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Novel carbohydrate blend enhances chemical and sensory properties of lobster (*Homarus americanus*) after one-year frozen storage

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ABSTRACT

It has previously been shown that a novel blend of carbohydrates could preserve lobster meat after 6 months of frozen storage. Increased year-round demand for high-quality lobster may make selling to the frozen seafood market an unintended option for some fishermen. Yet, the chemical and sensory changes that occur in lobster meat after one-year frozen storage in this cryoprotectant blend is not known. The objective of this study was to determine the chemical and sensory characteristics of lobster frozen in five different solutions: solution-1 (water); solution-2 (water + NaCl + STPP, sodium tripolyphosphate, 0.5%); solution-3 (water + NaCl + carbohydrate blend); solution-4 (water + NaCl + STPP, 0.25% + carbohydrate blend), and solution-5 (water + NaCl + STPP, 0.5% + carbohydrate blend). No difference ($P > 0.05$) existed among the treatments with regard to Malondialdehyde levels as a measure of lipid oxidation. Lobster frozen in the cryoprotectant showed increased tenderness, compared to the control which was frozen in water. The lobster meat treated with a combination of the carbohydrate blend and STPP had lower ($P < 0.05$) moisture content than the control. In addition, consumers preferred ($P < 0.05$) lobster frozen in the novel cryoprotectant blend and STPP with respect to flavour, texture, and overall acceptability compared to the control. Penalty analysis revealed that overall liking scores were positively associated with the attributes moist and sweet. In conclusion, the combination of the novel carbohydrate blend and STPP enhanced the sensory quality and the chemical properties of frozen lobster, which in turn extended the shelf-life of these products. These findings may have wide implications for the long-term preservation of frozen lobster meat.

1. Introduction

In 2018, Canada's lobster (*Homarus americanus*) exports to the United States (US) were valued at $1.52 billion Canadian Dollars (CAD). Among the products exported, frozen lobster accounted for 65%, compared to 34% for live lobster, making it an important component of the total lobster export shares (Fisheries and Oceans Canada, 2019). China and the European Union are also important export destinations for Canadian lobster with total export values of 298 and 173 million CAD, respectively (Fisheries and Oceans Canada, 2019). Although the best prices are obtained for live lobster, the increased demand for frozen lobster in the international marketplace shows that there is a need for these alternative product forms (Sackton, 2019). Moreover, the recent onset of the coronavirus pandemic which has disrupted the distribution chain, may make selling to the frozen seafood market an unintended option for some fishermen (Plaganyi, Murphy, Deng, Pascoe, & Hutton, 2020). Together, these changes have contributed to increased interest among key stakeholders in the seafood industry to develop new formulations or processes to improve the quality of frozen lobster products.

Currently, lobster for frozen export are prepared in either whole forms which are cooked, then frozen and sealed in vacuum-packed pouches containing brine; or the meat is removed from the shell, cooked and then frozen in cryoprotective solutions (CFIA, 2019; Wickins & Lee, 2003). Several parameters including slow freezing rates and moderate freezing temperatures (>-18 °C) can reduce the quality of frozen products due to protein denaturation or chemical or biochemical

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deterioration, respectively. Furthermore, the type of freezing method used can also directly impact the overall quality of frozen lobster meat (Rahman, 1999).

Among these methods, cryogenic freezing using sugars such as trehalose has gained importance because of their ability to maintain the quality of lobster meat over various short-term frozen storage periods (Calder, Bushway, Bayer, Davis-Dentici, & Camire, 2005). Although there is compelling evidence to suggest that disaccharides such as trehalose are effective cryoprotective agents, different theories have been proposed to explain their bioprotective effect (Olsson, Genheden, Sakai, & Swenson, 2019). For example, Green and Angell (1989) reported that the glass-forming characteristics of aqueous trehalose solutions contributed to their beneficial effect. On the other hand, Crowe, Leslie, and Crowe (1994) proposed that the ability of trehalose to reduce the amount of freezable water, was due to direct interactions between trehalose and the polar groups in biomolecules such as lipids and protein. By way of these mechanisms some of the purported benefits of using cryoprotectants in frozen seafood include reduced drip loss after thawing, improved moisture content, flavour and texture properties, and protein stabilization (Bland et al., 2018). Reduced lipid oxidation is often reported but by an alternate mechanism, that of cryoprotectants chelating heavy metal ions (Bland et al., 2018; Work, Bushway, Work, & Pelletier, 1997).

MacDonald and Lanier (1991, 1997) have also noted that blends of these cryoprotective agents may be more effective compared to when they are used individually. Furthermore, Lee (1984) showed that the cryoprotective effect of different sugars including sucrose and sorbitol are enhanced in the presence of polyphosphates. Our own observations have also shown improved consumer acceptability of lobster products when they were frozen for 6 months in solutions containing a blend of carbohydrates and sodium tripolyphosphate, STTP (English, Keough, McSweeney, & Razul, 2019). Although, the cryoprotective effects of this novel blend of carbohydrates and phosphates on lobster meat have been demonstrated over short-term frozen periods (≤six months), no studies to the best of our knowledge have evaluated their cryoprotective effect after one-year frozen storage. The experimental approach applied in the present study addresses this limitation by focusing on the sensory and chemical changes that occur in lobster meat after one-year frozen storage.

Accordingly, the objective of the present study was to investigate the changes in moisture content, texture, lipid oxidation, and salt-soluble protein concentrations in lobster meat that was preserved in sodium chloride (NaCl) and a novel blend of carbohydrates or STPP for one-year. Consumer acceptability of the frozen lobster products was also evaluated by using a check-all-that-apply (CATA) questionnaire and 9-point hedonic score ratings. The significance of this work lies in the fact that expanding our understanding of these chemical and sensory changes will facilitate the improvement of frozen lobster quality for the growing International seafood market.

2. Materials and methods

2.1. Materials

Food-grade carbohydrates, sodium chloride, phosphates and thiobarbituric acid were obtained from Sigma-Aldrich (Oakville, ON, Canada) and Alphachem Limited (Mississauga, ON, Canada). The experiments were performed with fresh, mature, hard-shell, male lobster (*Homarus americanus*) caught off the eastern coast of Nova Scotia (NS), Canada, in July 2017, and purchased from a grocery store in Antigonish, NS, Canada. The average weight of each lobster was between 1.25 and 1.5 pounds. Live lobsters were placed in large coolers containing ice and transported for 5 min to the food research laboratory in the Department of Human Nutrition, Saint Francis Xavier Univ., NS, Canada, for further processing.

2.2. Freezing of lobster meat

The preparation of lobster meat and the sensory evaluation studies were carried out as described by English et al. (2019). Briefly whole, hard-shell lobsters were boiled for 35 min in pots containing 20 L of 3% (w/v) saline solution. The samples were cooled for 30 sec in a saline solution 2% (w/v), and the entire portion of the tail meat, and meat contained in the knuckles, the crusher and pincher claws were removed and mixed together in a large bowl. All the samples were treated in a similar way due to the potential to affect meat texture.

Five different types of solutions were prepared to freeze the samples. Solution-1, (control), contained water, Solution-2, contained a typical industrial blend of NaCl and STPP, Solution-3, contained NaCl and a novel blend of carbohydrates (major component a disaccharide plus other minor food carbohydrates), Solutions 4 and 5, contained NaCl, varying concentrations of STPP and the same novel blend of carbohydrates used for the tubes containing Solution-3. Polypropylene tubes (VWR) containing lobster meat (20 g) and 20 mL of each solution sat for 2 min to allow diffusion of the cryoprotective solutions into the meat. Following which all the samples were frozen with liquid nitrogen. A reference tube containing NaCl was used to monitor freezing temperatures, and freezing was stopped when the temperature in this tube was between −55 °C and −57 °C. All the tubes were then placed upright in Styrofoam trays and stored in a −20 °C freezer for 12 months.

2.3. Chemical properties of lobster meat

2.3.1. Moisture content determination

To determine the moisture content (%) of lobster meat that was frozen for one year in the five treatment solutions, 5 g of each sample was blotted on a block of paper towel to remove excess cryoprotective solution following which they were placed in labelled, pre-weighed beakers. The beakers with the samples were weighed and then placed in a convection oven (VWR Scientific) set at 80 °C for 24 h. After the drying period, the weights of the dehydrated lobster meat and the beakers were determined. The percent moisture content (wt/wt) for each sample was then determined.

2.3.2. Lipid oxidation

The Thiobarbituric acid (TBA) assay was used to determine the presence of lipid peroxides (Thiobarbituric acid-reactive substances TBARS), in lobster meat that was frozen in solutions 1 to 5 for one year. A modified version of the method outlined by (Calder, 2003; Rhee & Watts, 1966) was used. Frozen lobster samples were thawed in running water (20 °C) for 20 min. To homogenize the thawed lobster samples, 4 g of lobster meat and 12 mL of a TBARS buffer (containing 0.1% EDTA w/v, 0.1% n-propyl gallate, and 50 mM phosphate buffer, pH 6.2) were placed in 50 mL centrifuge tubes and vortexed for 30 sec. Following which, 4 mL of 30% Trichloroacetic acid was added to the tubes and the samples were vortexed for an additional 60 sec. The samples were then vacuum filtered through fluted Whatman filter paper (15 cm, Fisher Scientific; Pittsburgh, PA) and collected in glass test tubes. For the TBA assay, 4 mL of the collected filtrate and 2 mL of 20 mM 2-thiobarbituric acid were vortexed together in a test tube and placed in a boiling water bath for 20 min. Afterwards, the tubes were placed on ice to stop the reaction, and the absorbance of the contents was measured at 750 nm. Oxidative rancidity was determined as the ratio between the absorbance values of the frozen samples and freshly cooked lobster. Assays were performed in triplicates.

2.3.3. Salt-soluble protein

To determine the concentration of salt-soluble proteins, a modified version of the Lowry assay described by Calder (2003) was used. Ten grams of lobster meat from each sample was homogenized (Brinkmann Poltron homogenizer) for 1.5 min with 90 mL of a NaCl solution (5%, w/v). Then the homogenate was centrifuged for 20 min at 12,000 rpm, at

4 °C, and the supernatant was collected as the salt soluble fraction. The samples were diluted (50 μL + 0.5 mL dH_2O) and then 5 mL of a solution containing Na_2CO_3 (2%, w/v), NaOH (0.4%, w/v), cupric sulfate (1%, w/v), and sodium potassium tartrate (2.7%, w/v) were added to all test tubes which were incubated at room temperature (RT) for 10 min. Afterwards, 0.5 mL of phenol reagent (0.2 N) was added to each tube and the latter were incubated for 20 min at RT. The absorbance values of the contents of each tube was determined at 750 nm, and the protein concentrations (mg/mL) were determined using a BSA standard curve.

2.3.4. SDS-PAGExxx

Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on fresh and frozen lobster meat using a modified version of the method described by English, Viana, and McSweeney (2019). Due to insufficient amounts of frozen lobster meat, protein was extracted from a composite sample of lobster meat that was frozen in solutions-3, 4, and 5. Protein extracts (20 μg and 50 μg) were mixed with 5 μL of protein sample buffer and heated for 10 min at 100 °C. Fifteen microlitres of each denatured protein extract was loaded onto a 12% Mini-PROTEAN® TGX™ gel (BioRad, Mississauga, ON). Gels were loaded into a Bio-Rad Mini-PROTEAN® Tetra Cell (Mississauga, ON) and the system was run for 40 min at 170 V and 30 mA. The gels were stained for 2 h with 0.1% Coomassie Brilliant Blue (R-250) solution and de-stained, and then gel images were captured using a BioRad Chemic Doc™ MP imaging system. A pre-stained protein marker (New England BioLabs, P77066) was used to estimate the molecular weights of proteins in the samples.

2.3.5. Texture Profile Analysis (TPA)

Lobster tail meat firmness/hardness was determined using a TA XTPlus texture profile analyzer (model TAXT2i, Texture Technologies Corp., MA). A modified version of the methods outlined by Xu, Xia, Yang, Kim, and Nie (2010) and Singha, Guizani, Al-Alawi, Claereboudt, and Rahman (2013) was used. The lobster samples for TPA were thawed exactly the same as those for sensory analysis. Briefly, the samples were thawed in a refrigerator overnight to a surface temperature of ~25 ± 2 °C. The load cell was calibrated with a 2.5 kg weight following which each sample (20 g) was placed in a 150 mL glass beaker and the latter was placed on the platform of the analyzer. Parameters for the texture analysis for two compressions are as follows: strain = 50%, test speed = 1 mm/sec, time between cycles = 5 sec.

Force–time deformation curves were obtained by applying a 25 kg load cell at 1 mm/sec and the hardness (firmness) of the samples were calculated using the Texture Expert program. Seven replicates were carried out for each sample, and all experiments were conducted at room temperature. In the present study hardness (firmness) was defined as the maximum force (expressed in Newtons, N) necessary to attain a given deformation at a 50% compression.

2.4. Sensory evaluation of lobster meat

2.4.1. Preparation of lobster meat

The freezing of the lobster samples for the sensory evaluation studies are described in Section 2.2. Before carrying out sensory evaluation studies, the lobster samples were removed from frozen storage and thawed at 4 °C for 24 h.

2.4.2. Recruitment of participants for sensory evaluation studies

The sensory evaluation studies were reviewed and approved by the Acadia University Research Ethics Board (REB # 13-72). Participants similar those reported by English et al. (2019) were recruited and invited to participate in the present study. Male and female participants (18 to 65 years), who consumed lobster at least once a month, were recruited for the study. Participants were recruited by printed advertisements and included students, faculty and staff, and residents of the Annapolis Valley region in NS. Participants with shellfish allergies or a dietary restriction that included shellfish were excluded from the study. Once the screening questionnaire and invitation to participate were completed, informed consent was obtained from each participant, and all participants received a small gift for their participation.

2.4.3. Data collection

The methods for the sensory trials (n = 107) were adapted from Lado, Vicente, Manzzioni, and Ares (2010). For each trial, a completely randomized design was followed and testing was conducted in sensory booths using white, fluorescent lighting. The five lobster samples were taken from the fridge (4 °C) and immediately served to the participants. The lobster samples were presented to the participants on odourless plastic containers labelled with random three-digit numbers, and water and unsalted crackers were given to the panelists to cleanse their palate between each sample. The testing was completed on computers using Compusense Cloud software (Guelph, Ontario Canada). Participants were asked to score their overall liking of the appearance, flavour, texture, and overall liking of lobster samples on nine-point hedonic scales ranging from 1 = Dislike extremely to 9 = Like extremely. Participants were also asked to answer a CATA question with 27 literature-informed terms related to the sensory characteristics of the lobster samples (Calder et al., 2005; Camire, Ismail, & Bayer, 1997). The following terms were considered in the CATA question: moist, chewy, tender, white, fishy, soft, off-white, sweet, red, salty, fibrous, stringy, mild, bland, firm, tough, pale pink, mushy, flakey, briny, pale yellow, dry, ragged, bright pink, coarse, bitter, and bright yellow. After completing the CATA task, the trial concluded with demographic questions including age, gender, and income.

2.4.4. Data analysis

Two-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc analysis was done to determine if there were any significant differences in liking of the appearance, flavour, texture, and overall liking among the lobster samples. The frequency of each attribute was established by counting the number of participants that used that attribute to evaluate the lobster sample in the CATA questionnaire. The CATA and overall liking scores were analyzed by penalty analysis to determine the mean impact of each attribute on overall liking using XLSTAT software (Version 2018.2, New York, NY, USA) in Microsoft Excel™ (Santa Rosa, CA, USA). One-way ANOVA tests and Tukey's post-hoc test with GraphPad Prism (version 8.4.1 for MacOS, GraphPad Software, La Jolla California USA, www.graphpad.com) were performed on the chemical data to find significant differences in the reported results. To evaluate correlations in the chemical (firmness) and sensory data Pearson's correlation test was also performed using GraphPad Prism version 8.4.1 for MacOS. Correlation coefficients were calculated, and significance level was set at $p < 0.05$.

3. Results and discussion

This work was focused on evaluating the chemical and the sensory changes that occurred in lobster meat that was frozen for one-year in water (solution-1, control), and various treatments. Solution-2, contained water, NaCl and 0.5% STPP; solution-3, had water, NaCl and a novel blend of carbohydrates; solution- 4, had water, NaCl, 0.25% STPP, and the novel blend of carbohydrates; and solution-5, contained water, NaCl, 0.5% STPP and the novel blend of carbohydrates.

3.1. Moisture content of lobster meat

The moisture content for the lobster samples after one year of frozen storage in the various solutions are shown in Table 1. Compared to the control (76.5 ± 0.6), in which samples were frozen in water, the samples that were preserved in solutions containing the novel carbohydrate blend registered moderately lower moisture contents, 75.5 ± 0.9 in

Table 1
Chemical properties of lobster meat frozen for one year in various treatments. Solution-1 (water); solution-2 (water + NaCl + STPP, 0.5%); solution-3 (water + NaCl + carbohydrate blend; solution-4 (water + NaCl + STPP, 0.25% + carbohydrate blend), and solution-5 (water + NaCl + STPP, 0.5% + carbohydrate blend).

Parameters tested	Solution-1	Solution-2	Solution-3	Solution-4	Solution-5
% Moisture content (wt/wt)	76.5 ± 0.6[a,b]	76.7 ± 0.9[a,b]	75.5 ± 0.9[a,b,]	75.3 ± 0.6[a,b]	72.2 ± 0.2[a,b,c]
Malondialdehyde conc. (μg/g)	0.27 ± 0.002	0.28 ± 0.01	0.26 ± 0.01	0.30 ± 0.01	0.27 ± 0.01
[SSP] in lobster tail (μg/mL)	7.6 ± 0.3	6.4 ± 0.3[a]	8.4 ± 0.1[b]	6.9 ± 0.4	8.6 ± 1.4[b]
[SSP] in lobster claw (μg/mL)	8.4 ± 0.8[a]	11.0 ± 0.4[b,c]	10.9 ± 0.5[b,c]	8.2 ± 0.9[a]	8.7 ± 0.2[a]

[1] SSP = salt soluble protein.
[2] [a-c]Means with different superscript letters in the same row indicate significant differences.
[3] Data are expressed as means ± standard deviation. Significant differences ($P < 0.05$) were tested with ANOVA followed by Tukey's test.

solution-3; 75.3 ± 0.6 in solution-4; and 72.2 ± 0.2 ($P < 0.05$) for solution-5, respectively. Thus, it appears that the concentration of the solutes, or even the solutes themselves (salt, ionic vs. sugars non-ionic) played a role in the bioprotective effect, which was observed in the sensory evaluation studies.

Similar values for moisture content (~78%) have been previously reported for lobster samples frozen for 6 months in 0.1% STPP in NaCl (0.9%) or 0.3% STPP (Calder et al., 2005). In the present study, moisture content represented a measure of the total amount of water in the lobster samples. Even though hard-shell lobsters were used in the present study, the relatively high moisture contents can be attributed to accumulation in their tissue during moulting (Thakurkthakur et al., 2017). This water acts as a solvent and is important for chemical and enzymatic reactions, and also contributes to the texture and overall acceptability of these products.

Although it might seem contradictory that the control samples with the highest moisture content (76.5 ± 0.6%) registered the lowest liking scores for texture (5.6 ± 1.9), Johnston, Nicholson, Roger, and Stroud (1994) noted that during the freezing of seafood, free water readily forms ice crystals which can damage cell walls resulting in decreased water holding capacity and the loss of fluid upon thawing, which in turn results in undesirable texture. Indeed, participants in this study selected terms such as fibrous and chewy to describe some of the lobster samples, which had negative impacts on overall product liking. On the other hand, the lower moisture content in samples preserved in solutions 3, 4 and 5 (Table 1), can be attributed to the presence of the salts (STPP and NaCl) as well as the novel blend of carbohydrates. It has been postulated that when solutes accumulate in seafood tissue, they bind to polar water molecules, thus, the tissue becomes more moist (MacDonald & Lanier, 1997). Therefore, it is not surprising that in the present study, samples preserved in solutions 3, 4 and 5 also received the highest scores for liking of flavour and texture, 7.2 ± 1.3, 7.1 ± 1.4, and 6.9 ± 1.6, respectively.

One parameter that was not measured in the lobster samples in the present study was water activity. Similar to moisture content water activity is also a measure of the water in food, but in this instance, water activity measures the energy of the water in food, and can be used to predict the stability of foods. Water activity is also affected by the concentration of solutes present in the food, and this value decreases as the solute concentration increases (Damodaran, Parkin, & Fennema, 2008). Sampels (2014) and Chao, Bin, Lu-Kai, and Ji-Peng (2017) also reported that decreases in water activity slowed down microbial growth and chemical reactions, and low rates of these processes resulted in improve seafood quality.

3.2. Lipid oxidation in lobster meat

The TBARs assay was used to measure malonaldehyde (MDA) concentration produced as a by-product of lipid oxidation in the lobster samples frozen for one year in solutions 1–5. The data obtained are shown in Table 1. Overall, the MDA concentrations ranged from 0.26 ± 0.01 to 0.30 ± 0.01 (μg/g) and ANOVA analyses indicated that the various treatments had no effect ($P > 0.05$) on the amount of lipid oxidation observed. Calder et al. (2005) reported MDA levels of 0.2 to 2 μg/g in lobster tail and claw meat during 6 months of frozen storage. Among the samples tested, lobster meat that was preserved in 0.1% STPP registered the lowest MDA values. However, in the present study, the presence of cryoprotectants did not appear to impact the MDA levels as similar values were obtained for lobster meat preserved in water (solution-1), vs. NaCl, STPP or the novel carbohydrate blend (solutions 2 to 5).

Yang and Boyle (2016) have reported several parameters that can impact the reliability and accuracy of data from TBARs assays, these include the sample preparation method, interfering agents, and triglyceride concentration. The lower MDA values reported for all the samples in the present could be related to the fact that our modified sample preparation method excluded a hydrolysis step which resulted in the measurement of free TBARs as opposed to total TBARs in the lobster samples. Moreover, similar to the Calder et al. (2005), we have also incorporated the use of the antioxidant n-propyl gallate which lowered the possibility of measuring erroneously formed MDA. However, Papastergiadis, Mubiru, Van Langenhove, and De Meulenaer (2012) caution that substances such as sugars and oxidized proteins may also interfere with TBA colour complex formation and thus overestimate results of the TBARs assay. With regard to the interfering effect of sugars, no difference in MDA values reported in solutions 2 to 5 containing salt vs. carbohydrates (Table 1), which eliminated our concerns over obtaining overestimated values. In addition, other parameters such as environmental factors, and lipid concentration can also affect the results of the TBARs assay. MDA concentrations of 2.7 ± 0.3 (μg/g) were reported for fresh salmon which is known to be a high-fat fish ~ 9% triglycerides compared to 1% in lobster (Lobster Council of Canada, 2014; Papastergiadis et al., 2012). Thus, the data obtained for these experiments appear reasonable.

3.3. Hardness/firmness

TPA experiments were done to obtain quantitative assessments of the

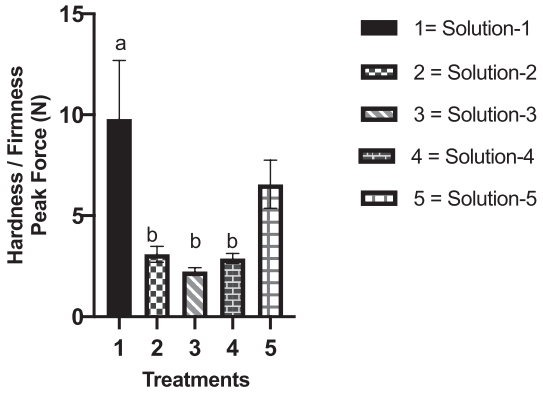

Fig. 1. Hardness (firmness) of lobster samples preserved for one year in various treatments. Solution-1 (water); solution-2 (water + NaCl + STPP, 0.5%); solution-3 (water + NaCl + carbohydrate blend; solution-4 (water + NaCl + STPP, 0.25% + carbohydrate blend), and solution-5 (water + NaCl + STPP, 0.5% + carbohydrate blend). There was a significant difference ($P < 0.05$) in hardness/firmness between samples preserved in solution-1 compared to those in solutions-2, 3, and 4.

firmness or hardness of the lobster samples frozen for one year, the data are shown in Fig. 1. These experiments consisted of determining the force required to compress the lobster meat between the probe and the plate. This force was also indicative of sample hardness or firmness. Samples that were frozen in solution-1 (water) exhibited significantly higher values for firmness (9.8 ± 7.7, $P < 0.05$) compared to the samples that were frozen in solutions containing NaCl, STPP or the novel carbohydrate blend. For example, the firmness of samples frozen in solutions-2, and 3, were 3.1 ± 1.0 and 2.2 ± 0.5; the firmness recorded for samples in solutions-4, and 5 were, 2.9 ± 0.7, and 6.6 ± 3.2, respectively (Fig. 1).

Other researchers have also tried to correlate TPA measurements with the sensory evaluation of seafood texture. Calder et al. (2005) measured the force required to shear (penetrate) lobster meat samples, and observed significant increases in this force when samples were prepared in 0.9% NaCl (control), compared to those that were prepared in 0.1% or 0.3% STPP. Similarly, in the present study an increase in tenderness in the presence of STPP and the carbohydrate blend was also observed. However, lower compression forces are reported in this study, probably because different methods were used to determine the firmness/hardness measurement.

Another possible explanation for lower compression forces may be related to moisture present inside the lobster tissue. Hydrogen bonding of water molecules with polar groups on proteins in lobster tissue may increase water-holding capacity, and the presence of water likely contributed to less forces required for compression. Vacha et al. (2013) and Rosenthal (2010) also mentioned several factors including sampling technique, test parameters, and heterogeneity of the sample which can impact the reproducibility of TPA measurements. The heterogeneity of the lobster samples used for the TPA analyses may also have been a factor in contributing to the variability observed in the measurements (Fig. 1). Sigurgisladottir et al. (1999) also reported a similar limitation when using different attachment probes (flat cylinder, blade and a sphere) to measure the hardness of salmon fillets, but found no difference in the hardness when using the flat cylinder and the sphere. In contrast, hardness was different when samples were obtained from different locations (between the head and tail). Although the samples described here are different (salmon fillets vs. lobster meat), the results from the Sigurgisladottir et al. (1999) study emphasize the importance of homogeneity and sampling as key factors in determining the final hardness or texture result. For future texture measurement of lobster meat, it may be more feasible to apply measurements to representative portions of the tail and the claw separately, as opposed to the combined sample approach that was used in the present study.

Nevertheless, the penalty analysis of the overall liking and CATA terms identified hardness and chewy as attributes that negatively impacted the overall acceptability of the lobster samples (impact score, −0.6, and −0.7, respectively). Moreover, Pearson correlation results showed that hardness negatively impacted texture ($r = -0.7$) and overall acceptability ($r = -0.7$) of the samples. Texture is one of the most important attributes of lobster products, and firmness or hardness are terms that are often used to describe these products. Thus, in this regard, the sensory data seems to support the objective data obtained for firmness, since samples that were more preferred exhibited lower values for firmness (Fig. 1). MacDonald and Lanier (1997) also noted that the development of firmness is related to a decrease in moisture and pH, and the latter can also result in changes in protein conformation.

3.4. Salt soluble proteins

Salt soluble protein (SSP) concentrations determined from lobster claw and tail are shown in Table 1. Overall, in the lobster tail, solutions-3 and 5 had the highest concentrations of SSP, 8.4 ± 0.1 and 8.6 ± 1.4, respectively. Conversely, for the lobster claw, samples preserved in solutions-2 and 3 had the highest concentrations of SSP, 11.03 ± 0.4 and 10.9 ± 0.5, respectively. Calder et al. (2005) hypothesized that lower SSP concentrations would be an indication of tougher seafood texture, but their sensory results did not support this hypothesis. Conversely, in the present study samples with higher SSP values were among those that were more preferred, more so for the lobster tail samples with overall liking scores of 6.8 ± 1.4 and 7.0 ± 1.3 for solutions-3 and 5, respectively. However, it should be noted that the amount of protein recovered depends on the extraction method used, and the solubilizing conditions which includes ionic strength, pH, and dilution ratio (Lan, Novakofski, Carr, & McKeith, 2006). For example, Regenstein and Stamm (1980) used relatively high salt concentrations containing either 0.6 M NaCl or 0.6 M NaCl, plus (40 mM $NaHC0_3$ and 10 mM $NaCO_3$) to extract the salt-soluble or myofibrillar proteins found in lobster, and observed that the amount of protein extracted increased with ionic strength. Myofibrillar or salt-soluble proteins are structural proteins, and they impact the functional as well as the structural properties of muscle protein (Wan-Ling, Qing-Xiao, Zhi-Wei, & Guo-Sheng, 2012). Other factors including the diet of the lobster prior to cooking is also another important component that can change the ultrastructure of muscle proteins which in turn induces changes in texture (Lin, Zeng, & Zhu, 2009).

3.5. SDS-PAGExxx

In order to determine if there were any changes in the relative protein compositions between the fresh and frozen lobster samples, protein banding patters obtained on SDS-PAGE gels were compared. Fig. 2 shows the electrophoretic banding pattern using two different protein concentrations (20 μg and 50 μg). Overall, there was good separation across the molecular weight range, and the banding patterns appeared very similar in the fresh and frozen samples. Detectable bands of the main myofibrillar protein actin, were also present in both the fresh and

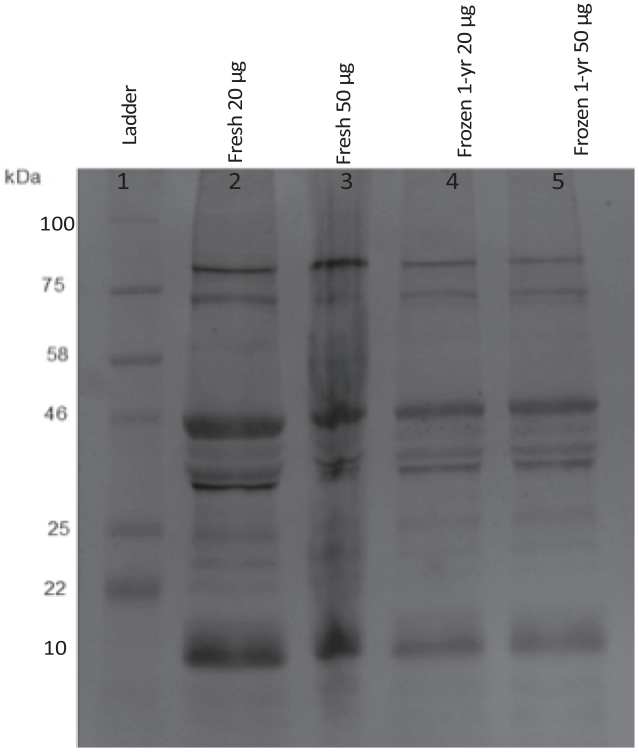

Fig. 2. SDS-PAGE showing molecular weights of protein extracted from fresh lobster vs. lobster meat frozen for one year. Overall, the protein banding patterns appeared similar in all the samples for the two concentrations used (lanes 2–5). All proteins samples were separated using a 2% polyacrylamide resolving gel and the latter was stained using a Coomassie Brilliant Blue stain (0.1%). Lane 1- shows a New England BioLabs pre-stained protein marker (P77066) with molecular weights ranging from 10 to 100 kDa.

frozen samples at the predicted molecular weight (~43 kDa). Ideally, it would have been interesting to compare the impact of the different solutions on the overall banding pattern, but due to insufficient amounts of each sample, one composite sample consisting of lobster meat preserved in solutions 3, 4 and 5 was used instead for the SDS-PAGE study. In a previous study, differences in protein composition were shown to impact the textural characteristics of muscle protein (Wan-Ling et al., 2012). However, the results in the present study show that the relative composition of the protein components in the composite sample remain essentially unchanged, with no breakdown of proteins in fresh or frozen samples confirming the cryoprotective effect of the carbohydrate blend and STPP (Fig. 3). The formation of ice crystals can result in the disruption of cells and consequently the release of proteinases which may degrade muscle protein (Nakazawa & Okazaki, 2020). Thus, it was hypothesized that protein degradation in the frozen samples would be observed as an increase in the amount of smaller molecular weight fractions on the SDS-PAGE gels. However, this was not observed when the fresh and frozen samples were compared. Reduction in the quantity of frozen water in the protein matrix may be one mechanism by which sugars stabilize muscle protein during storage (Chang & Regenstein, 1997).

3.6. Sensory evaluation of lobster meat

The results for the mean liking scores obtained for the lobster samples are presented in Table 2. Considering the ANOVA analysis, the presence of the cryoprotectants did not seem to impact the appearance of the samples, as no significant differences ($P > 0.05$) were noted between the control, solution-1 (mean score, 6.6 ± 1.7) as compared to the other solutions that contained salt and either STPP or the novel blend of carbohydrates (mean score, 6.6 ± 1.7). Conversely, for flavour and texture, the lowest scores were obtained for the samples preserved in solution-1 (control), whereas the flavour and texture of lobster meat preserved in solutions 3 and 5 were rated significantly higher ($P = 0.001$). Mean scores for flavour and texture in solution-1 were 5.6 ± 1.9 and 5.6 ± 1.9, respectively vs. 6.9 ± 1.5 and 6.8 ± 1.5 for solution-3, and 7.2 ± 1.3 and 7.0 ± 1.3 for solution-5.

Table 2
Mean liking scores for lobster meat preserved for one year in various treatments. Solution-1 (water); solution-2 (water + NaCl + STPP, 0.5%); solution-3 (water + NaCl + carbohydrate blend; solution-4 (water + NaCl + STPP, 0.25% + carbohydrate blend), and solution-5 (water + NaCl + STPP, 0.5% + carbohydrate blend).

Treatments	Mean Scores: Appearance	Mean Scores: Flavour	Mean Scores: Texture	Mean Scores: Overall Acceptability
Solution-1	6.7 ± 1.7[a]	5.6 ± 1.9[a]	5.6 ± 1.9[a]	5.4 ± 1.9[a]
Solution-2	6.6 ± 1.7[a]	6.7 ± 1.6[b]	6.5 ± 1.7[b]	6.4 ± 1.8[b]
Solution-3	6.8 ± 1.9[a]	6.9 ± 1.5[b]	6.8 ± 1.5[b]	6.8 ± 1.4[b]
Solution-4	6.4 ± 1.7[a]	7.1 ± 1.4[b]	6.7 ± 1.7[b]	6.7 ± 1.6[b]
Solution-5	6.7 ± 1.6[a]	7.2 ± 1.3[b]	6.9 ± 1.6[b]	7.0 ± 1.3[b]

[1] Data input on a 9-point hedonic scale where 1 = Dislike extremely and 9 = Like extremely.
[2] Means in the same column with the same letter are not significantly different ($p < 0.05$).
[3] N = 107.

Similarly, the overall liking scores were significantly higher ($P < 0.05$) for lobster samples preserved in solutions-2, 3, 4, and 5 (mean score, 6.7 ± 1.5) compared to the control in solution-1 (mean score, 5.4 ± 1.9). Yet, it appears the type of cryoprotectant (STPP, ionic vs. carbohydrate, non-ionic) seemed to have marginal impacts on the overall liking of the samples. For example, the overall acceptability was marginally higher for samples containing the novel carbohydrate blend found in soultions-3, 4, and 5, which were 6.8 ± 1.4, 6.7 ± 1.6, and 7.0 ± 1.3, respectively compared to the ratings observed from samples in solution-2, 6.4 ± 1.8 (Table 3). It is also noteworthy that the samples preserved in soltion-3, which contained NaCl and no STPP also had marginally higher overall liking ratings (6.8 ± 1.4) than samples that were preserved in solution-2 (6.4 ± 1.8) which contained a blend of NaCl and 0.5% STPP. This seems to suggest that the novel cryoprotectant blend used in solutions-3, 4, and 5 provided similar bioprotective effects on lobster meat during frozen storage for one year compared to

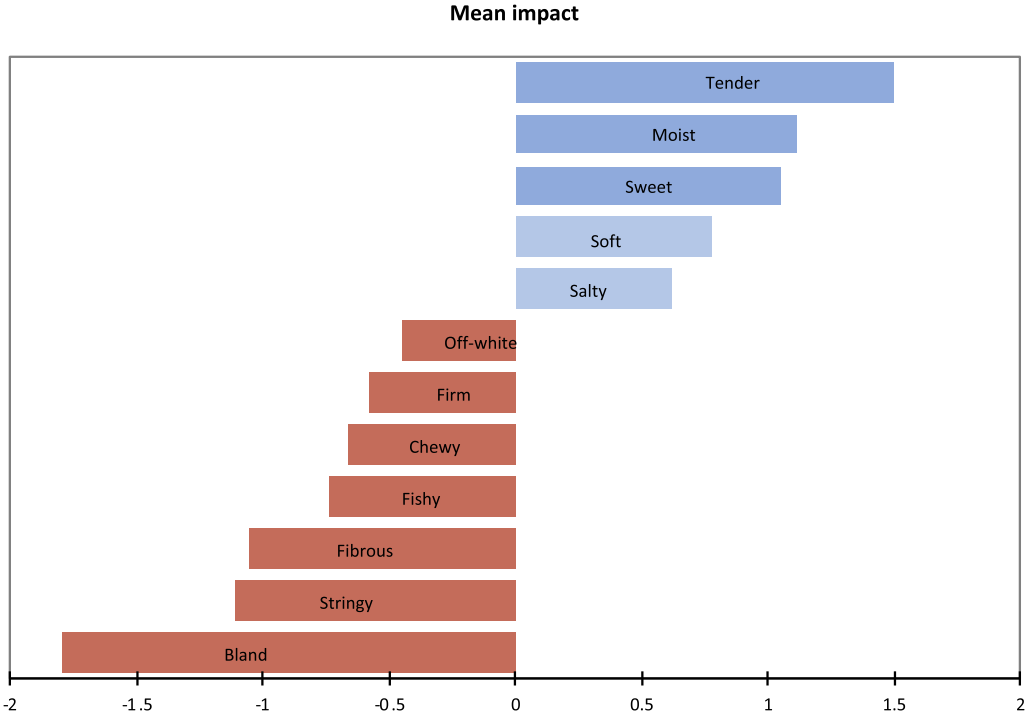

Fig. 3. Penalty analysis of CATA terms and overall liking scores. The values represent the difference between the average liking across all observations for which one term was selected minus the average liking across all observations for which the same term was not selected.

Table 3
CATA terms and the frequency of those terms used to describe lobster samples frozen for one year in various treatments. Solution-1 (water), solution-2 (water + NaCl + STPP, 0.5%); solution-3 (water + NaCl + carbohydrate blend; solution-4 (water + NaCl + STPP, 0.25% + carbohydrate blend), and solution-5 (water + NaCl + STPP, 0.5% + carbohydrate blend).

Category	Number of Mentions	Frequency (%)
Moist	245	60.5
Chewy	244	60.3
Tender	144	35.6
White	142	35.1
Fishy	136	33.6
Soft	136	33.6
Off-white	128	31.6
Sweet	119	29.4
Red	115	28.4
Salty	112	27.7
Fibrous	101	24.9
Stringy	100	24.7
Mild	96	23.7
Bland	88	21.7
Firm	84	20.7
Tough	77	19.0
Pale pink	75	18.5
Mushy	65	16.1
Flakey	60	14.8
Briny	53	13.1
Pale yellow	35	8.6
Dry	32	7.9
Ragged	25	6.2
Bright pink	23	5.7
Coarse	20	4.9
Bitter	19	4.7
Bright yellow	4	1

the traditional industrial STTP used in solution-2. A similar observation was also noted in our previous work which evaluated overall liking of lobster meat that was frozen for six-months in the same solutions (English et al., 2019).

Although significant differences ($P = 0.002$) in moisture contents were observed in samples preserved in solution-2 (76.7 ± 0.9%) compared to those preserved in solution-5 (72.2 ± 0.2%), no difference ($P = 0.43$) in their registered hardness was observed (6.6 ± 3.2 in solution-5) versus (3.09 ± 1 in solution-2). The presence of more water was expected to increase the formation of ice crystals, damage cells and result in undesirable texture (Johnston et al., 1994). However, the hardness (firmness) data confirms the proposition that blends of cryoprotective agents (in this instance, carbohydrate blend and STTP in solution-5) may be more effective compared to when they are used individually (as demonstrated with the use of STPP only in solution-2) (MacDonald and Lanier, 1991, 1997). Direct interactions between disaccharides and polar groups in proteins have been reported to be important for stabilizing biomaterials during freezing (Crowe et al., 1994). However, the lower SSP contents in lobster tail samples frozen in solution-2 (6.4 ± 0.3) compared to (8.6 ± 1.4) for samples preserved in solution-5 may have contributed to the slightly lower scores registered for texture (6.5 ± 1.7 in solution-2) versus (6.9 ± 1.6 in solution-5). During freezing or frozen storage, myofibrillar proteins may unfold and expose non-polar amino acids which can interact with similar groups and lead to protein aggregation (Dey & Dora, 2010). Nevertheless, carbohydrates act as cryoprotectants by covering protein molecules and increase their hydration, which in turn prevents protein aggregation (Parvathy & George, 2014). Collectively, these findings further strengthen our initial hypothesis that the novel blend of carbohydrates could be a suitable potential alternative for long-term preservation of lobster meat.

Participants selected terms from the CATA question that best described the lobster samples. The terms used to describe the samples and how often they were selected (expressed as frequency, %) are shown in Table 3. It is interesting that the terms related to texture, specifically moist, chewy, and tender registered the highest frequencies, 60.5%, 60.3%, and 35.6%, respectively. Some of the terms with the lowest ranking frequencies included coarse and bitter, with frequency scores of 4.9% vs. 4.7%, respectively.

A penalty analysis was also conducted to determine how each attribute selected impacted the overall liking scores (Fig. 3). Terms describing attributes such as tender, moist and sweet had the greatest positive impact on the overall liking scores obtained for the lobster samples (between 1 and 1.5). On the other hand, terms such as bland, stringy, and fibrous had the greatest negative impact (between −1 to −1.7) on overall liking scores (Fig. 3).

Taken together, the findings of this study reveal a strong association between the presence of the novel carbohydrate blend and STPP with improved overall acceptability of lobster meat frozen for one-year. This observation is based on the minimal loss in moisture, little or no evidence of lipid oxidation, and the increase in tenderness observed in lobster meat frozen in the presence of the novel carbohydrate blend, all of which contribute to extension of shelf-life. What then are the implications of these findings for the Lobster industry? We propose that the use of the novel carbohydrate blend could be beneficial in the following scenarios. First, current Health Canada regulations recommend that the total amount of phosphates allowed in frozen lobster cannot exceed 0.5% (Canada, 2020). Gonçalves and Ribeiro (2008) proport that exceeding this limit could result in excessive moisture in frozen products, and the risk of adulteration and economic fraud. On the other hand, for processors who want to reduce the amount of phosphates used in their frozen lobsters without the risk of reduced product quality and decreased shelf-life, the novel carbohydrate blend could be a suitable alternative to combine with STPP. In addition, the use of the novel carbohydrate blend could potentially be a 'clean-label' strategy for lobster processors who want to reduce the amount of phosphates in their products. This change in formulation would support the recent food industry trend to use more natural ingredients in keeping with recent consumer requests for ingredient transparency (Label Insight, 2016, 2017). Future research in this area should consider characterizing the microbial quality of the frozen lobster samples, which would not only strengthen the chemical and sensory data but would also provide more product specific evidence to support regulatory approval and industry acceptance of the carbohydrate blend. Another potential focus for future studies would involve characterizing the sensory and chemical differences between fresh and the one-year frozen lobster meats.

4. Conclusion

We have demonstrated that under the conditions reported in the present study, a combination of STPP and a novel carbohydrate blend can effectively preserved lobster meat for one year in frozen storage. The benefits of using these cryoprotectants resulted in little change in the chemical properties of the frozen products and improved overall liking of the products. Future studies should focus on characterizing the effect of this novel blend on the microbial quality of lobster frozen for short period (6 months or less) as well as over long-term (one year) frozen storage. The findings from the present study may have wide implications for the use of natural cryoprotectants for long-term preservation of lobster meat in Canada.

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CRediT authorship contribution statement

Marcia M. English: Writing - original draft, Writing - review & editing, Conceptualization, Data curation, Formal analysis, Supervision, Resources, Methodology. **Pablo M. Scrosati:** Investigation,

Methodology. **Anthony J. Aquino:** Investigation, Methodology. **Matthew B. McSweeney:** Investigation, Resources, Writing - review & editing. **M.S. Gulam Razul:** Funding acquisition, Supervision, Resources, Conceptualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

M. English interpreted the results and drafted the original manuscript, P. Scrosati and A. Aquino carried out the chemical experiments, M. McSweeney collected the sensory data and analyzed the results and proofread the manuscript, and M. S. Gulam Razul developed the idea for the study, assisted in the design of the objective experiments and proofread the manuscript.

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