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Use Opportunities of Hydrated Fullerene Nanoparticles for Hypothermic Storage of Industrial Oysters †

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Abstract: Hydrated fullerene (C60FWS) as an aqueous colloidal solution exhibits a wide range of biological activity at low concentrations. The purpose of this work was to study the effect of hydrated fullerene nanoparticles on the safety of Pacific oysters (*Crassostrea Gigas*) during hypothermic storage. Sensory characteristics, physicochemical and biochemical properties of the control group (CG) without addition and the experimental group (EG) with the addition of 10^{-8} M aqueous solution of C60FWS were used to assess the effect of hydrated fullerene nanoparticles on the preservation of oysters during storage at a temperature of 5 °C. The content of volatile nitrogen compounds as products of accumulation of protein degradation during autolytic and microbiological processes for CG and EG was 3.36 and 2.51 mg/10 mL of extract, respectively. This sensitive and objective indicator of negative changes in mollusk tissues during storage confirms the antioxidant properties of hydrated fullerene as an antioxidant protection tool for damage and destruction of biomolecules. This conclusion is confirmed by experimental data on changes in protein concentration in the tissues of mollusks in the adaptation processes of oysters during storage. So, on the 9th day of the experiment, the tissues of the animals of the experimental group contained 19% less protein in comparison with the control animals. Changes in physicochemical and biochemical parameters correlate with changes in the organoleptic characteristics of oysters. Preliminary storage of oysters in sea water with the addition of hydrated fullerene nanoparticles slows down the process of autolysis and allows it to be used as a tool to prevent changes in the quality of this food product, doubling the storage time of mollusks during transportation under anoxic and hypothermic conditions.

Keywords: hydrated fullerene; hypothermic storage; oysters; food quality; anoxia; sensory characteristics

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1. Introduction

Currently, nanomaterial's are used in food technologies due to their ability to create new or improve the properties of food products [1]. Of considerable interest is the possibility of using the antioxidant properties of nanoparticles in the production and preservation technologies of various food products [2–4]. Due to their special physicochemical properties, nanoparticles, such as fullerenes, have a unique biological activity: they are able to participate in biological processes as a regulator of reactive oxygen species and as acceptors

of free radicals, to protect against oxidative stress [5,6]. It is obvious that long-term hypothermic storage of biological material as a food raw material is accompanied by oxidation processes that contribute to a decrease in the potential of antioxidant protection, biological damage and the destruction of biomolecules. Therefore, the antioxidant properties of hydrated fullerene [7–9] were considered as the main factor for increasing the resistance of oysters to adverse effects that occur during their long-term storage. The purpose of this study was to evaluate the effect of C60FWS antioxidant properties on the survival of *Crassostrea gigas* oysters under hypothermia and anoxia according to their physicochemical and biochemical parameters and sensory characteristics (Figure 1).

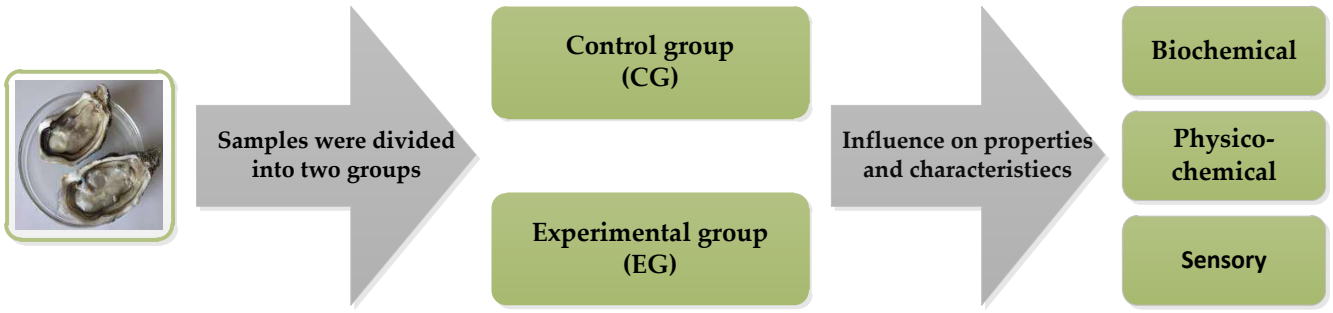

Figure 1. A graphical scheme of study approach.

2. Materials and Methods

2.1. Chemicals

Hydrated fullerene C60FWS with a concentration of 10^{-4} M was obtained from the Institute of Physiologically Active Compounds Ltd., Kharkiv, Ukraine. Reagents were obtained from the Sigma-Aldrich, Darmstadt, Germany. All reagents were of analytic grade.

2.2. Samples

The samples of Pacific oysters (*Crassostrea Gigas*) FINE DE CLAIRE™ (Marennes-Oléron, Charente-Maritime, France) was obtained from local distributor (Oyster Farm Ltd., Kharkiv, Ukraine). Oyster's samples were divided into two groups: experimental group and control group. The CG group of mollusks was kept for one day in seawater with the addition of fullerene C60FWS solution at a final concentration of 10^{-8} M. The EG group was kept in similar conditions, but without fullerene C60FWS. Further, oysters of both groups were stored under conditions of hypothermia and anoxia in a household refrigerator in a humid chamber (without water) at a temperature of 5 ± 1 °C for 9 days.

2.3. Methods

The state of preservation and moisture of oyster tissues, nitrogen amount and pH of oyster tissues extract were determined as described in [10]. The protein amount in mollusk tissues was determined by photometry using a ERBA CHEM 7 biochemical analyzer (Erba Lachema s.r.o., Brno, Czech Republic) and the appropriate test system TP245 (Randox Laboratories Ltd., Crumlin, UK) on the supernatant, which was obtained as follows: A fragment of the mantle and muscle tissue was separated from mollusks, weighed and homogenized using a glass homogenizer. Next, the homogenate was filtered through a nylon filter and centrifuged for 5 min at $840\times$ *g*.

An preliminary acceptance test was used to evaluate the following attributes in the samples: appearance, signs of vital activity, color, consistency, smell and taste by the ISO's methods [11,12]. The weighting of each of the above indicators in the overall assessment of the quality of the samples was determined by introducing a weighting factor: for appearance, signs of vital activity and color equal to 0.10; for consistency equal to 0.20; and for smell and taste equal to 0.25. The intensity of each sensory characteristic was recorded

on a 5-point hedonic scale after 1 h orientation sessions. The coded samples were shown simultaneously and evaluated in random order. The sensory analysis was performed on the laboratory of sensory analysis at State Biotechnological University (Kharkiv, Ukraine). The panelists stayed in the room with temperature 25 ± 2 °C and the relative humidity 52 ± 3%.

2.4. Statistical Analysis

All experiments were conducted at least in duplicate, and the corresponding results were statistically analyzed by Minitab v. 19 software (Minitab LLC, State College, PA, USA). The significant differences between means were evaluated by using one way ANOVA, and a Turkey's multiple range tests was applied for the multiple comparisons among experimental means ($p < 0.05$).

3. Results and Discussion

3.1. Physicochemical Properties

According to [13], autocatalytic and microbiological processes proceed in parallel in seafood after catching and subsequent storage time. Autolysis leads to the destruction of the structure of the meat tissue. Also, this process contributes to the penetration of bacteria into the meat and to the development of the bacterial reproduction process. It is practically impossible to distinguish the processes of autolysis and putrefaction. Proteins undergo the most significant changes during autolysis. The main typical indicators of autolysis of seafood meat (indicators of the beginning of spoilage) are volatile nitrogenous bases, ammonia and trimethylamine.

Accumulation of protein degradation products can be the most sensitive and objective indicators of negative changes in mollusk tissues during storage. An important point is that a change in physicochemical properties can occur earlier than the deterioration of biological material is noted according to sensory attributes. The use of only sensory analysis does not provide an opportunity to fully resolve the issue of preservation of mollusks.

The obtained results of the content of amino-ammonia nitrogen in the meat of mollusks during the hypothermic storage of oysters coincided with indicators of monitoring the quality and safety of mussels [14]. During storage, a certain trend of growth of protein degradation products was observed (Table 1).

Table 1. Physicochemical properties and nitrogen amount in oyster meat during hypothermic storage.

Sample Group	Storage Time, Days	Nitrogen Amount, mg/10 mL of Extract	Moisture of Oyster Meat, %	pH of Extract
CG	0	2.35 ± 0.02^{b}	81.52 ± 0.04^{c}	6.39 ± 0.01^{e}
	4	3.27 ± 0.03^{c}	73.93 ± 0.04^{e}	6.46 ± 0.01^{d}
	7	3.36 ± 0.03^{b}	79.89 ± 0.04^{e}	6.49 ± 0.01^{d}
	9	-	-	-
EG	0	2.11 ± 0.01^{e}	84.13 ± 0.04^{a}	6.83 ± 0.01^{b}
	4	2.16 ± 0.03^{d}	81.45 ± 0.04^{c}	6.92 ± 0.01^{a}
	7	2.35 ± 0.03^{b}	83.57 ± 0.04^{b}	6.66 ± 0.01^{c}
	9	2.51 ± 0.05^{a}	82.33 ± 0.04^{d}	6.63 ± 0.02^{c}

[a–e] Values with different superscript Roman letters in the same row are significantly different according to the Turkey's test ($p < 0.05$).

It should be noted the tendency of higher water content in the muscle tissue of mollusks of the experimental group compared to the control (Table 1). This fact had a positive effect on the degree of preservation of oysters in the presence of fullerene C60FWS.

3.2. Biochemical Properties

Oysters are littoral animals and are used as food when alive. Therefore, it is advisable, together with the determination of nutritional suitability, to evaluate possible changes in

adaptation processes. These processes could primarily affect the level of protein synthesis or degradation in mollusk tissues. In the study of changes in protein concentration in general, an increase in its amount in the tissues of mollusks was revealed both in the control and in the experimental group (Table 2).

Table 2. Protein amount in mollusk tissues during hypothermic storage.

Sample Group	Storage Time, Days	Protein Amount, mg/g of Mollusk Tissues
CG	0	25.8 ± 1.6^{bc}
	4	28.7 ± 3.3^{b}
	7	27.4 ± 4.1^{b}
	9	34.7 ± 3.7^{a}
EG	0	25.8 ± 1.6^{bc}
	4	23.8 ± 2.6^{cd}
	7	28.6 ± 2.9^{b}
	9	28.3 ± 1.2^{b}

[a–d] Values with different superscript Roman letters in the same row are significantly different according to the Turkey's test ($p < 0.05$).

However, the dynamics of the change in the amount of protein in both research groups had significant differences. In the experimental group, a slight decrease in protein concentration was observed on the 4th day of storage. Additionally, on the 9th day of the experiment, the animals of the experimental group contained 19% less protein in their tissues compared to the control animals.

It is known that the majority of metabolic processes, the course of which requires significant energy expenditure, are inhibited in the body under the influence of adverse factors, such as hypothermia and anoxia [15]. Such processes include the process of protein synthesis. According to studies [16], bivalve mollusks under conditions of anoxia, along with carbohydrates, usually use proteins as an energy resource, and lipids are not recognized as sources of energy. It is possible that the oysters of the experimental group could maintain the processes of their own homeostasis for a longer time in unfavorable environmental conditions due to the use of proteins as an energy substrate.

Under the conditions of the experiment in the control group on the 9th day, the processes of "food spoilage" of mollusks occur, which are based on the activation of the processes of autolysis and necrotic changes. The observed increase in protein concentration in the control group compared with the experimental group may indicate the activation of additional destructive processes and tissue lysis.

Some authors in their studies note the protective properties of fullerene under the negative influence of the external environment. The data they obtained indicate that fullerenes have antioxidant and radioprotective properties and significantly reduce the harmful effects of ionizing radiation [8]. Fullerene C60FWS captures free radicals in vitro and in vivo in a model of ischemic muscle damage [7]. At the same time, it acts as a kind of sponge, which accumulates and neutralizes free radicals [9].

3.3. Sensory Analysis

The results of sensory analysis of samples from both groups of oysters are presented in Table 3.

Table 3. Results of the sensory evaluation of oysters samples.

Attribute	Weight Factor	Evaluation during Hypothermic Storage, Days							
		CG				EG			
		0	**4**	**7**	**9**	**0**	**4**	**7**	**9**
Appearance	0.10	5	5	5	5	5	5	5	5
Signs of vital activity	0.10	5	5	3	3	5	5	4	4
Color	0.10	5	4	3	3	5	5	4.5	4.5
Consistency	0.20	5	3.5	3	3	5	4.8	4.8	4.8
Smell	0.25	5	4	3	3	5	5	5	4.5
Taste	0.25	5	4	3	3	5	5	5	5
General evaluation	1.00	30	25.5	20.0	20.0	30	29.8	29.3	28.8

With an equal initial assessment of the samples of both groups on the 4th day of storage, the experts noted the best overall assessment for the samples of the experimental group. This differentiation increased with increasing shelf life. The difference was especially noticeable for consistency, color and smell indicators. Changes of sensory attributes were fully corrected with changes of biochemical properties.

4. Conclusions

Analysis of the results of physicochemical, biochemical and organoleptic tests as markers of food safety of mollusks allows us to conclude that the use of hydrated fullerene C60FWS allows for the doubling the shelf life of *Crassostrea gigas* at a temperature of 5 °C, thereby maintaining their food quality. The results obtained will help reduce losses during the transportation of shellfish and their storage in a retail chain and restaurants.

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References

1. Zhou, H.; McClements, D.J. Recent Advances in the Gastrointestinal Fate of Organic and Inorganic Nanoparticles in Foods. *Nanomaterials* **2022**, *12*, 1099. [CrossRef] [PubMed]
2. Eswaran, S.V. Water Soluble Nanocarbon Materials:A Panacea for All? *Curr. Sci.* **2018**, *114*, 1846. [CrossRef]
3. Goltsev, A.; Bondarovych, M.; Babenko, N.; Gaevska, Y.; Dubrava, T.; Ostankov, M. Use of Nanomaterials in Cryobiology and Cryomedicine. *Probl. Cryobiol. Cryomed.* **2020**, *30*, 313–330. [CrossRef]
4. Sforzini, S.; Oliveri, C.; Barranger, A.; Jha, A.N.; Banni, M.; Moore, M.N.; Viarengo, A. Effects of Fullerene C60 in Blue Mussels: Role of MTOR in Autophagy Related Cellular/Tissue Alterations. *Chemosphere* **2020**, *246*, 125707. [CrossRef] [PubMed]
5. Barzegar, S.; Asri Kojabad, A.; Manafi Shabestari, R.; Barati, M.; Rezvany, M.R.; Safa, M.; Amani, A.; Pourfathollah, A.; Abbaspour, A.; Rahgoshay, M.; et al. Use of Antioxidant Nanoparticles to Reduce Oxidative Stress in Blood Storage. *Biotechnol. Appl. Biochem.* **2022**, *69*, 1712–1722. [CrossRef] [PubMed]
6. Sims, C.M.; Hanna, S.K.; Heller, D.A.; Horoszko, C.P.; Johnson, M.E.; Montoro Bustos, A.R.; Reipa, V.; Riley, K.R.; Nelson, B.C. Redox-Active Nanomaterials for Nanomedicine Applications. *Nanoscale* **2017**, *9*, 15226–15251. [CrossRef] [PubMed]
7. Kartal, H.; Küçük, A.; Kılıçarslan, A.; Polat, Y.; Süngü, N.; Kip, G.; Arslan, M. The Effect of Fullerenol C60 on Skeletal Muscle after Lower Limb Ischemia Reperfusion Injury in Streptozotocin-Induced Diabetic Rats. *J. Surg. Med.* **2020**, *4*, 451–455. [CrossRef]

8. Gudkov, S.V.; Guryev, E.L.; Gapeyev, A.B.; Sharapov, M.G.; Bunkin, N.F.; Shkirin, A.V.; Zabelina, T.S.; Glinushkin, A.P.; Sevost'yanov, M.A.; Belosludtsev, K.N.; et al. Unmodified Hydrated C60 Fullerene Molecules Exhibit Antioxidant Properties, Prevent Damage to DNA and Proteins Induced by Reactive Oxygen Species and Protect Mice against Injuries Caused by Radiation-Induced Oxidative Stress. *Nanomed. Nanotechnol. Biol. Med.* **2019**, *15*, 37–46. [CrossRef] [PubMed]
9. Yang, X.; Ebrahimi, A.; Li, J.; Cui, Q. Fullerene-Biomolecule Conjugates and Their Biomedicinal Applications. *Int. J. Nanomed.* **2013**, *9*, 77. [CrossRef]
10. Falko, O.V.; Yevlash, V.V.; Piliugina, I.S.; Gazzavi-Rogozina, L.V.; Chizhevskiy, V.V. Changes in Quality Characteristics of Crassostrea Gigas Oysters during Hypothermic Storage. *J. Drug Deliv. Ther.* **2022**, *12*, 58–62. [CrossRef]
11. *ISO 8586:2012*; International Organization for Standardization. Sensory Analysis: General Guidelines for the Selection, Training and Monitoring of Selected Assessors and Expert Sensory Assessors. International Standards Meat and Meat Products: Genève, Switzerland, 2016; p. 34.
12. *ISO 13299:2016*; International Organization for Standardization. Sensory Analysis. Methodology. General Guidance for Establishing a Sensory Profile. International Standards Meat and Meat Products: Genève, Switzerland, 2016; p. 34.
13. Bykowski, P.; Dutkiewicz, D. Freshwater Fish Processing and Equipment in Small Plants. *FAO Fish. Circ.* **1996**, *509*, 1–59.
14. Melnyk, P.I. Quality Control of Edible Mussels in the Conditions of State Laboratories Vetsanexpertise in the Markets. *Sci. Bull. Natl. Agrar. Univ.* **2005**, *89*, 171–173.
15. Larade, K.; Storey, K.B. Characterization of a Novel Gene Up-Regulated during Anoxia Exposure in the Marine Snail, Littorina Littorea. *Gene* **2002**, *283*, 145–154. [CrossRef] [PubMed]
16. Shulman, G.E.; Abolmasova, G.I.; Stolbov, A.Y. The Use of Protein in Energy Exchange of Hydrobionts. *Usp. Sovrem. Biol.* **1993**, *113*, 576–586.