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RESEARCH

Edible Coatings Based on a Furcellaran and Gelatin Extract with Herb Addition as an Active Packaging for Carp Fillets

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Abstract

This is the first such study in which a gelatin extract obtained from carp skins enriched with dry herbs (thyme or rosemary) has been prepared. Extracts prepared in such a manner were added to furcellaran coatings. Coatings were tested for their mechanical properties and the obtained results showed that the control coatings, and those with the addition of rosemary, had the best strength-related parameters. A new ready-to-cook product was evaluated with regard to the preservative effects of carp skin gelatin coatings containing rosemary and thyme extracts in terms of pH, biogenic amine formulation, microbial changes and sensorial characteristics. The coatings with added rosemary proved effective in inhibiting the formation of biogenic amines, and slowing down the microbial deterioration of carp fillets (reduction by 0.53 and 0.29 log cfu/g). The evaluated herb coatings changed the characteristic taste of fish. Interestingly, the coatings emphasized the natural saltiness of fish meat.

Keywords Edible coatings · Fish waste · Preservation · Shelf-life

Introduction

According to scientific reports, the main trends on the food market in the coming years will include products characterised as 'convenient and practical foods', but, at the same time, 'healthy' and 'natural'. This is related to the change in lifestyle among modern consumers who declare that they have less and less time to prepare meals. The convenience of preparing foods does not necessarily lead to fast-food consumption. Authentic, simple products, which additionally allow to support local producers and preserve traditions, have created the so-called 'Slow Food' movement (Werner et al., 2021). Plant extracts and other natural additives are generally accepted. This trend, in combination with the convenience that consumers expect, is a challenge for food producers, but it also presents an opportunity for them (Dhir & Singla, 2019).

Despite the fact that there are many existing fish products, the fish sector, in its proposal for the convenient food market, does not have much to offer consumers. Observing market trends, it may be assumed that in the future, the most popular products will be those simultaneously having functional features, especially dietary ones, and being convenient to prepare. At present, the production of convenient products is already increasingly targeted at specific groups of recipients, such as children, the elderly, diabetics or athletes (Asioli et al., 2017). Fish, as products with high dietary value, fit perfectly into the trend of a healthy lifestyle. Therefore, it is necessary to search for new preservation techniques so that the obtained product is of high quality and, moreover, is easy to further prepare (Samoggia & Castellini, 2018).

Common carp is the 3rd most frequently produced fish species in the world, with 97.3% of its production taking place in aquaculture (Karnai & Szűcs, 2018). Global common carp production is dominated by China. In the EU, the

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leading carp producer is Poland. However, the consumption of carp in Poland is currently experiencing a gradual decrease; i.e. it amounts to 0.56 kg per year/person. In research, it has been shown that it is not the price that is the main obstacle for consumption, but the demand for carp is rather unyielding (Raftowicz, 2022).

As mentioned above, increasingly noticeable is emphasis on a healthy lifestyle combining healthy, ecological food and outdoor activities in valuable natural areas, along with self-realisation and passion. Food should also be convenient. However, this does not go hand in hand with the consumption of carp in Poland, which, for most young consumers, is associated mainly with a traditional Christmas dish and not a healthy and convenient food. Carp is commonly available at supermarkets and discount retailers only in the 1st half of December. At other times, consumers have to go directly to the carp producer. The short supply chain within the carp market is still insufficiently developed, even if the origins of the production and the carp freshness are very important for young adult populations. This means that the development of the carp market in Poland and in Europe requires diversification of its offer. Furthermore, the market of carp producers in Poland is very fragmented. At present, there are about 300 carp farms run by private breeders (Raftowicz, 2022).

Finding ways to consume and waste as little as possible is another popular trend that will continue to shape consumer thinking about food over the next decade. Therefore, both producers of foods and of food additives are already looking for newer and newer ways to extend the shelf-life of their products. Spices and herbs have been used for centuries to impart appropriate organoleptic qualities to food. In addition, they are a source of many substances with antioxidant and antimicrobial properties, and thus, they extend the shelf-life of food products (Chua et al., 2019). The use of herbal and spice extracts as antioxidant food ingredients has been known in the scientific literature for many years (Amarowicz et al., 2009; Żegarska et al., 1998). However, not all of the known natural extracts can be freely used in combination with fish as they need to fulfil a set of requirements, such as, e.g. compatibility with fish flavour and other fish constituents (Estévez, 2021). Additionally, the use of biopolymer matrices as carriers for herbal extracts aimed at controlled release of these compounds is still a new and not fully explored issue. Different matrices, including animal fat, protein and carbohydrates, such as those proposed in our research, may have an unexpected effect on the product and its storage stability. Most of the existing research data characterise edible coating in the form of dry film obtained by a casting method, which is wrapped into the food products (Pluta-Kubica et al., 2021; Cai & Wang, 2021; Chen et al., 2016). The use of coating as a solution with active properties is a quite unusual approach (Areújo et al., 2018).

Waste from fish processing offers great potential in the recycling of by-product materials and conversion into relevant products with nutritional or functional value. Fish collagens in the industry of food processing are very interesting as they are employed in the production of gelatin extracts taken from collagen (Tkaczewska et al., 2018). Bio-based coating as well as the preservation of films are currently viewed as effective and eco-friendly methods in the maintenance of food product freshness, while contributing to shelf-life extension. In the existing literature data, usually, freeze-dried commercial gelatin is used as a component of the active coating (Tosati et al., 2017). The use of a fresh gelatin solution from fish waste with the omission of the drying process is a very innovative approach.

Gelatins have broad application in the preparation of edible coatings as well as films; however, those derived from pure gelatin demonstrate weak biological features, i.e. antioxidant- and antimicrobial-related abilities (Sun et al., 2019). So far, various polysaccharides have been used as components of gelatin-polysaccharide films and coatings (Jimenez et al., 2012; Poverenov et al., 2014). However, the use of furcelleran as a component of such coatings has not yet been investigated. Furcellaran is a negatively charged polysaccharide that is extracted from *Furcellaria lumbricalis* red algae. This sulphate polysaccharide can be used as components of films unnecessary and coatings (Kulawik et al., 2019). Furcellaran has excellent gel-forming properties and can form complexes with proteins (Jamróz et al., 2018). In addition, it is a very neutral film-forming base; therefore, it can be enriched with various active ingredients (Marangoni et al., 2020).

Therefore, the aim of our work was to develop a technology for the production of coatings based on protein waste from carp processing, which can be an a nutritious part of a healthy convenience-food product. Carps fillets were covered with innovative coatings and their quality was tested during storage. It was assumed that the developed technology should be as simple as possible, so that it could be used in small fish processing plants at fish farms. It is noteworthy that in this work, we were the first to use gelatin obtained from carp skin as a solution to extract dry herbs from thyme or rosemary. We added dry herbs to the extract, which is a new idea. The procedure does not require the preparation of additional herbal extracts. It can be used with dry herbs and by implementing them into a gelatin solution. There are many works in which a prepared herbal extract, e.g. water or alcohol, was added to the prepared biopolymer coatings (Mehdizadeh & Mojaddar Langroodi, 2019; Moreno et al., 2020; Salem et al., 2021). However, it is noteworthy that in the case of this solution, we have additionally reduced the time and costs of preparing such an extract.

Material and Methods

Coating Preparation

Gelatin Solution The carp skins (*Cyprinus carpio*) were supplied by a local fish processing plant (Sona Sp. z o. o. Koziegłówki, Poland). The applied gelatin was formed by implementing an extraction procedure. Three hundred grammes of carp skins was minced and then soaked in a 1500-mL volume containing the following water solutions: 0.05 M sodium hydroxide, 0.004 M hydrochloric acid and 0.05 M citric acid. This was carried out in room temperature. For each of the treatments using solutions, carp skins were soaked for a period of 90 min. There was an intermediate washing step using tap water. Following this stage, the skins were washed with water once more, and then mixed with 1500 mL distilled water. The incubation period lasted overnight and was performed at 45 °C. The material achieved during the process was subjected to sieving (sieve diameter totalling 72 μm) to remove all remaining skin from the gelatin-based solution.

Thyme and Rosemary Polyphenol Extraction Thyme (*Thymus pulegioides*) and rosemary (*Rosmarinus officinalis*) were cultured in Poland during the summer of 2021. In September 2021, they were picked, dried and vacuumed packed. The gelatin solution having a 5% addition of dry thyme or rosemary (g/mL) was prepped and then incubated at a temperature of 85 °C for a 2-h period. Following incubation, the solution was subjecting to filtration which was done using a sieve (with 72-μm diameter). The solution's pH was adjusted to 7. After adjustment, the solution was applied as a component for coating.

Coating Fabrication Furcellaran type 7000, M_w 2.951×10^5, was chosen and bought from Est-Agar AS (Karla village, Estonia). The furcellaran product, comprising 79.61% of carbohydrates, 1.18% of protein and 0.24% of fat, was implemented in the study. The solution containing herb-gelatin/furcellaran was prepared in the following manner: 3.5% of the furcellaran (g/mL) sample was mixed with the gelatin at a ratio of 3:7 and the herb addition. This was carried out in defined conditions (i.e. 400 rpm mixing rate, temp. at 50 °C, for 30 min). As a plasticiser, glycerine was utilised at a 1-mL volume, which was further added to 100-mL sample of the solution consisting of furcellaran and gelatin.

Mechanical Properties of Coatings

In plastic boxes, we placed 30 g of the film-forming solution, which were further put into a refrigerator at 4 °C for 48 h. For the compression test, the samples were removed from the containers, while for the penetration test, they were not removed.

Mechanical parameters of coatings were determined using EZ-test Shimadzu (Japan) equipment, via TrapeziumX software.

Compression was carried out using a cylinder with the dimension of 30 × 30 mm (diameter × height), at a rate of 1 mm/s and having a plate with a diameter of 40 mm, until a 50% deformation rate was obtained.

The following parameters were tested using this method: fracture force, elastic modulus, fracture strain.

Penetration of the measuring element, in the form of a 20 × 20 mm cylinder at a rate of 1 mm/s into the gel located in a container with a diameter of 30 mm, was analysed. The following parameters were tested with this method: firmness, strength and adhesiveness. Each parameter was specified in 5 replicates. The scheme of mechanical processing is shown in supplementary material Fig. S1.

Effects of Tested Coatings on Refrigerated Carp Fillet Safety and Quality Levels

Sample Preparation

The innovatively created coatings were put on raw fish fillets to carry out efficacy evaluation and to make a product that is of ready-to-cook type. The fresh carp (*Cypirnius carpio*) was procured from a local fish processing plant (Gospodarstwo Rybackie, Przyborów, Poland). Three of the carp fillets were randomly chosen for each analysis day. Moreover, 50 g of fillets was dipped into the coating solution twice for 4 s and was then subjected to air-drying. On average, the coating constituted 17.28% of the mass of the sample.

The fillets enclosed in the coatings were situated on a plastic tray. It was closed and then sealed in a heat sealer using synthetic foil.

The fish samples under study were appointed to 4 individual groups: uncovered sample (K); that covered in furcellaran/carp gelatin coating without the addition of herbs (G); the sample covered with furcellaran/carp gelatin coatings, including 5% thyme addition (T); and finally, the specimen covered with furcellaran/carp gelatin having a 5% rosemary addition (R).

In Fig. 1, the scheme is shown for obtaining the coatings and their application on carp fillets.

Carp Fillet pH Analysis During Storage

From the fish samples, 10 g was homogenised using 10 mL of distilled water. This process was conducted for 30 s. The pH value for the fish homogenate was measured with

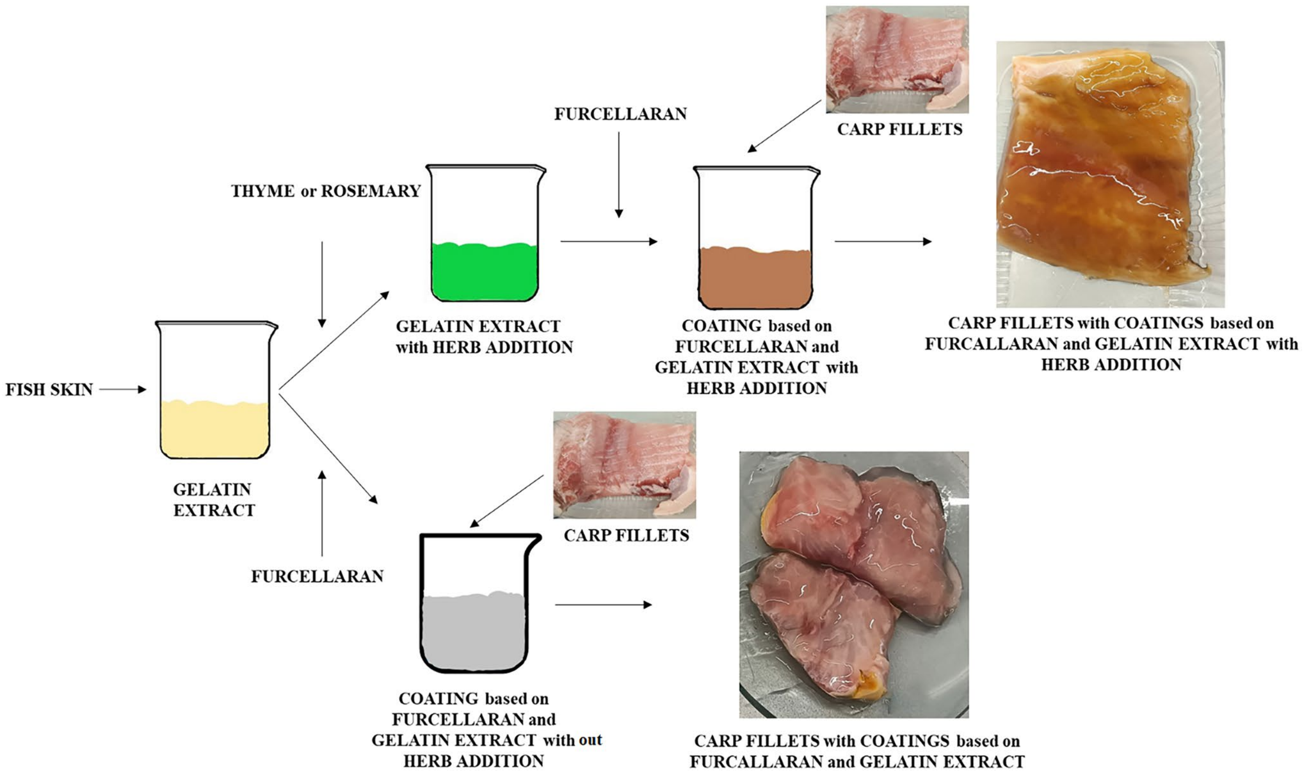

Fig. 1 The procedure of obtaining active coatings

a digital pH metre and then standardised via a buffer at the pH levels equalling 4 and 7.

Carp Fillet Microbiological Analysis During Storage

In total, 10 g was selected from each fish sample to be mixed with 90 mL of maximum recovery diluents (Oxoid, Basingstoke, UK). They were further homogenised for a 120-s duration applying a Stomacher blender. Total viable count (TVC) analysis was carried out via the pour plate method with a Plate Count Agar (Biomaxima, Warsaw, Poland). The analysed plates were incubated for 48 h at 30 °C. Yeast and mould (YM) counts were provided using the spread plate procedure performed on a DRBC agar (Biomaxima, Poland). The incubation conditions for the plates were 25 °C for 120 h.

Carp Fillet Biogenic Amine Analysis During Storage

Biogenic amine analysis was conducted utilising the method of HPLC (additionally employing dansyl chloride derivatisation). Sample preparation was done according to the procedure given by Kulawik et al. (2018). The Dionex Ultimate 3000 UHPLC (Thermo Scientific, Waltham, USA) was used for separation, FLD 3400RS 4-channel fluorescent detector (Thermo Scientific) on a Kromasil 100–5-C18 4.6 × 250 mm column (Akzo Nobel, Amsterdam, the Netherlands). The noted chromatographic conditions have been earlier detailedly described in the work by Jamróz et al. (2022). As for references, the Supelco biogenic amine standards were applied (Sigma-Aldrich, St. Louis, USA).

Sensory Analysis of Carp Fillets Having Selected Coatings

The examined carp fillets were split into small portions, each weighing 100 g. The portions then underwent furcellaran/carp gelatin coating without the addition of herbs (G) or with a furcellaran/carp gelatin coating comprising 5% rosemary addition (R), or a furcellaran/carp gelatin coating having a 5% thyme addition (T). The fillets were subsequently wrapped in parchment paper. Following this process, the next step was placing the samples on baking plates, which were additionally covered using aluminium foil. The fillets were subjected to baking for a duration of 30 min. Post-baking, the samples were uncovered for a 10-min period. Directly after baking, the still-hot fillets underwent testing. The sensory evaluation was conducted by a cyclically trained sensory panel, recruited and selected from the employees of the Faculty of Food Technology. This group included 15 selected assessors who were trained at least 16 h before the carp fish quality evaluation according to PN-EN ISO 5495:2007/A1:2016, PN-EN ISO 5492:2009, PN-ISO 3972:2016–07, PN-EN ISO 11132:2017–08, PN-EN ISO 10399:2018–03 and PN-EN ISO 8586:2014–03 regulations, in conditions specified in the standard PN-EN ISO 8589:2010. Moreover, the panellists were familiar with the fish test assessment procedure. By the leader, the team was introduced to the attributes, terminology, scale method and quantitative descriptive analysis technique in relation to the fish sample. Just before the sensory analysis, the panellists were provided with cards having detailed descriptions of typical characteristics regarding the baked carp fillets. The cards were designed by using a quantitative descriptive profile (a description of the sample including both attribute- and intensity-related values). They concerned assessment regarding fillet's general appearance (form as well as surface condition, sauce appearance), smell (fishy, rosemary, salty, sweet, sour, bitter, fish oil and rancid), texture with fork (juiciness, elasticity and cohesiveness), texture evaluated while in the mouth (juiciness, hardness and chewiness) and taste (fishy, rosemary, muddy, fish oil, rancid). With regard to smell and taste as well as texture, we requested the panellists suggest a degree for attribute intensity of an ideal carp fillet. This was done according to the following scale: 0—'imperceptible', 1—'the least intense' to 5—'the most intense'. General fillet acceptance was rated on a 5-point hedonic scale (1—'dislike very much' to 5—'like very much').

The samples were presented to the panellists on white plastic plates and marked with a randomly designated 3-digit code. Distilled water and sugar-free rusk were provided for neutralisation of the following each sample tasting. This was a requirement for minimising error as well as masking sensory attributes.

Statistical Analysis

Each experiment was carried out using 3 independent replicates and triplicate repetitions for each replicate, unless otherwise stated. The results were provided using mean ± standard deviation. Statistical analysis was performed using Statistica v. 13.0 software (Tibco, Palto Alto, USA). Result normality was assess via the Shapiro–Wilk test. Variables demonstrating non-normal distribution were conversed using the Box-Cox data method for conversion. One-way ANOVA was employed to assess the results of coating property. The results from fish preservation testing underwent to two-way analysis of variance, the independent variables assumed as storage duration and coating type. The discovered differences between individual groups were determined by applying Tukey's test for post hoc analysis. The null hypothesis was tested and all statistical analyses were discarded when $P < 0.05$.

Results and Discussion

Coating Mechanical Properties

The mechanical properties of coatings based on gelatin with herb addition and furcellaran are presented in Table 1. The compression and penetration raw curves are presented in Fig. S2 (Supplementary Data).

The fracture force values for the control coatings were 6.64 N, while the addition of 5% rosemary extract did not change this parameter. A different situation occurred in the case of adding the 5% thyme extract. Here, a clear decrease in fracture force was observed. A similar trend was noted for the elastic modulus parameter. However, the addition of 5% rosemary extract increased the value of this parameter (without statistically significant differences).

The decrease in value of the elastic modulus parameter may be related to the reduction of inter- and intra-molecular interactions between polymer chains. It may further be influenced by the presence of micropores during the addition of an extract, which prevents elasticity of the gels. In addition, the discontinuity of the hydrogel phase reduces flexibility by preventing the mobility of the polymer chains (Jaiswal et al., 2019). The presence of plant extracts may interfere with the formation of the gel structure and simple, steric hindrance which may be important here, especially when the chains have branches. In addition, degradation of polymers during processing of the material may have occurred, forming particles with shorter chains and of weaker gel structures.

The decrease in the value of the elastic modulus parameter may be related to the reduction of inter- and intramolecular interactions between polymer chains and functional groups in plant extracts. An analogous phenomenon was observed for the fracture strain parameter, where the addition of rosemary extract was irrelevant, while the addition of thyme extract significantly decreased the values.

Table 1 The mechanical properties of coatings based on furcellaran, gelatin and plant extracts

	Control	Rosemary	Thyme
Fracture force [N]	$6.64^b \pm 0.52$	$6.87^b \pm 0.79$	$1.28^a \pm 0.20$
Elastic modulus [kPa]	$18.78^b \pm 1.82$	$20.32^b \pm 0.90$	$10.03^a \pm 0.94$
Fracture strain [%]	$23.52^b \pm 1.39$	$21.90^b \pm 1.40$	$14.65^a \pm 1.61$
Firmness [N]	$3.21^c \pm 0.20$	$2.78^b \pm 0.17$	$1.55^a \pm 0.03$
Strength [N]	$1.38^b \pm 0.04$	$1.43^b \pm 0.13$	$0.50^a \pm 0.17$
Adhesiveness [N/mm]	$-16.47^b \pm 2.71$	$-17.59^b \pm 2.08$	$-9.00^a \pm 1.34$

Results are expressed as mean ± standard deviation. Different lettering in the rows indicates significant differences $p < 0.05$. All gel samples had the same shape, size and thickness

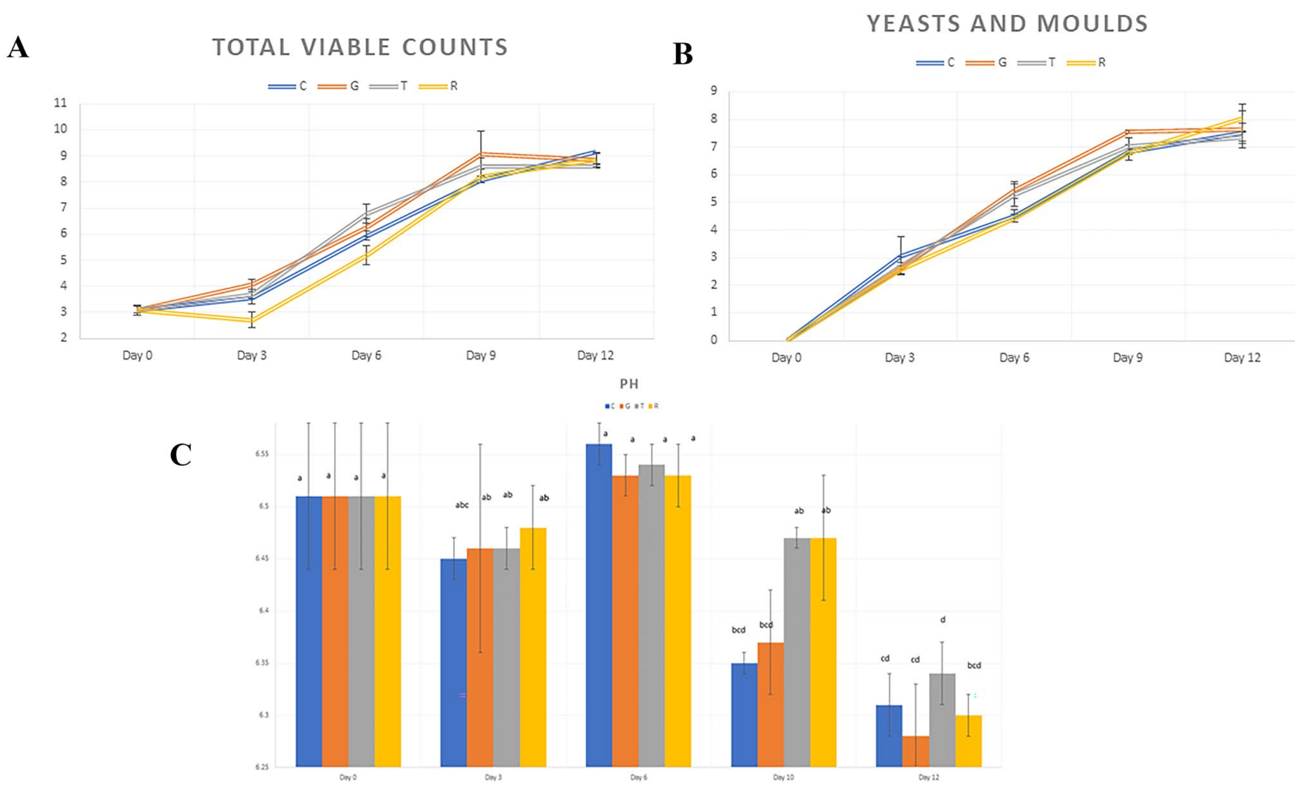

Fig. 2 Microbiological contamination and pH changes in carp fillets during the storage period at a temperature of 4 °C. **A** Total viable counts in carp fillets during storage, **B** yeasts and moulds in carps fillets during storage, C pH of carps fillet during storage, **C** carp fillets without any coatings, G samples preserved by coatings without herbs, T samples preserved by coatings with thyme extract, R samples preserved by coatings with rosemary extract. [a,b,c]Different letters in columns indicate significant differences between means ($p < 0.05$)

To a large extent, the addition of plant extracts caused a loss in firmness of coatings; however, the greatest decrease in value was observed in the case of coatings with the added thyme extract. Addition of the rosemary extract did not significantly affect the parameters of strength or adhesiveness; nonetheless, this was a different case with the thyme extract, where the changes in the values of these parameters were significant.

Thus, the rosemary extract did not change the mechanical parameters of coatings based on gelatin and furcellaran, which may suggest its potential use as active coatings for food products.

Effects of Tested Coatings on Refrigerated Carp Fillet Safety and Quality Levels

Carp Fillet Microbiological Analysis During Storage

The TVC of carp fillets covered with different coatings during storage at 4 °C is shown in Fig. 2.

The results indicate that, over time, during 12 days of storage, the value of TVC in all samples increased and differences between the samples occurred during the whole storage period. From days 3 to 9 of storage, surprisingly, the highest TVC value was observed for the sample covered using the coating without herbs and not for the control one. Such a result is startling, as it was expected that even the gelatin/furcellaran coating without the addition of herbs will limit the development of microorganisms by restricting the availability of oxygen to the product. These assumptions were confirmed by data from literature on the subject, indicating a beneficial effect of gelatin coating without active additives on the microbiological quality of fish fillets (Andevari & Rezaei, 2011; Sun et al., 2019). However, it may be assumed that due to the fact that the gelatin from the carp skin contains a large amount of protein (Tkaczewska et al., 2018), it was an excellent medium for the development of microorganisms, which resulted in deterioration concerning the quality of carp fillets.

Coatings with the addition of rosemary extract showed the highest efficacy in inhibiting the growth of bacteria on the fish surface—the samples covered with the coatings during the initial storage period (days 3, 6 and 9) demonstrated a lower total viable count compared to the control group, as well as those coated fillets without the addition of herbal extracts. The changes of yeast and mould counts showed similar trend as in the case of TVC.

The obtained results allow to indicate that the use of gelatin/furcellaran coatings is conducive to the development of microorganisms, but the use of rosemary extract caused antibacterial and antimicrobial properties in gelatin coatings, which could have been caused by the presence of antimicrobial agents in the extract, such as phenolic compounds. No analogous effects were noted in the case of the extract with thyme. It is not easy to attribute the antimicrobial influence of herb extracts to one or even a few active principles. This is due to the fact that extracts always comprise a mixture of varying chemical compounds. As well as major components, minor ones may also significantly contribute to extract antimicrobial activity of extracts (Jiang et al., 2011). Moreno et al. (2006) indicated that the antimicrobial efficacy of rosemary extract is connected with their exact phenolic composition. Carnosic and rosmarinic acids might be the chief bioactive antimicrobial compounds found in rosemary extracts.

Interestingly, according to data from literature, essential oils derived from thyme also show strong antibacterial effects (Sakkas & Papadopoulou, 2017); however, coatings with thyme extract do not demonstrate antibacterial influence on carp fillets. Therefore, it may be assumed that the interactions between rosemary phenol compounds and the gelatin/furcellaran functional groups are stable (this has been confirmed by mechanical tests), which results in the obtained coatings showing in vivo biological activity. On the other hand, in coatings with the addition of the thyme extract, the formation of hydrocarbons could be unstable, which results in an excessively rapid release of active ingredients, and thus, their antimicrobial activity cannot be observed.

Salem et al. (2021) evaluated the effects on the microbial properties of a model food product of a coating consisting of gelatin that was enriched with *Lepidium sativum* extract. They noted that the number of microorganisms found in the food experienced a meaningful decrease because of applying the coating. This was ascribed to the fact that the *Lepidium sativun* extract antimicrobial substances may inhibit microbial growth.

These results revealed that gelatin/furcellaran coatings containing rosemary extract had a good preservative effect on the carp fillet, especially in the first period of storage. It has been found that of the 2 tested herbs, rosemary is more suitable as an active ingredient in the designed innovative coatings.

Carp Fillet pH During the Storage Period

pH values can be good indicators of freshness, although there are authors who disagree with such a statement (Abbas et al., 2008). Sun et al. (2019) reported that pH is not reliable as an indicator of fish quality evaluation. The characteristic pH of live fish muscle is 7.0, and it decreases to around 6.2 due to glycogen decomposing and lactic acid increase. The changes in pH of carp fillets with different coatings during storage at 4 °C for 12 days are shown in Fig. 2c. The initial pH was 6.51, a value in agreement with that for fish meat reported by other researchers (Gimenez et al., 2002; Volpe et al., 2015). In the study conducted by Kyrana et al. (1997), pH values of freshly caught fish were low (6.1–6.2) and indicated their good natural state. After 3 weeks of storage, this value increased to 6.6. This increase was associated with the accumulation of alkaline bacterial metabolites in fish meat. Moreover, pH differences observed in fresh fish flesh are usually due to the dissociation of carbonic acid in general, which brings about the pH increase as the storage time progresses. In the post-mortem period, decomposition of nitrogenous compounds leads to an increase in pH, which affects the quality of the product during storage; especially, sensorial characteristics such as odour, colour and texture are negatively affected. As indicated by Susanto et al. (2011), pH changes in fish during storage may have different patterns depending on the fish species. Nonetheless, it always increases during storage. The same increase in pH was observed in our study, in all the samples, up until the 6th day of storage. Still, the pH measured after 12 days of storage decreased for all samples. Despite the fact that these differences were not statistically significant, it was interesting to note them. One of the reasons can be the formation of lactic acid by lactic acid bacteria that were used as a carbohydrate (furcellaran) added to the coating as a source of energy. Similar results were obtained by Dehghan Tanha et al. (2021) who studied the effect of *Portulaca oleracea L.* extract in the gelatin coating on the quality properties of fish sausages during refrigerated storage. Summarising, pH can be an indicator of fish freshness, but only in the case of fresh fish not being subjected to any additional treatments or additives. Our results allow to indicate that measuring pH may even be misleading, and should not be recommended as a freshness indicator of fish products with unconventional additives. Therefore, in our research, further quality studies were conducted.

Concentration of Biogenic Amines Among Carp Fillets During the Storage Period

In seafood, there is a risk of food quality deterioration due to contamination with decarboxylase-positive microorganisms. The enzyme decarboxylase produced by the microorganisms converts available amino acids to biogenic amines (BA). On the other hand, the tissue of scombroid fish itself contains a high level of free histidine that can be easily converted to histamine by associated microorganism. The accumulation of a greater amount of histamine causes scombroid fish poisoning (Halasz & Barath, 1994). Other biogenic amines are also health-risk agents, such as tyramine, that are identified as a mutagen precursor, putrescine and cadaverine, which can react with nitrite to form carcinogenic nitrosoamines, but can also have impact on toxicity of histamine and tyramine (Bulushi et al., 2009; Prester, 2011). Additionally, 2-phenyloamine can raise the toxicity of histamine (Özogul & Özogul, 2019). Besides the hygienic quality of raw materials and availability of free amino acids, the critical factors contributing to the formation of BA are pH level, water activity, NaCl concentration, production temperature, packaging, storage or activity of present proteases that can degrade proteins towards free amino acids (Restuccia et al., 2015).

In the present trial, all study groups that were subjected to storage at 4 °C resulted in an increasing trend of biogenic amine accumulation across the storage duration (Supplementary data: Table S1). The kinetic increase in biogenic amines, e.g. histamine, putrescine and cadaverine as indicators for the spoilage of fish (Stute et al., 2002), is illustrated in Fig. 3.

Histamine content was not significantly different among the examined samples on days 3, 6 or 9. However, it was significantly lower in all samples covered with coatings compared to the unwrapped control on day 12. There was no impact of coating type on histamine content, resulting in 9.84 ± 8.85, 10.65 ± 4.19 and 8.47 ± 1.88 mg/kg for the coating without herbs, and the coating with thyme and rosemary extract, respectively. The content of putrescine was not significantly different among all the materials on days 3, 6 or 9 of storage. However, its content was significantly reduced on day 12 when wrapped in the gelatin/furcellaran coating (5.87 ± 3.94 mg/kg), compared to its unwrapped control (22.31 ± 9.36 mg/kg). Furthermore, the rosemary-rich coatings demonstrated a small but significant reduction of that amine (13.92 ± 6.22 mg/kg). The contents of cadaverine but also spermidine and spermine were significantly reduced in gelatin coating samples on day 12 compared to their unwrapped controls. Similarly, the rosemary film sample also had significantly reduced content of spermine on the final day. There were no effects of the applied coatings or their type on the content of tryptamine and 2-phenylethylamine on the final day of storage, their range being low but still between 0.14 ± 0.04–0.19 ± 0.08 and 0.80 ± 0.17–0.99 ± 0.74 mg/kg, respectively.

The tyramine content in the samples showed no significant differences during the storage on days 3 and 6, but on day 9, it was significantly reduced in the material covered by rosemary-rich films in comparison to the control. Nevertheless, the content of tyramine on the final day resulted in non-significant differences (3.02 ± 0.90–4.14 ± 0.64 mg/1 k g) between all examined samples, indicating a lack of film or herb inclusion effect.

The mechanism of biogenic amide formulation is based on the activity of endogenous and microbial decarboxylating enzymes in the tested conditions (Sun et al., 2016; Mah

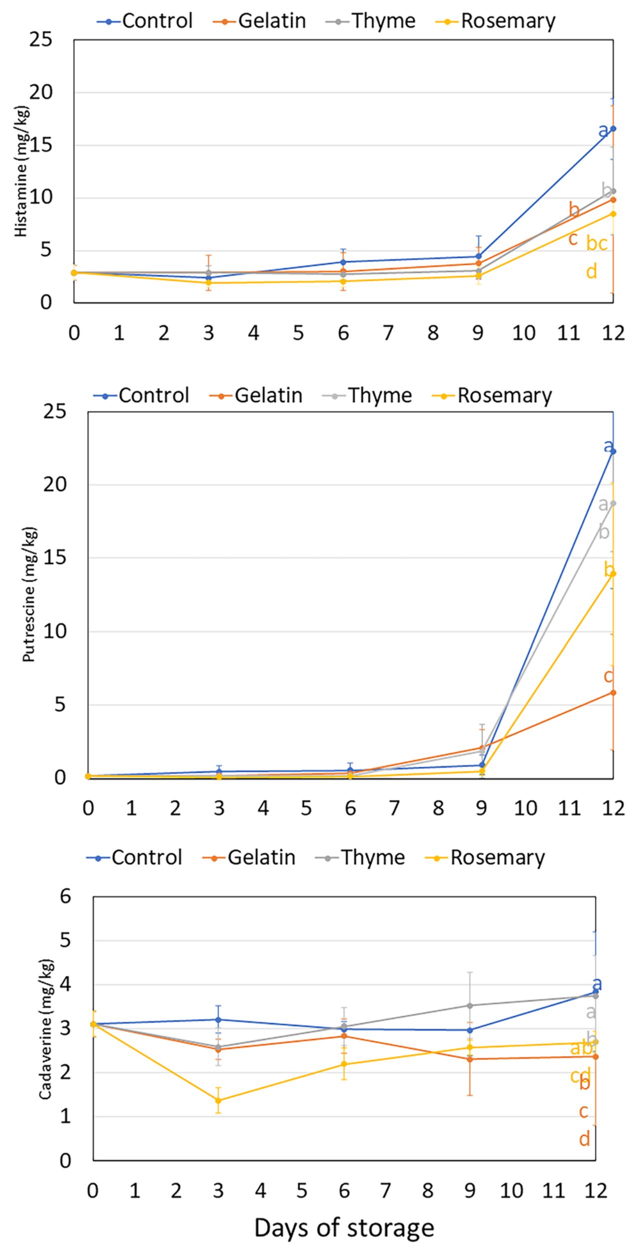

Fig. 3 Contents of histamine, putrescine, cadaverine in carp during the storage period at a temperature of 4 °C (mg/kg), different letters in columns indicate significant differences between means at day 12 ($p < 0.05$)

et al., 2019). As the removal of oxygen in active packaging of Seer fish led to a reduction of biogenic amines during storage, as indicated in the study by Mohan et al. (2009), our gelatine film might also possibly protect the fish carcass from abundance of oxygen access, reducing the decarboxylate enzyme activity followed by reduced content of accumulated amines. Polyphenols present in rosemary or thyme may potentially contribute to a reduction in the growth of microorganisms (theoretically, producing decarboxylating enzymes) due to the polyphenol antimicrobial activity, derived from eugenol or thymol constituents (Naila et al., 2010).

None of the examined materials exceed safety limits, such as 100–500 mg histamine per 1 kg of sample (Lehane & Olley, 2000). The analysed contents of biogenic amines was substantially lower in the current experiment on carp carcasses compared to the blue tuna slices (Jiang et al., 2019), reporting a level of histamine between 59.47–94.54 mg/kg or tyramine at 5–6 mg/kg on day 10 of storage at 4(± 1) °C, when examining the effect of gelatin-chitosan films with Maillard peptides. In a study by Tkaczewska et al. (2021), the impact was investigated of gelatin hydrolysate films with and without Ala-Tyr peptide on Atlantic mackerel when stored for 15 days 4 °C. They reported the levels of accumulated histamine to be at 3.58 mg/kg in the control without wrapping in films, 4.20 mg/kg in fish covered with the hydrolysate gelatin-furcellaran film and 6.11 mg/kg in fish packed with the Ala-Tyr peptide rich film based on hydrolysate gelatin-furcellaran. Similarly, the contents of putrescine, cadaverine, tyramine, spermidine and spermine on the final day storage of Atlantic mackerel was substantially lower compared to the currently examined carp carcasses. This implies that the type of fish as well as used film has some general influence on the accumulation of BA.

Biogenic amines are typically formed during seafood storage. This is particularly true when storage is carried out at heightened temperatures (> 8 °C) (Prester, 2011). From a toxicological perspective, histamine is an important amine due to it being a causative agent of Scombroid fish poisoning as well as food intolerance. Predominant biogenic amines are formed only at the late stage of storage (on day 12), while histamine could be noted in all wild fish during the whole period of iced storage. This is demonstrated in Supplementary Data in Table S1. According to microbial analysis, it therefore appears that determining biogenic amines at a temperature of 4 °C may not be considered a measure of health regarding fresh fish (Bulushi et al., 2009).

In summary, the application of gelatin/furcellaran coatings alone may prevent the accumulation of putrescine, cadaverine, histamine, spermidine and spermine. Incorporation of herbs into coatings demonstrated a lower decrease strength of amine accumulation, but still indicated a reduction in putrescine, histamine and spermine by coating with the rosemary addition.

Sensory Analysis Regarding the Carp Fillets Covered with Innovative Coatings

Sensory attributes are the key determinants of food products considered by human perception; therefore, the information obtained during professional sensory analysis is useful for food manufacturers. The first impression very often determines consumer choice and affects commercial success. Therefore, integration of the product development process with sensory analysis and consumer acceptability reduces the risk of commercial failure (Kemp et al., 2011).

Immediately after baking, the carp fillet samples were evaluated with regard to their shape and surface condition, as well as appearance of the sauce (Fig. 4A, B). In general, the coatings and baking process did not change the shape or surface condition of carp fillets to a great extent (> 50% of the responses—"maintained"). The panellists evaluated the shape and surface condition of carp fillets coated in gel without herb and with rosemary extracts slightly better; however, the sauce appearance was slightly worse for G and T treatments. This was due to the sauce (juice that has leaked during baking from the fish meat, and that resulting from coating) being a more yellowish brown colour for thyme treatments as compared to rosemary, and a bit blurred in case of the G coating.

In Fig. 4C–F, evaluation is shown concerning the intensity of attributes evaluated by panellists for the fish fillet coated in the gel without herbs and in gels with rosemary or thyme extracts. According to the panellists, the texture evaluated using a fork and assessed in the mouth was similar for all fillets. Only juiciness was evaluated slightly higher for carp fillets in herbs coatings as compared to G treatment.

In the case of the carp fillet smell and taste, their characteristic was different. According to the evaluators, the smell of carp coated in gel without herbs was more fishy and muddy than the samples coated in herb extracts. Some panellists also detected fish oil and rancid smell in G sample, which suggests that herbs can help mask undesirable odours. Thyme and rosemary smell and taste intensity were assessed at a 3 (on a 5-point scale) for R and T fillets. Herb coatings changed the taste characteristic of fish. Fillets coated in gels without herbs were less salty and less bitter, but were more sweet and had the taste of fish oil more than other samples; however, these differences were not very high. A rancid taste was found only in the G sample, similar to the smell attribute. It is interesting that the panellists could detect the salty taste in fish (2 score), because we did not used any salt for baking. This means that the coatings proposed in the study emphasized the natural saltiness of fish meat.

Taking overall acceptance of fish fillets into account, the fish coated in thyme extract obtained the highest score on the 5-point hedonic scale (4.14). Graded a bit below 4 was the sample coated in rosemary extract (3.98), and worse assessment was noted for fish in the gel without herbs, probably due to its slightly worse smell and taste, as well as lower juiciness. Our results are in accordance with the data reported by Altan et al. (2022) in the case of bonito fish prepared with different herbs (dill and garden cress). According to these authors, the raw bonito and bonito gravlax products made with herbs differed statistically only with regard to salty taste and aroma. There were no differences between

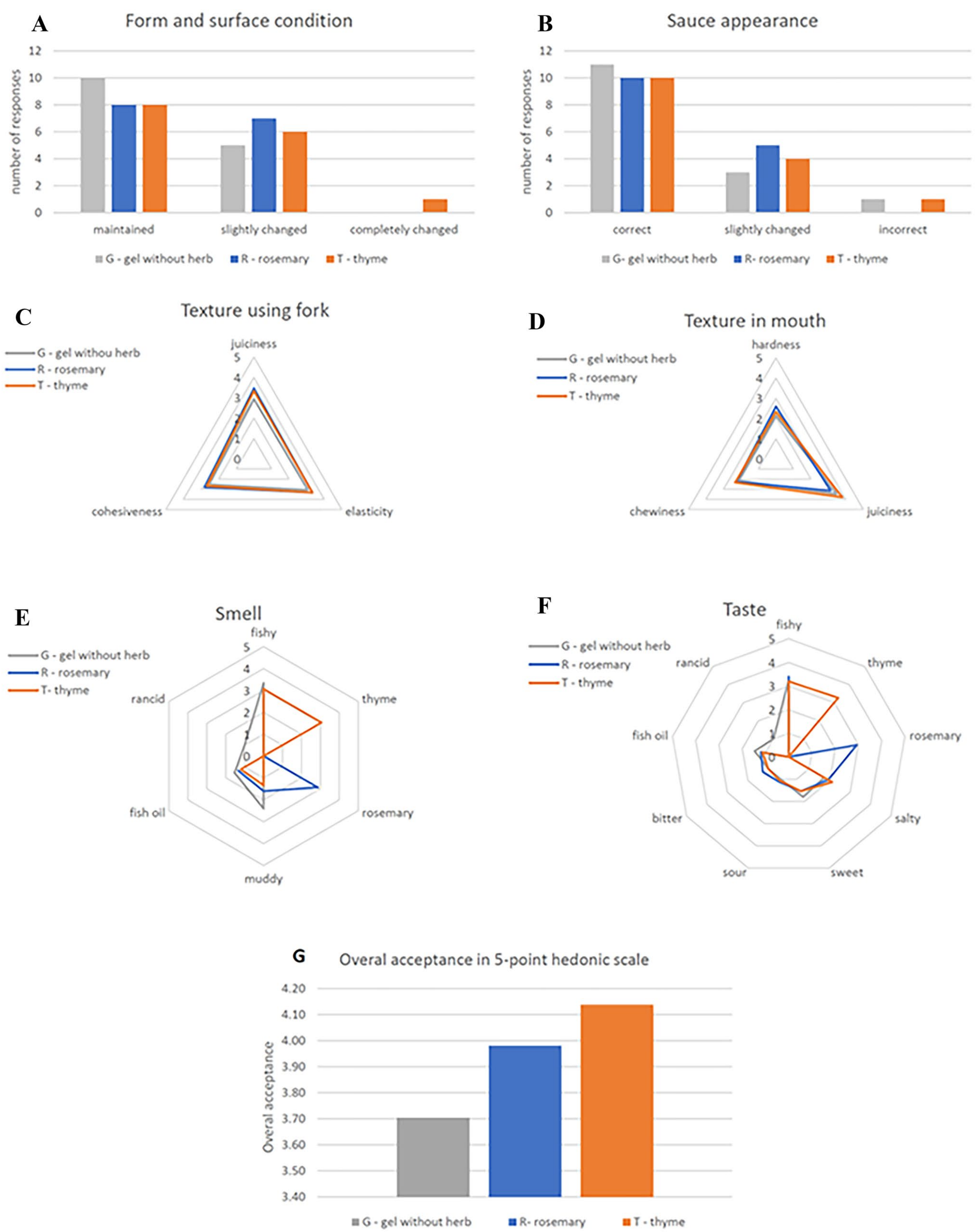

Fig. 4 Sensory evaluation regarding **A**, **B** external appearance of carp fillets without and with herb coatings; **C**–**F** descriptive sensory profile of carp fillets without and with herbs coatings; **G** sensory acceptability of individual attributes and overall acceptability of carp fillets without and with herbs coatings

the remaining variables studied. Kneifel et al. (1992) and Pinho et al. (2004) stated that a product should be accepted if it has attractive appearance (texture and colour), because this parameter is crucial for consumer decision. Thus, by using different herbal extracts and their concentration, we can modify the physical properties of baked carp fillets.

Conclusion

A simple and inexpensive technology has been developed for the production of edible active coatings based on furcellaran and carp skin extracts with the addition of herbs, which enable easy preparation of a ready-to-cook product, while having a preservative effect. The addition of rosemary to the coatings did not adversely affect the mechanical parameters of obtained hydrogels, unlike thyme, which changed their strength and adhesive parameters. It has been found that of the 2 tested herbs, rosemary is more suitable as an active ingredient in the designed innovative coatings. The coatings with the addition of rosemary proved effective in inhibiting the formation of certain biogenic amines and slowed down the microbial deterioration of carp fillets. In addition, the fillets covered with coatings from both herbs were positively assessed by the sensory panel, which means that such products are characterised by high sensory acceptability. The developed coatings show great potential for commercial use as a simple and cheap technology for stabilising carp fillets and preparing ready-to-cook products. The use of innovative active coatings may make the sensory features of the product more attractive and may find an audience among consumers who do not like the taste of fish or carp. Such research should be undertaken in the future, especially with regard to packaging and implementing longer storage durations.

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Author Contribution Joanna Tkaczewska: conceptualization; methodology; investigation; writing—original draft; project administration; funding acquisition. Ewelina Jamróz: conceptualization, methodology, investigation, writing—original draft. Mirosław Kasprzak: investigation; writing—original draft; writing—review and editing. Marzena Zając: investigation, writing—review and editing. Paulina Pająk: investigation; writing—original draft; writing—review and editing. Wiktoria Grzebieniarz: investigation. Nikola Nowak: investigation. Lesław Juszczak: investigation, writing—review and editing.

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Data Availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests.

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