones, Aguilera, & Brown [30] confirmed that gloss of chocolate surfaces diminished exponentially as roughness increased while lightness decreased linearly. All samples stored at 25°C were brighter than those at 7°C . This could be due to the high RH (82%) in the refrigerated storage [31].

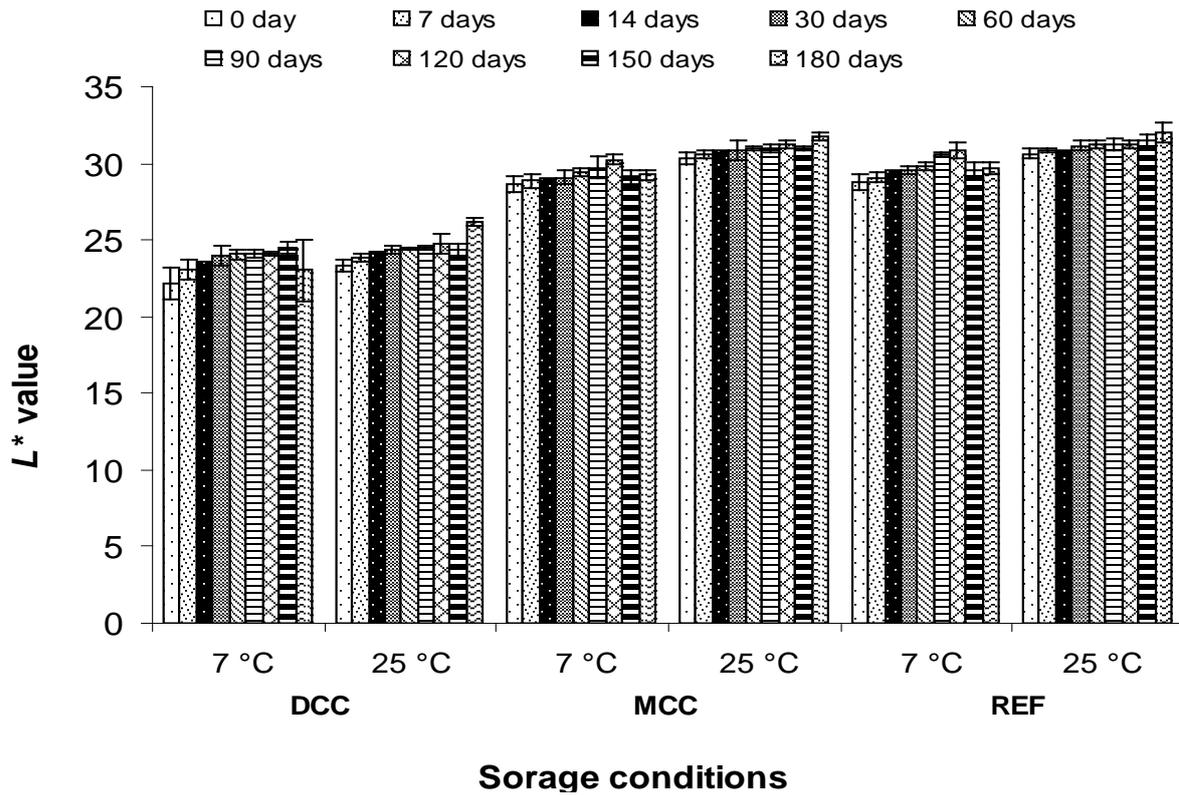


Figure 3. L* values of chocolate coated chestnut surface during storage as measured by a Minolta colorimeter

3.1.2. Chroma and Hue angle

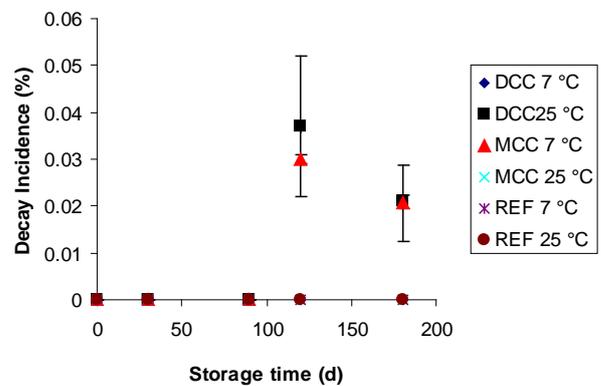
Chroma and Hue angle which are a combination of the two chromatic components *a** (redness) and *b** (yellowness) values, are colorimetric parameters extensively used to characterize the variation of colors in foods during processing and/or storage. Table 2 shows changes in surface color of chocolate-coated-chestnut during 180 days of storage at 7 and 25°C. The chroma value indicates the degree of saturation of color and is proportional to the strength of the color. Generally, chroma did not change in all samples. However a low decrease was observed in MCC 7°C and REF 7°C. Since REF was constituted of peanut-coated milk chocolate and used as control, the result showed that REF and MCC had similar behavior.

A decrease in chroma in MCC 7°C and REF 7°C during storage was generally accompanied by a decrease in colorimetric *b** value (data not shown), which indicated reduction in yellowness of samples and a decrease toward a brighter chroma. In other words, the differences in chroma might reflect the differences in yellow color which are likely due to the apparent color of the milk chocolate surface covering the nuts. Maskan [25] reported similar observations when investigating color change of kiwifruit during drying. The Hue angle values also did not show significant change in most of samples. As for chroma, Hue angle decreased in MCC 7°C during storage. The results confirm that major color differences were induced in samples as surface roughness increases. More so, dark or high-chroma objects are particularly affected by changes in gloss, whereas high-lightness objects are not. This indicates stability of surface color when glossing was applied [10,32,33].

All in all, the results presented in this work suggest that the changes in *L** and *b** values were small as compared to uncoated freeze-dried chestnut [18]. This may not contribute significantly to perception of color change.

3.2. Decay Incidence

The early signs of visible decay appeared in DCC 25°C and MCC 7°C after 120 days of storage (0.037% ± 0.015 and 0.03% ± 0.0011 respectively). At the end of 180 d of storage, 0.021 ± 0.0015% DCC 25°C and 0.0206 ± 0.008% MCC 7°C were infected by molds, while DCC 7°C, MCC 25°C and REF showed no visible signs of decay (Figure 4). 7°C and 25°C provided then the best storage conditions for dark chocolate and milky chocolate coating respectively.



Vertical bars represent standard deviation of the means.

Figure 4. Decay incidence of chocolate-coated chestnut and reference stored at 7°C, 82% RH and 25°C, 53% RH

The decay incidence was reduced significantly ($p < 0.001$) compared to WPI-Pul-coated freeze-dried chestnut [19]. The percentage of damaged fruits was 13.44% at the end of 120 d of storage (20°C, 53% RH); that is, 363 times higher than the corresponding value in case of DCC stored at 25°C 30% RH. This could be attributed to the nature of chocolate, which is traditionally known as microbiological stable and safe to eat food [21].

3.3. Sensory Evaluation

Sensory tests provide useful information about the human perception of products due to ingredients, processing, packaging, or shelf-life.

3.3.1. Sensory Profiles of Chocolate-coated Chestnuts

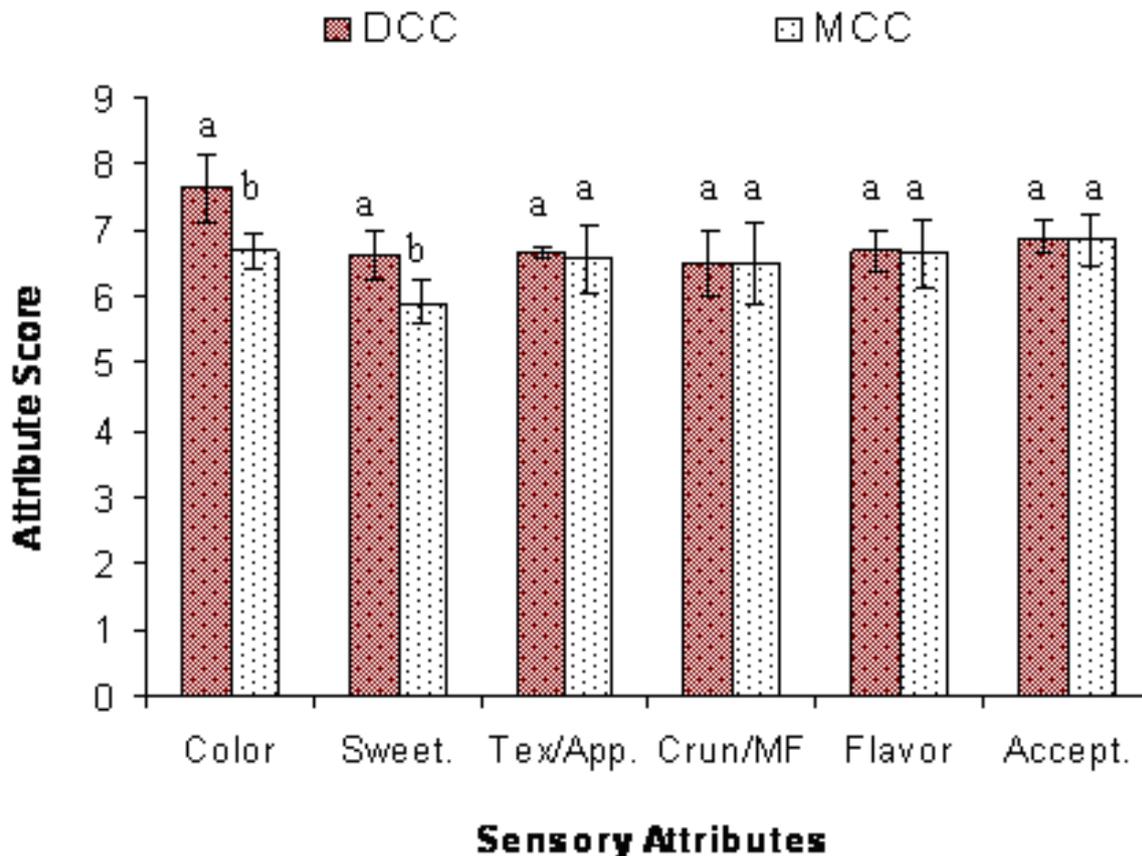
Mean sensory ratings for dark chocolate and milk chocolate coated chestnuts subjected to sensory evaluation are shown in Figure 5. Dark chocolate-coated chestnuts (DCC) and milk chocolate-coated chestnuts (MCC) received similar liking scores for texture/appearance, crunchiness/mouth feel, flavor and overall acceptability. However analysis of variance showed a significant effect ($p < 0.05$) of chocolate-coating for 2 attributes (Table 3), suggesting that color and sweetness differed with respect to both original and compound chocolate. The darker color of DCC was liked significantly more ($p < 0.05$) than the color of MCC. Also, the sweetness level of DCC was found to be strongly liked than the chestnut coated with

milk chocolate. These results suggest that consumers may be able to perceive a difference in the sweetness levels of the 2 products.

Assuming that the panelists were asked to judge the whole product, as mentioned previously, the freeze-dried chestnut, whether coated with original chocolate or compound chocolate, was found to be sweet. That could be due to the sugar content in both chocolate formulations, in addition to the sweet taste of roasted chestnut [34]. The result agrees with the findings of Künsch et al. [32] who reported that original chocolate, whether white or dark, is sweeter than milk chocolate. The consumers were also naturally more attracted by the dark original color of chocolate.

Chocolate texture and appearance are key attributes in consumer choice and acceptability even though flavor is frequently judged important in product identification [35]. The likeness related to the texture/appearance of the 2 samples was not significantly different ($p > 0.05$) with total score of 6.91 (DCC) and 6.86 (MCC). However chocolate coatings affected greatly the acceptance of RFDC when compared to the WPI-Pul-coatings [20].

Proper analysis of the data is a critical part of sensory testing. Data generated from human observers is often highly variable. There are many sources of variation in human responses that cannot be completely controlled in a sensory test. Table 2 presents the LSD result of all sensory attributes. There was a high significant difference in color for DCC and MCC ($p < 0.001$) while difference was only observed at 0.01 in sample's sweetness.



Different letters above bars signify significant ($P < 0.05$) differences in means.

Figure 5. Consumer acceptance scores (n = 120) of chocolate-coated chestnut

Table 2. Analysis of variance and LSD parameters for all attributes^a

Source of variation	DF	Sensory Attributes					
		Color	Sweetness	Texture/ Appearance	Crunchiness/ Mouth feel	Flavor	Acceptance
Mean Square	1	54.626	29.751	0.477	0.0042	0.817	0.15
Error	238	3.194	5.481	3.929	0.748	4.801	4.479
F value	-	17.10	5.43	0.12	0.0	0.17	0.03
P value	-	< 0.001	0.0207	0.728	0.976	0.680	0.855
LSD ^b	-	0.381*	0.449*	0.423*	0.464*	0.467*	0.451*
		0.455**	0.595**	0.504**	0.554**	0.557**	0.538**
		0.599***	0.785***	0.664***	0.730***	0.734***	0.709***

^aEach value represents the mean value of 120 determinations (N = 120).

^bLSD values at different significance levels *10%; ** 5%; ***1%

Table 3. Demographic questions and distribution percentage of the answers

Demographic questions	Answer and distribution percentage				
	Often	Occasionally	Rarely	Never	
Q1. Do you buy chestnuts out of season?	5	46.7	35	13.3	
Q2. Would you prefer a ready to eat chestnut to a non peeled one?	Yes	No		Not sure	
	56.7	25		18.3	
Q3. Would it affect your buying decision to know that the chestnuts were dipped in natural ingredients for the purpose of extending shelf-life?	Yes	No		Don't care	
	42.5	29.2		28.3	
Q4. When you find in the market chestnut coated with chocolate, would you buy because of:-	Chestnut	Chocolate		Both	
	10.8	20.8		68.4	
Q5. Which factor (Attribute) does affect more your buying decision?	Color	Sweetness	Texture	Crunchiness	Flavor
	10.5	13.3	26.7	32.4	39
Q6. Would you prefer chocolate-coated chestnut with a milk taste?	Yes	No		Don't care	
	60.8	17.5		21.7	

3.3.2. Demographic Survey Results from Consumers

Of the total 120 consumer panelists who participated in this study, 45% were women. This is not significant proportion knowing that, in China, women consume chestnut more than men. The nut is very much appreciated in university environment, especially by students. When asked whether they buy chestnut out of season, 51.7% of the consumers responded either "often" or "occasionally," 35% responded "rarely," and 13.3% responded "never." There were 6 categorical questions, which are listed in Table 3. For the 6 categorical questions, there was generally a significant difference in the distribution of the number of consumers who chose one category over the others. The results revealed that a significant majority of the consumers appreciated the chocolate-chestnut so the majority of them were favorable in their liking of this product. This resulted in "Both" in their purchase intent for chocolate (only) or chestnut (only) or both of them. Also, a significant majority of the consumers expressed "strong preference" for the product associated with milk taste, thus their purchase intent was significantly high. The results from the product survey indicate that the information collected from the panelists could be used for

advertisement and marketing purposes to promote the new processed product.

All in all, it is important to note that the results presented here could be considered satisfactory estimates of the sensory profiles of the products. They may be therefore used in Principal Component Analysis (PCA).

4. Conclusion

The investigation of color change, decay incidence and sensory attributes, as the main factors that affect selection of food, has been successfully carried out and provides information about physical characteristics of chocolate-coated chestnut as a new product. The results showed a wide range of values for most of the elements studied which depended on the type of coating materials (original or compound chocolate). It can be concluded from this study that, chestnut, as other nuts and nut products, could be coated with chocolate for commercial use hence it has the potential for technological applications in confectionary industries. This is a pioneer study and the information obtained is very important in order to develop a kind of product from food which is consumed by a large

proportion of the population, since sensory information reduces risk in decisions about product development and strategies for meeting consumer needs. Further studies should focus deeply on the investigation of nutritional and microbial evaluations of the product for commercial use at industrial level.

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