A CHITOSAN-GELATIN BLEND AS A COATING FOR FISH PATTIES

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Abstract

A coating made in cold from a blend of a chitosan and a gelatin solution was applied to patties made of chilled cod, and its preservative effect was assessed by colour measurements, rheological measurements (hardness, elasticity, cohesiveness, chewiness, gumminess, and adhesiveness), biochemical determinations (total volatile bases and thiobarbituric acid as measures of rancidity) and microbiological assays (total bacterial counts, luminiscent bacteria, enterobacteria, pseudomonas, lactic acid bacteria, and *Staphylococcus aureus*). The effect of dry powdered chitosan mixed into the patties was tested as well. The use of chitosan either as a coating or as a powdered ingredient did not affect lightness at the end of the storage period considered but did result in an increase in the value of yellowness. The coating increased patty elasticity, whereas adding powdered chitosan to the patty mixture increased the other rheological parameter values. The findings on the effect of the chitosan on rancidity were not conclusive due to the low values recorded in the cod. However, the coating did prevent spoilage of the cod patties as reflected by a decrease in total volatile basic nitrogen and in the microorganism counts, in particular counts of gram-negative bacteria. In contrast, none of these effects on spoilage were observed when the chitosan was added to the patty mixture in powdered form. Coatings prepared in cold from a blend of gelatin and chitosan offer a promising alternative for preserving fish patties.

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Key words: Coating, chitosan, gelatin, patty, fish

Introduction

The use of edible coatings and films is rapidly growing, especially on highly perishable unmodified and/or fresh foods as a means of preventing or delaying spoilage. This is the case of hamburgers made from meat and especially fish patties.

Many different substances have properties suitable for use as a coating or film. Most of these act as barriers to oxygen but not to water, this being one of the factors limiting the compounds apt for such use. Other important characteristics include antioxidant, binding, and texturizing properties.

Antimicrobial activity by certain substances is another extremely important factor, and coatings active in this way hold out considerable interest. Chitosan, a fibre of animal origin, is one such substance. The precursor of chitosan is chitin, which is naturally present in crustacean exoskeletons. Chitin is a polysaccharide composed of N-acetylglucosamine units and glucosamine linked by beta bonds (1→4). Chitosan is relatively insoluble in water but soluble in acid because of the positive charge on the C2 of the glucosamine monomer at pH 6 below.

Chitosan has been reported to have a number of functional properties that make it technically and physiologically useful in nutrition (Shahidi, Arachchi & Jeon, 1999; Gallaher, Gallaher, Mahrt, Carr, Hollingshead, Hesslink & Wise, 2002). Technically, these include its antimicrobial activity and its ability to form protective films (Cuero, 1999; Jeon, Kamil & Shahidi, 2002), its texturizing (Benjakul, Visessanguan, Phatchrat. & Tanaka, 2003), and binding action (No, Lee & Meyers, 2000); and its antioxidant activity (Kamil, Jeon & Shahidi, 2002). Other possible applications for chitosan in the food industry include, for example, use in water purification, enzyme immobilization, and encapsulation of nutraceuticals (Shahidi, Arachchi & Jeon, 1999). There are various types of chitosan, the differences mainly relating to molecular weight and/or the viscosity value and the degree of deacetylation. Chitosan is also soluble in acid media, and there is a body of work dealing with the benefits it offers both in powdered form and in solution (Lin & Chao, 2001; Jeon, Kamil & Shahidi, 2002). It therefore appears to be a good candidate for future applications in the food industry.

Storage of fresh fish fillets and mince calls for minimum processing that avoids warm or high temperature treatments, which act to alter the fresh characteristics. One way to achieve this is to apply a coating or film prepared using a gelatin gel stable at cold temperatures, a novel method of coating preparation. The properties of gelatin molecules make this substance particularly well suited to making flexible coatings and films, though the remaining properties will depend on the type of gelatin and the coating composition and application procedure (Sobral & Habitane, 2001; Sobral, Menegalli, Hubinger & Roques, 2001). Gelatin made from fish skins

offers an alternative to the more commonly used mammalian gelatin that is highly suitable for coating seafood products. Fish gelatins with a variety of attributes can be prepared for this purpose, depending on the raw material, i.e., source species and body parts used, and the manufacturing process (Gómez-Guillén, Sarabia & Montero, 2000, Gómez-Guillén, Turnay, Fernández-Diáz, Ulmo, Lizarbe & Montero. 2002).

Consequently, a mixture of fish gelatin and chitosan, both derived from marine sources, would seem to be especially suitable for use in the preparation of seafood products. The object of the present experiment was therefore to evaluate the preservative ability of a chitosan-gelatin blend used as a coating to cover fish patties and of chitosan alone in the form of a dry powder mixed in with the patty ingredients.

Materials and methods

Preparation of the blended chitosan-gelatin solution: A chitosan solution and a gelatin solution were prepared separately. A 4 % chitosan solution was made using 97 % deacetylated chitosan from shrimpshells (Guinama, Valencia, Spain) in 0.5 M acetic acid. The mixture was held at room temperature for 16 h, stirred for 5 min, and then degassed ultrasonically for 15 min. Separately, dry gelatin was obtained from megrim skins according to Gómez-Guillén *et al.* (2002). The dry fish

gelatin (8 %) was dissolved in water, first being allowed to swell at 7 ºC for 15 min and then warmed to 55 ºC for 30 min. An amount of 25 % glycerol was added to the gelatin solution, and warming at 55 ºC continued for a further 30 min.

The blended chitosan–gelatin solution was then prepared with 70 parts of the gelatin/glycerol solution and 30 parts of the chitosan solution.

Preparation of the fish patties: Cod fillets were purchased at a local market, and batches of 4 Kg were cut into small pieces and comminuted at low speed (1500 rpm) at 2 ºC in a Stephan homogeneizer (Model UM5; Stephan und Söhne GmbH & Co., Hameln, Germany) for 2 min. A portion of the mince was reserved and the other was homogenized at high speed (3000 rpm) for 3 min. with the following ingredients: 3 % NaCl (Panreac, Barcelona, Spain) and 5 % crushed ice. Next, 2 % egg white (Sanofi, Barcelona, Spain) and 10 % starch (Clearam CH 20, Laisa, Valencia, Spain) were added and homogenization continued for a further 5 min. The resulting batter was blended with the mince reserved (without ingredients) in the relation 40:60 (batter/mince) and divided into three different batches. The first batch without added chitosan was directly moulded into patties and used as a control (batch designation: control). Powdered chitosan (1.5 %) was blended into the mixture in batch number two (batch designation: powder), and patties were formed. The third batch was made into patties, which were dipped into the chitosan-gelatin solution for about 20 s (batch designation: coating). The coating layer gave a weight gain of about 7 %. All patties weighed around 30 g and were moulded with a geometry of 1.5 cm in height and 5.2 cm in diameter. Patties in all

three batches were kept chilled in a forced-air cold room at 2 ºC pending analysis. At least four patties per batch were used. All analyses were performed after three days in storage, which was used as the first sampling date for all the analyses except the microbiological assays and the total volatile basic nitrogen.

Proximate analysis of the raw cod was performed according to the procedures of the Association of Official Analytical Chemists for moisture (method 24003), ash (method 1821), and protein (method 24024) [AOAC, 1989)]. Crude fat was determined according to the method of Bligh and Dyer (1959). Proximate analysis results were: total protein 16.47 ± 0.25 %; moisture 82.34 ± 0.23 %, total fat 0.82 ± 0.06 %, and ash 1.05 ± 0.01 %.

pH: The initial pH of the cod (7.69 ± 0.03) was measured using a pH meter (MeterLab pHM 93, Radiometer Analytical, Denmark) using a mixture of 10 g of muscle in 100 mL of distilled water.

Colour: The colour parameters lightness (L) and yellowness (b) were measured using a Hunter Lab colorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA).

Viscoelastic properties of the chitosan-gelatin blend. Dynamic viscoelastic study was performed on a Bohlin CSR-10 rheometer rotary viscometer (Bohlin Instruments Ltd., Gloucestershire, UK) using a cone-plate geometry (cone angle

4º, gap=0,15 mm). Cooling of the chitosan-gelatin blend was performed from 40ºC to 6ºC at a scan rate of 1ºC/min, frequency 0.5 Hz, and oscillating applied target strain of 0.02 mm. The elasticity modulus (G'; Pa), viscosity modulus (G''; Pa), and the phase angle (δ; º), were represented as a function of temperature. Several determinations were performed, being the experimental error always below 6%.

Texture profile analysis (TPA): Fish patties were placed on the flat plate of the texturometer. Compression was applied using a cylindrical plunger (58 mm in diameter) connected to a 5 kN load-cell at a deformation rate of 50 mm/min. The samples were compressed to 30 % of height. The parameter values determined were hardness (N), elasticity (%), cohesiveness (adimensional), chewiness (N.mm), gumminess (N), and adhesiveness (g/cm).

Stress-relaxation test: Elasticity was also determined by means of a stress-relaxation test after relaxation for 1 min. Percentage relaxation was calculated as $Y_T=100\cdot(F_0-F_1)/F_0$, where F_0 was the force registered at the onset of relaxation immediately after sample compression and F_1 was the force registered after relaxation for 1 min. Thus, $100-Y_T$ was taken as a percentage index of gel elasticity.

Thiobarbituric acid (TBA) index was determined using a modified version of the method of Vyncke (1970) with a glass fibre filter (Type A/E, 1 µm, Pall Corporation, NY, USA) and incubation at 15 ºC for 20 hours. The method is based on the great

reactivity of the thiobarbituric acid with carbonyl compounds (aldehydes and ketones) which may arise in fish products as a consequence of lipid oxidation. Results have been expressed as mg malondialdehyde per Kg of sample.

Total volatile basic nitrogen (TVBN): The TVBN determination was based on the method of Antonacopoulos and Vyncke (1989). Portions of 10 g cod patties were homogenized with 90 mL perchloric acid (6 %) to precipitate the muscle proteins. This was followed by centrifugation at 4,000 x *g* at 5 ºC for 5 min and distillation of the supernatant (Tecator AB, Kjeltec System, model 1002, Höganäs, Sweden). The distillate was collected in boric acid (3 %) and titrated with hydrochloric acid (0.05 N). Results have been expressed in mg of nitrogen per 100 g of sample.

Microbiological assays: A total amount of 10 g from several patties was collected and placed in a sterile plastic bag (Sterilin, Stone, Staffordshire, UK) with 90 mL of buffered 0.1 % peptone water (Oxoid, Basingstoke, UK) in a vertical laminar-flow cabinet (mod. AV 30/70 Telstar, Madrid, Spain). After 1 min in a Stomacher blender (model Colworth 400, Seward, London, UK), appropriate dilutions were prepared for the following microorganism determinations: (i) total bacterial counts (TBC) and luminescent bacteria (LM) on spread plates filled with modified Long & Hammer's medium (L&H) (Van Spreekens, 1974) incubated at 15 ºC for 5 days; (ii) *Pseudomonas* on spread plates filled with Pseudomonas Agar Base (Oxoid) with added CFC supplement for *Pseudomonas* spp. (Oxoid) incubated at 25 ºC for 48 h; (iii) enterobacteria on double-layered plates filled with Violet Red Bile Glucose

agar (VRBG, Oxoid) incubated at 30 °C for 48 h [after first adding 5 mL of Tryptone Soy Agar (Merck, Darmstadt, Germany) and incubating at room temperature for 1 h]; (iv) lactic acid bacteria on double-layered plates filled with MRS Agar (Oxoid) incubated at 30 °C for 72 h; (v) *Staphylococcus* coagulase-positives on pour plates filled with Baird Parker RPF Agar (bioMérieux, Marcy l'Etolile, France) after incubation at 37 ºC for 48 h without subsequent confirmation (ISO, 1999). All microbiological counts have been expressed as the log of the colony forming units per gram (log cfu/g) of sample.

The day the fish patties were prepared was taken as day 0. Sampling took place on days 3, 7, 11, and 14 of storage (microbiological and TVBN analyses were also performed on day 0 as well). At least three replications of all analyses were carried out.

Statistical analysis

Two-way analysis of variance was run. The computer program used was the Statgraphics Plus (Rockville, MD, USA.) statistical program. Pairwise comparison of the differences between means was performed using Duncan's test with confidence intervals set for a level of significance of $p \leq 0.05$.

Results and discussion

In order to partial characterise rheological properties of the chitosan-gelatin blend used as a coating of fish patties, changes in viscoelasticity parameters upon cooling from 40ºC to 6ºC were determined (Fig.1). The blend at 40ºC behaved as a colloidal dispersion where the modulus of viscosity, *G''* (0.51 Pa) was considerably higher than the modulus of elasticity, *G'* (0.13 Pa). As a consequence, the value attained by the phase angle at this temperature was high (75.7 º). Upon cooling, changes in phase angle denoted a relatively broad thermal transition, taking place at around 18-27 ºC. This range of temperatures will define the gelling point of the coating, indicating that it may be stable even at room temperature. Values of G', G'' and phase angle at 22ºC were, respectively, 11.01 Pa, 5.79 Pa, and 27.8º. As temperature still decreased the blend exhibited a noticeably increase in G' and G'' achieving at 6ºC values of 3390 Pa and 74.9 Pa, respectively, with a phase angle of 1.3. It is clear from these results, that although at room temperature the coating will not disintegrate, at refrigerated temperatures, which in fact is the recommended storage condition for fish patties, this blend is able to form a strong gel, acting as a thin protective barrier.

The fish patty coating had done completely in under 6 h, by which time the chitosan-gelatin blend had gelled and become firm and non-sticky. It was quite natural looking and entirely translucent, with a slight sheen from the gelatin, producing a more uniform and smooth surface. In the case on cooking the fish patties, the coating would turn liquid as the gelatin melted from the heat, leaving the mince patty completely uncoated with no remnants that could be seen or tasted.

The lightness value in the colour measurements was initially different in the three different types of patty, being highest in the patties that contained powdered chitosan in the mixture (Fig. 2). The value of the coated patties was intermediate between the control batch and the powdered chitosan batch. However, this relationship changed during storage, and the batches with the lower initial lightness values ended up having higher values.

Because the patties were made from cod and the blended chitosan-gelatin solution could take on a yellow tone, the value of yellowness (b) was also evaluated. For the most part the values were rather similar and quite uniform, with slight differences ($p \leq 0.05$), and the patties that contained the powdered chitosan in the mixture tended to yellow more.

No large differences in colour were reported in others studies on pork sausages, just as slight increased in lightness and yellowness when adding 0.2 % chitosan oligomer (Jo, Lee & Byun, 2001) and also no great differences when using 0.1 % chitosan dissolved in acetic acid (Lin & Chao, 2001). Moreover, Darmadji and Izumimoto (1994) found out no significant modification on lightness of beef minced meat during incubation at 30 °C for 24 h containing 0.2-1 % chitosan.

Hardness was similar in the control patties and in the coated patties early in storage (Fig. 3) and higher somewhat in the powdered chitosan batch, though it was not significantly different ($p \geq 0.05$). Cohesiveness was also similar in the

control batch and in the coated batch during 11 days in storage (Fig. 3). The fish patties containing the powdered chitosan had higher cohesiveness values initially ($p \leq 0.05$), but exhibited similar values over the rest of storage.

Adhesiveness and gumminess (Fig. 4) were similar in behaviour and also similar to hardness. The fish patties containing the powdered chitosan displayed a tendency towards higher adhesiveness values, though this tendency was not always significant, since adhesiveness increased appreciably in the coated patty batch towards at the end of chilled storage. Gumminess, being the product of adhesiveness multiplied by hardness, the differences for this parameter was slightly more pronounced.

Elasticity (Fig. 5) as measured by TPA was quite similar in the control batch and the powdered chitosan batch until the end of storage, when elasticity in the powdered chitosan batch decreased. The coated fish patties exhibited higher elasticity at the beginning of storage ($p \leq 0.05$). Chewiness was the product of elasticity times gumminess and was influenced particularly by the latter (Fig. 5).

The values of elasticity measured by the stress-relaxation test after compression for 1 min (Fig. 6) were higher than those recorded previously by TPA. The three batches of fish patties initially displayed significant differences. The control batch had the lowest elasticity values ($p \leq 0.05$). At the end of storage the coated patties exhibited a certain decrease in elasticity (Fig. 6), that was also reflected by the

other elasticity measurement (Fig. 5). A noticeable increase in elasticity by stress-relaxation test was also found out in pressurized cod sausages with added 1.5 % chitosan (López-Caballero, Gómez-Guillén, Pérez-Mateos & Montero, 2004).

On the whole, the coated patties tended to be more like the control patties than the patties that contained the powdered chitosan in the mixture, which means that application of the coating did not bring about large changes in the rheological properties.

Some studies (Jo, Lee & Byun, 2001) carried out in pork sausages with addition of 0.2 % chitosan oligomer reported that texture (hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness) did not change greatly at 25 % of compression. Even at higher rate of compression (70 %), there was not great changes in texture of low-fat pork sausages after the incorporation of 0.1 % chitosan with various molecular weights dissolved in 1 % lactic (Lin and Chao, 2001). However other studies reported that chitosan increased the gel formation of surimi but it depends on the quality of the surimi, the type of chitosan, the concentration and the gelling treatment (Benjakul, Visessanguan, Phatchrat. & Tanaka, 2003).

Cod is a lean fish species, but oxidative changes in the lipids contained in the flesh may still take place, since the lipids are highly vulnerable to oxidation. Table 1 presents the TBA values for the different batches during storage at 2 ºC, that represents the aldehyde level in the lipid-oxidised fraction. The batches were

practically devoid of rancidity, the only significant increase being after 7 d. No antioxidative effect of the chitosan coating was observable, possibly because the initial TBA values were so low in all the batches.

Jeon *et al.* (2002) found lower contents of TBARS in chitosan-coated herring and cod samples than those of the uncoated samples throughout the storage time. These authors found the higher inhibitory effect when using chitosan with the highest viscosity values, probably due to the presence of a large number of ionic functional groups, which create strong polymers interactions that restrict the chain motion in high-viscosity chitosans, resulting in good oxygen barrier properties. In addition, Xue, Yu, Hirat, Terao & Lin (1998) reported that the antioxidant mechanism of chitosan could be by chelant action of ion metals and/or the combination with lipids.

The total volatile bases nitrogen (TVBN) present in a fish patty are also an index of spoilage. The fish fillets were purchased at a local market and at the time of purchase had a total of 12.19 ± 0.92 mg of TVBN/100 g of flesh. On day 3 of storage (Fig. 7) the control patties had the highest TVBN values ($p \leq 0.05$). In contrast, the protective chitosan-gelatin coating lowered values distinctly and hence slowed spoilage. The powdered chitosan played a minor role and a slight protective effect was exerted only in the early stages. Using whole cod fillets and different types of soluble chitosan coatings, Jeon *et al.* (2002) reported reduction of 33-50 % in the formation TVBN at the end of a 12-day storage period. They

observed somewhat lower TVBN values probably because the fillets were used whole and as a result did not acquire the same microbial load during handling and thus did not spoil as quickly as the our patties. In addition, the coated patties did not undergo any heat treatment, unlike the fillets in the study by Jeon *et al.* (2002).

Initial total bacterial counts (TBC) in the cod fillets were 4.2 log cfu/g, within the normal range given that they were purchased at a local market and hence were subjected to handling during preparation of the fillets. This value was lower than the value found by Devebere and Boskou (1996) for cod fillets at storage time 0 (10^6 cfu/g). *Pseudomonas* spp., at 3.5 log cfu/g, made up a major share of the total flora. The lactic acid flora was present at lower concentrations (≈ 2 log cfu/g). Both enterobacteria and staphylococci were below the detection threshold (<1 log cfu/g). Staphylococci were not detected during the storage period considered (14 days), probably because of the low storage temperature (2 ± 1 ºC) employed. Luminiscent *Photobacterium phosphoreum* has been reported in fresh and spoiled cod, saithe, and place (Dalgaard, Mejlholm, Christiansen & Huss, 1997), but in this experiment luminescent bacteria were not detectable either in the raw material (detection threshold: 2 log cfu/g) or in any of the three batches of patties during storage.

Microorganism counts during storage are depicted in Fig. 8. Counts increased during preparation of the patties. All three batches followed similar trends, but the coating inhibited microbial growth to a certain extent. Thus, there was a difference of around 2 log cycles between the control and the coated batches for TBC,

pseudomonas and enterobacteria (Figs 8 a, b, c, respectively) at 8-11 days in storage.

The antimicrobial properties of chitosan have been discussed previously. Jeon *et al.* (2002) described how bacterial growth (total counts on plate count agar at 20 ºC) reached the stationary phase in all chitosan-coated cod and herring samples after 6 days and also how there was a reduction of up to 3 log cycles between coated batches and controls after 12 days of chilled storage. Various factors affect the antimicrobial action of chitosan (Cuero, 1999), and its mechanism of action appears to be related to disruption of the lipopolysaccharide layer of the outer membrane of gram-negative bacteria (Nikaido, 1996; Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades & Roller, 2001) and to its function as a barrier against oxygen transfer (Jeon *et al.*, 2002). The coating, a blend of chitosan dissolved in acid acetic and gelatin, was observed to exert an inhibitory effect on the gram-negative flora in this study (Fig. 8a-c).

At the same time, the presence of the chitosan coating seemed to stimulate growth of the lactic acid bacteria very slightly, probably because surface pH on the coated patties was lower thanks to the acid solution in which the fibre was dissolved (Fig. 8d). Lee, Park, Jung & Shin (2002) reported that chitosan oligossacharide showed a bifidogenic effect at concentrations between 0.1 and 0.5% and it had growth stimulatory effect on *Lactobacillus casei* and *Lactobacillus brevis* at 0.1 %.

The addition of powdered chitosan to the mixture of patty ingredients had no effect on bacterial growth (Fig. 8a-d). This finding can be explained because of the poor insolubility of chitosan at neutral pH and the presence of a significant proportion of uncharged amino groups (Sudharshan, Hoover & Knorr, 1992). The permeabilizing effects of chitosan were demostrated at slightly acid conditions, in which it is protonated, and the carboxyl and phosphate group of the bacterial surface are anionic and offer potential sites for electrostatic binding of chitosan. (Helander *et al*., 2001). Ouattara, Simard, Piette, Bégin and Holley (2000) used antimicrobial films with a chitosan matrix designed to release antimicrobial agents (organic acids) gradually at the product surface and thereby were able to inhibit the growth of enterobacteria.

Presumably, the antimicrobial effect would have been more readily discernible if the initial microbial load in the present experiment had been lower. The effect of coatings chitosan-gelatin of fresh fish products on the lag phase of microbial growth and the efficacy of edible chitosan coatings containing different organic acids applied to fish patties will be the subject of further study.

The microbial counts (Fig. 8) were related to the high TVBN levels recorded. High levels of such active spoilers as *Pseudomonas* sp., *Shewanella putrefaciens*, etc. break down compounds like trimethylamine oxide (TMAO), peptides, amino acids, etc. (Gram and Huss, 1996), resulting in an increase in the basic nitrogen fraction (Fig. 7).

In conclusion, the chitosan-gelatin solution employed allowed cold preparation of a coating that was suitable for preventing fish spoilage and could be applied without need for heating. The coating had good sensory properties, melted away on cooking and hence did not impart any taste to the product, and provided partial protection by delaying spoilage.

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FIGURE LEGENDS

Figure 1. Changes in elastic modulus (G'), viscous modulus (G'') and phase angle (δ) of the chitosan – gelatin blend, monitored upon cooling from 40 ºC to 6 ºC at a rate of 1 °C/min.

Figure 2. Colour parameter measurements for the patties. C: control, P: powdered chitosan, C: coating. Different letters (a, b, c, d) indicate significant differences ($p \leq 0.05$) on each lot as a function of storage time; different letters (x, y, z) indicate significant differences ($p \leq 0.05$) among lots on each sampling date.

Figure 3. Hardness and cohesiveness of the patties. Labels are the same as those in Figure 2.

Figure 4. Adhesiveness and gumminess of the patties. Labels are the same as those in Figure 2.

Figure 5. Elasticity and chewiness of the patties. . Labels are the same as those in Figure 2.

Figure 6. Elasticity (stress-relaxation test) of the patties. Labels are the same as those in Figure 2.

Figure 7. Total volatile basic nitrogen (mg TVBN/100 g) of the patties. Labels are the same as those in Figure 2.

Figure 8. Microbiological counts (log cfu/g) for the patties: a (total bacterial counts), b (*Pseudomonas* sp.), c (enterobacteria), d (lactic acid bacteria).

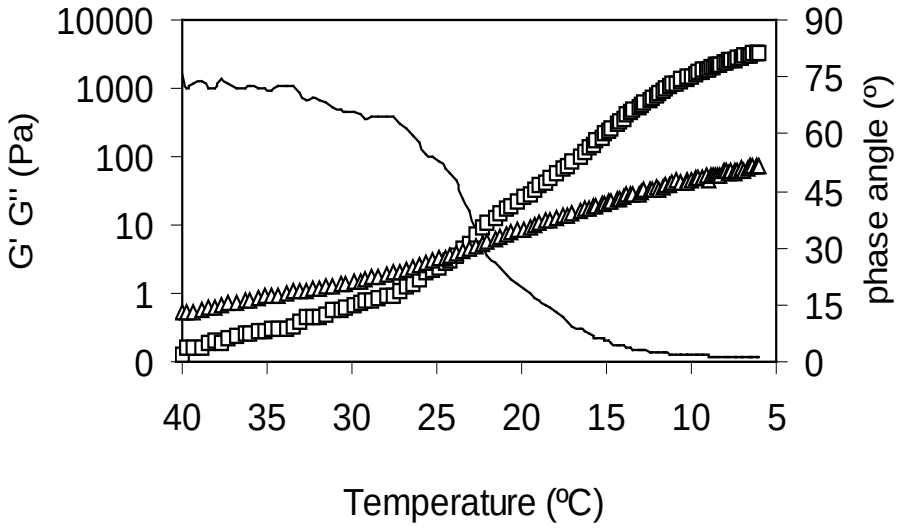
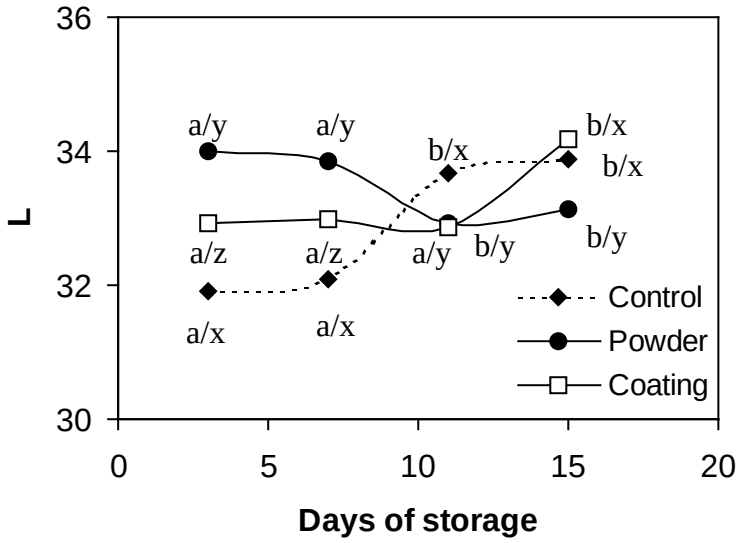

10000
1000
100
10
1
0
G' G'' (Pa)
90
75
60
45
30
15
0
phase angle (°)
40
35
30
25
20
15
10
5
Temperature (ºC)

Figure 1



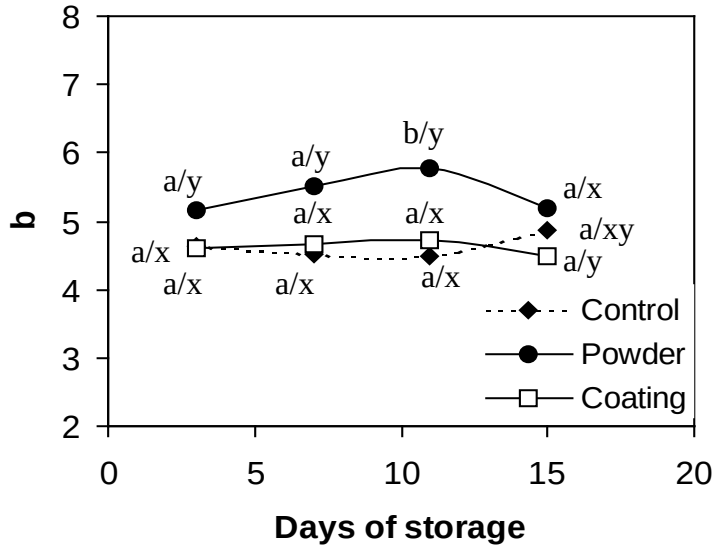
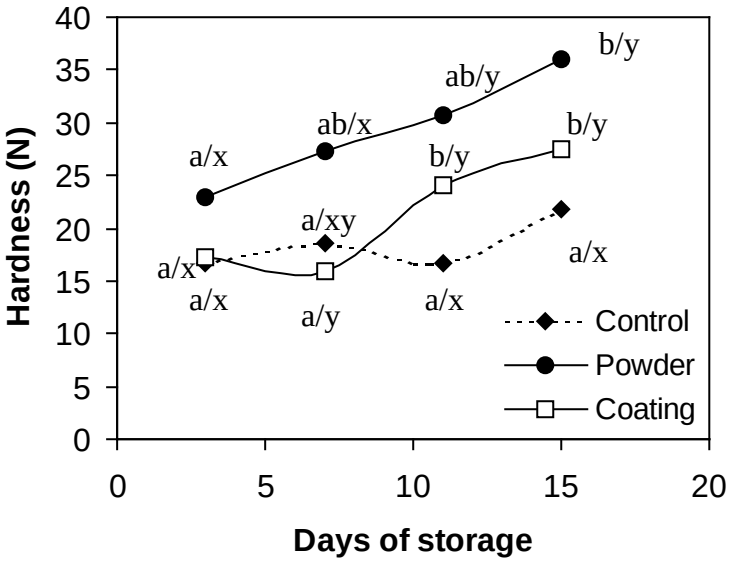


Figure 2

Food Hydrocoll. 2005;19(2):303-311


Hardness (N)
Days of storage
Control
Powder
Coating
a/x
ab/x
ab/y
b/y
a/xy
a/y

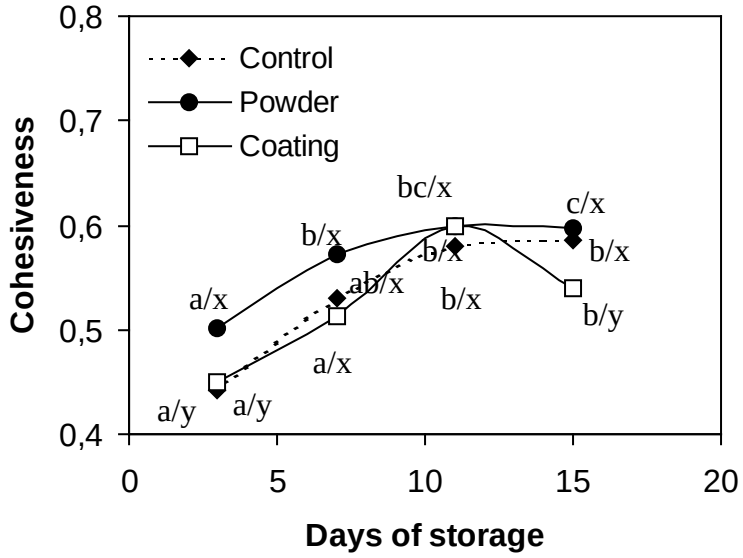
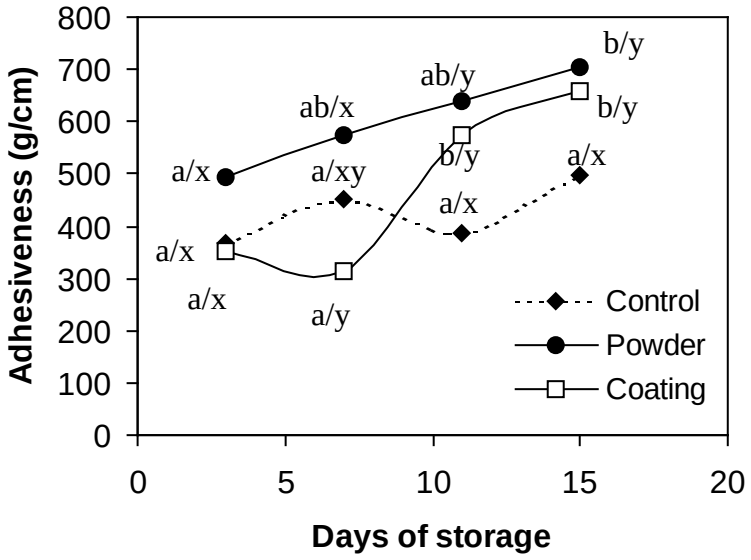

Cohesiveness
Days of storage
Control
Powder
Coating
a/x
b/x
bc/x
c/x
ab/x
a/y
b/y

Figure 3


Adhesiveness (g/cm)
800
700
600
500
400
300
200
100
0
0
5
10
15
20
Days of storage
a/x
ab/x
ab/y
b/y
a/x
a/xy
b/y
b/y
a/x
a/x
a/x
a/y
Control
Powder
Coating

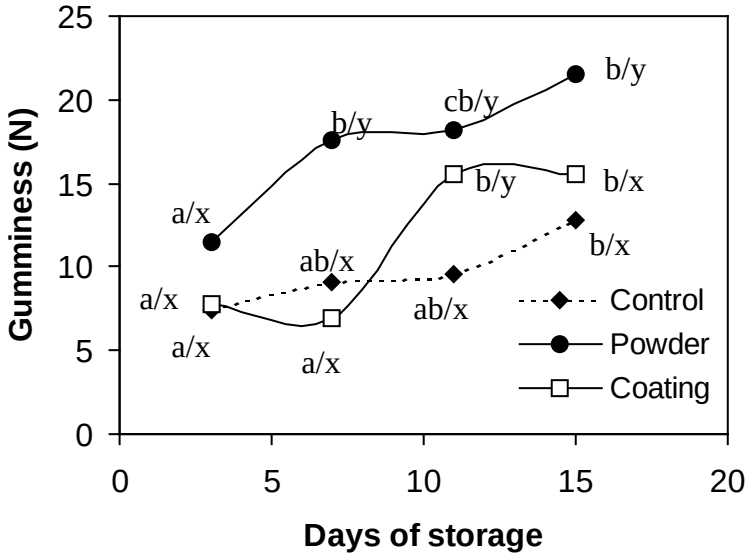
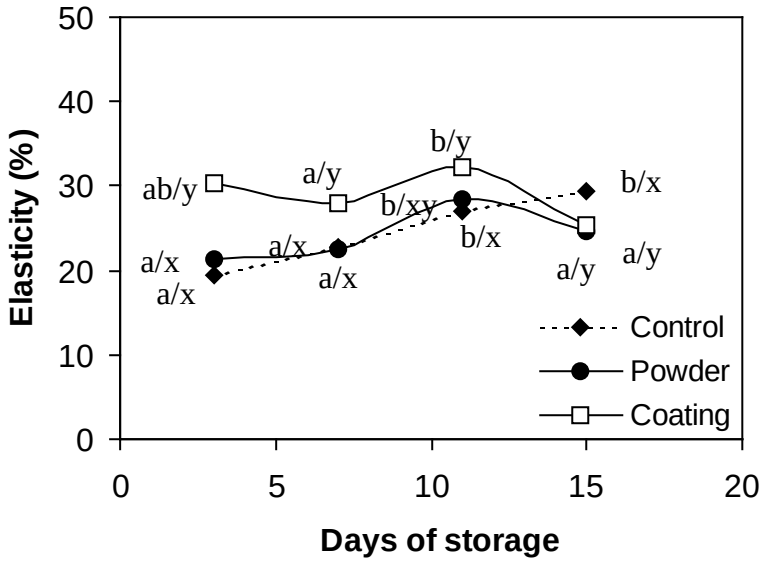

Gumminess (N)
25
20
15
10
5
0
0
5
10
15
20
Days of storage
a/x
b/y
cb/y
b/y
b/y
b/x
a/x
b/x
ab/x
a/x
ab/x
a/x
a/x
Control
Powder
Coating

Figure 4


Elasticity (%)
Days of storage
Control
Powder
Coating
ab/y
a/y
b/y
b/xy
b/x
a/x
a/x
a/x
b/x
a/y
a/y

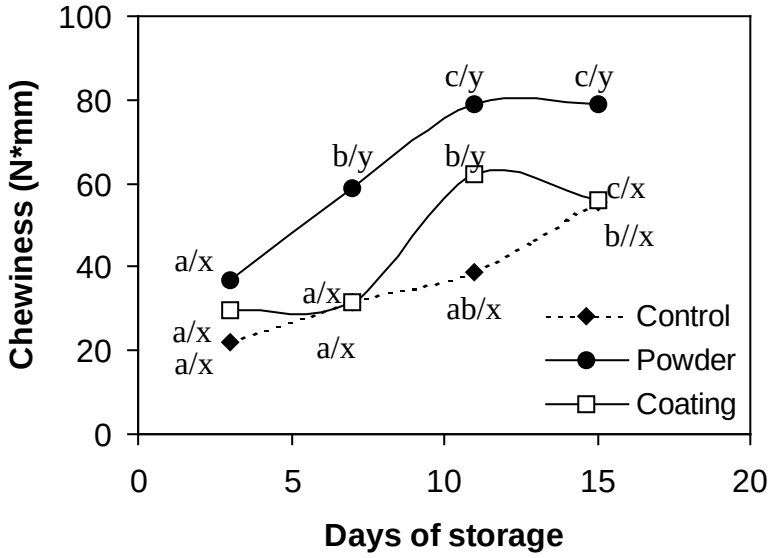

Chewiness (N*mm)
Days of storage
Control
Powder
Coating
c/y
c/y
b/y
b/y
c/x
b//x
a/x
a/x
ab/x
a/x
a/x
a/x

Figure 5

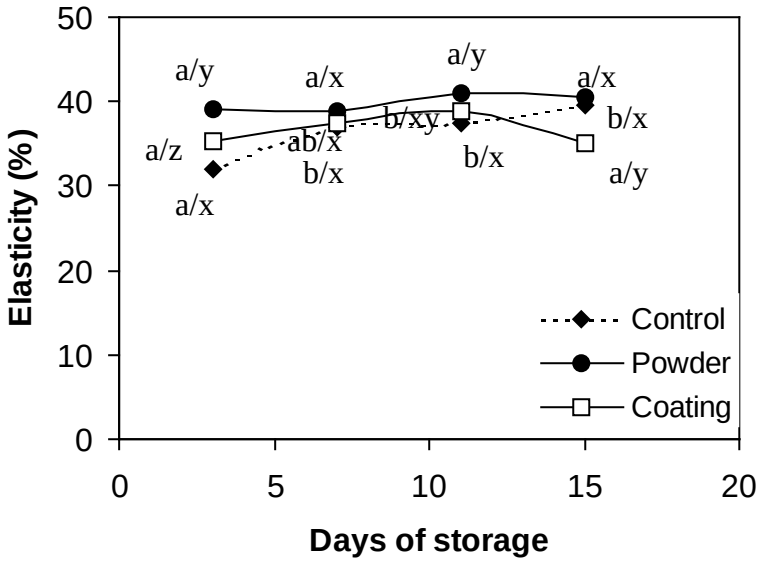

50
40
30
20
10
0
Elasticity (%)
0
5
10
15
20
Days of storage
a/y
a/x
a/y
a/x
b/x
a/z
ab/x
b/xy
b/x
a/y
b/x
a/x
Control
Powder
Coating

Figure 6

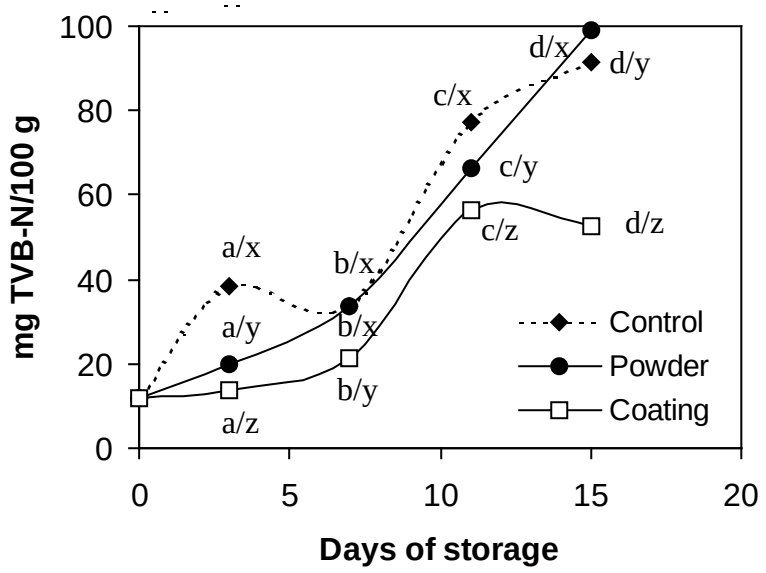

mg TVB-N/100 g
Days of storage
0
20
40
60
80
100
0
5
10
15
20
a/x
a/y
a/z
b/x
b/x
b/y
c/x
c/y
c/z
d/x
d/y
d/z
Control
Powder
Coating

Figure 7

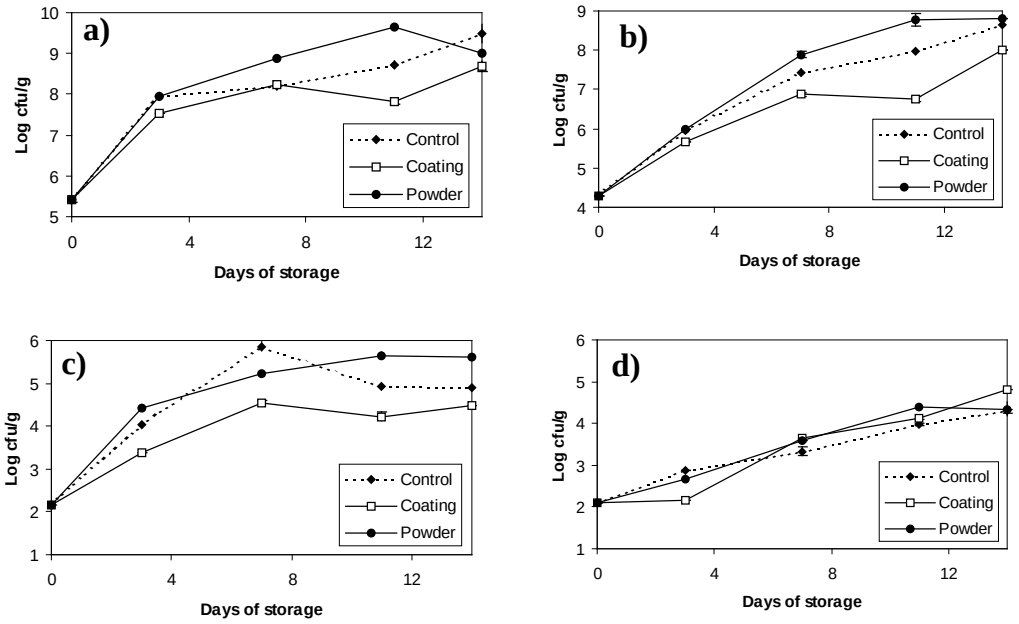


Figure 8

Table 1. Thiobarbituric acid index values for the patties. Different letters (a, b) indicate significant differences ($p \leq 0.05$) on each lot as a function of storage time; different letters (x, y) indicate significant differences ($p \leq 0.05$) among lots on each sampling date.

5

Batch	Storage (days)			
	3	7	11	14
Control	0.00 *a/x*	0.11 ± 0.00 *b/y*	0.12 ± 0.02 *b/y*	0.10 ± 0.02 *b/y*
Coating	0.02 ± 0.01 *a/x*	0.12 ± 0.00 *b/y*	0.11 ± 0.01 *b/y*	0.11 ± 0.00 *b/y*
Powder	0.03 ± 0.02 *a/x*	0.15 ± 0.02 *b/y*	0.11 ± 0.01 *b/y*	0.12 ± 0.04 *b/y*