https://doi.org/10.17221/310/2019-CJFS

Effects of monoacylglycerols and chitosan on the biogenic amine formation in the flesh of rainbow trout (*Oncorhynchus mykiss*)

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Citation: Matějková K., Křížek M., Dadáková E., Vácha F., Pelikánová T. (2020): Effects of monoacylglycerols and chitosan on the biogenic amine formation in the flesh of rainbow trout (*Oncorhynchus mykiss*). Czech J. Food Sci., 38: 164–170.

Abstract: Contents of eight biogenic amines (putrescine, cadaverine, spermidine, spermine, histamine, tyramine, tryptamine and phenylethylamine) were determined in vacuum-packed fillets of rainbow trout (*Oncorhynchus mykiss*). Fish flesh was treated using a solution of chitosan (2%, w/v) or monoacylglycerols (monocaprylin C8, monocaprin C10, 5%, w/v). The control and treated packs were stored at 3.5 °C for up to 25 days. Samples of good quality did not contain more than 30 mg kg^{-1} of either putrescine or cadaverine. Exceeding this limit was usually followed by a worsening of the sensory properties of samples. Chitosan was found to be the most potent additive, prolonging the storage time of fillets by approximately four times, compared to control samples. Histamine was not found in any sample treated with chitosan. of the monoacylglycerols, C8 was more efficient compared to C10. All additives are easily applicable to the surface of fish flesh.

Keywords: polyamines; putrescine; histamine; fish quality; shelf life

Biogenic amines (BAs) including putrescine (PUT), cadaverine (CAD), tyramine (TYM), histamine (HIM), spermidine (SPD), spermine (SPM), 2-phenylethylamine (PEA) and tryptamine (TRM) are ubiquitous compounds present in all tissues. In fresh fish, the contents of amines are usually low, in stored flesh their contents may increase considerably due to the action of bacteria. In the case of freshwater fish, information on BAs formation is limited (Chytiri et al. 2004; Li et al. 2012; Matějková et al. 2013; Křížek et al. 2018).

Fish are known for their susceptibility to microbial contamination, so finding a cost-effective preservation method with antimicrobial potential is important (Hassanzadeh et al. 2018). Coating of food with edible materials is just such a type of active packaging (Elsabee & Abdou 2013; Yuan et al. 2016).

Chitosan is known for its antimicrobial activity. It is a commercially produced polysaccharide with prospective applications in the food industry (Moradi et al. 2011; Kanatt et al. 2013; Fu et al. 2016). Moreover, chitosan can enhance product quality, as its molecule is able to carry some special compounds with antimicrobial properties (Siripatrawan & Noipha 2012; Qiu et al. 2014; Ramezani et al. 2015).

Supported by the Czech Science Foundation GACR, Project No. 17-09594S.

https://doi.org/10.17221/310/2019-CJFS

Fatty acid esters, e.g. monoacylglycerols (MAGs), are naturally occurring non-toxic substances (Buňková et al. 2010; Hauerlandová et al. 2014). MAGs are natural compounds produced by the decomposition of medium-chain triacylglycerols. They have the potential to reduce the proliferation of microorganisms. The number of carbon atoms and the number of double bonds in the fatty acid molecule are the key factors upon which depends the MAG ability to kill bacteria (Buňková et al. 2011).

This study was undertaken to assess the effectiveness of the application of a surface coating of monoacylglycerols on the chemical prolongation of shelf life for fish flesh food products when compared to the use of chitosan.

MATERIALS AND METHODS

Fish samples. The trout (*Oncorhynchus mykiss*) samples were obtained from a fish farm in Třeboň (southern Bohemia). The fish were of an average body mass of 380 g. Portions of about 20 g of muscles from the dorsal to abdominal side were used as samples. Fillet samples were divided into four groups: uncoated (control, C); immersed in a chitosan solution (CH); immersed in a solution of caprylic acid (C8); immersed in a solution of capric acid (C10). Prior to packaging, samples were immersed for 10 s in 500 mL of the coating solution. Samples were let drip for about 5 seconds. All samples were prepared in triplicate for each treatment. They were wrapped in PA/PE film (thickness 80 μm), sealed under vacuum (Mini Jumbo; Henkelman B.V., Netherlands) and placed in a thermostat set at 3.5 °C.

Preparation of coating solutions. Chitosan (medium molecular weight) was purchased from Acros Organics (Belgium). The solution was prepared with 2% (w/v) chitosan in 1% (v/v) acetic acid. After opening the samples for analysis, 1% of acetic acid in chitosan coating was hardly detectable by smell.

MAGs (1-monocaprylin, 1-monocaprin) were prepared according to Janiš et al. (2000) and Buňková et al. (2010). The solutions were prepared at the department of the Faculty of Technology, Tomas Bata University in Zlín, by dissolving 0.5 g of MAG in 500 mL of 1% ethanol (according to Buňková et al. 2011). Coatings are water soluble and they form a thin layer on the meat surface. We recommend rinsing the fish flesh before eating.

Sampling. Samples were analysed in triplicate after 0 (fresh meat), 3, 7, 10, 14, 17, 21 and 25 days of storage. As 10 trained panellists are required for a fully valid evaluation, our sensory results can be understood only as complementary to the main objective of this study – the determination of chemical changes in flesh – and were simplified to three levels:

good (1): odour: meat, neutral,
appearance: white, tightly elastic flesh;
acceptable (2): odour: neutral, slightly spicy,
appearance: grey, solid flesh;
poor (3): odour: fishy, repulsive,
appearance: grey, muddy flesh.

A sensory panel consisting of three panellists evaluated the meat samples; each panellist tested all samples twice.

Analytical method. The samples (20 g) were homogenised with an Ultra-Turrax T25 homogeniser (Ika Labortechnik, Germany) in 50 mL of diluted perchloric acid, p.a. (0.6 mol L^{-1}). After filtration, the volume was made up to 150 mL with perchloric acid (0.6 mol L^{-1}). The amines were determined after derivatisation with dansyl chloride by UPLC (Agilent 1200 Series Rapid Resolution LC System; Agilent Technologies, USA). The procedure was described in detail by Dadáková et al. (2009).

Samples were prepared in triplicate from one batch of fish and each sample was analysed twice. The statistical parameters and ANOVA (Analysis of variance) were calculated using Statistica (data analysis software system) 9.0 (StatSoft, Czech Republic).

RESULTS AND DISCUSSION

Samples of trout flesh were analysed in triplicate. The initial contents of BAs in fresh meat (day 0) were: PUT: 6.4 ± 0.35 mg kg^{-1}, CAD: ND, SPD: 6.4 ± 0.99 mg kg^{-1}, SPM: 3.6 ± 0.19 mg kg^{-1}, HIM: ND, TYM: ND, TRM: ND, PEA: ND. The mean amine contents for control and treated samples at 3.5 °C are given in Tables 1 and 2.

Putrescine, cadaverine and tyramine. The stability of the vacuum-packed samples that were not treated with any additive was approximately 3 days (control samples at the selected temperature of 3.5 °C). The first sensory signs of decay were observed after approximately 7 days of storage. In subsequent samplings, the increase of PUT, CAD and TYM content and the worsening of sensory indicators showed similar trends (Table 1). The influence of the additives on the stability of samples was significant, as evidenced by both the MAG and the chitosan-treated samples. During the whole experiment (until the 25th day of storage), no sample treated with chitosan reached sensory level 3.

In the case of chitosan treatment, the first sensory signs of decomposition appeared on the 25th day of storage, whereas in the case of MAG they were

Czech Journal of Food Sciences, 38, 2020 (3): 164–170

https://doi.org/10.17221/310/2019-CJFS

Table 1. Content of putrescine, cadaverine and tyramine (mg kg^{-1}) in trout meat (T = 3.5 °C; n = 3; mean ± SD)

	Time (days)						
	3	7	10	14	17	21	25
Putrescine							
Control	7.0 ± 0.90^{Aa}	*7.8 ± 0.84*^{Aa}	*8.9 ± 1.58*^{Aa}	***36.3 ± 5.94***^{Ba}	***63.1 ± 13.7***^{Ba}	***70.9 ± 27.5***^{ABabc}	***94.1 ± 27.5***^{Ba}
C8	6.5 ± 0.79^{Aa}	7.1 ± 1.22^{Aa}	8.0 ± 1.28^{Aa}	17.6 ± 10.6^{ABabc}	28.4 ± 4.81^{Babc}	*40.0 ± 6.42*^{BCabc}	***47.8 ± 3.09***^{Cab}
C10	7.1 ± 1.46^{Aa}	8.2 ± 1.53^{ABa}	11.8 ± 2.96^{ABa}	*18.5 ± 8.14*^{ABabc}	*29.5 ± 8.76*^{BCbc}	*51.6 ± 2.17*^{Ca}	***84.3 ± 20.6***^{Ca}
CH	5.3 ± 0.71^{Ba}	5.8 ± 1.03^{ABCDEa}	6.6 ± 0.42^{Ca}	5.2 ± 0.55^{Ac}	7.9 ± 1.14^{Dd}	18.0 ± 12.2^{ABCDEc}	*25.9 ± 21.0*^{ABCDEb}
Cadaverine							
Control	ND	*7.2 ± 2.80*^{Aa}	*8.6 ± 3.54*^{Aa}	***96.8 ± 10.7***^{Ba}	***146 ± 26.2***^{BCa}	***171 ± 49.7***^{BCab}	***218 ± 36.3***^{Cab}
C8	ND	2.6 ± 1.21^{Aa}	8.3 ± 1.57^{Ba}	54.4 ± 25.1^{ABCDab}	85.2 ± 12.1^{DEab}	*111 ± 13.5*^{EFb}	***130 ± 12.9***^{Fbc}
C10	ND	0.8 ± 0.66^{Aa}	22.9 ± 12.2^{ABa}	*50.7 ± 19.0*^{Bb}	*63.6 ± 23.0*^{Bbc}	*131 ± 15.0*^{Cb}	***188 ± 23.6***^{Da}
CH	ND	ND	ND	3.1 ± 2.19^{Ac}	27.0 ± 15.6^{ABc}	50.9 ± 19.8^{ABa}	*87.1 ± 32.4*^{Bc}
Tyramine							
Control	0.9 ± 1.47^{ABCa}	*1.7 ± 0.59*^{BCa}	*1.6 ± 1.50*^{ABCa}	***27.6 ± 6.77***^{Da}	***37.7 ± 23.9***^{CDab}	***74.9 ± 34.0***^{CDab}	***115 ± 39.4***^{Da}
C8	2.5 ± 0.69^{Aa}	0.9 ± 1.63^{Aa}	3.2 ± 0.36^{ABa}	10.3 ± 11.1^{ABCab}	11.3 ± 3.44^{BCa}	*24.8 ± 7.62*^{CDa}	***42.2 ± 2.54***^{Da}
C10	1.5 ± 0.42^{Aa}	0.9 ± 0.77^{Aa}	2.4 ± 2.33^{Aa}	*7.4 ± 3.80*^{ABb}	*13.8 ± 6.60*^{ABab}	*48.0 ± 11.4*^{Ca}	***101 ± 38.4***C^{Da}
CH	1.2 ± 0.15^{Aa}	ND	2.2 ± 1.93^{Aa}	ND	1.2 ± 0.49^{Ab}	2.9 ± 3.05^{Ab}	*4.1 ± 4.08*^{Ab}

ND – not detected; means indicated by different capital letters in the same row differ significantly (P < 0.05); means indicated by different lowercase letters in the same column differ significantly (P < 0.05); values in italics indicate sensory level 2, values in bold italics indicate sensory level 3

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Table 2. Content of spermidine, spermine, and histamine (mg kg^{-1}) in trout meat (T = 3.5 °C; n = 3; mean ± SD)

	Time (days)						
	3	7	10	14	17	21	25
Spermidine							
Control	4.4 ± 0.41^{Ca}	*3.5 ± 0.42*^{Ca}	*4.6 ± 1.37*^{ABCa}	***2.4 ± 0.26***^{Ba}	***2.9 ± 0.90***^{BCab}	***1.3 ± 1.44***^{ABCab}	***1.7 ± 0.36***^{Ba}
C8	4.7 ± 1.59^{DEa}	5.0 ± 0.63^{Eb}	4.7 ± 0.54^{Ea}	2.3 ± 0.12B^{Ca}	2.1 ± 1.79^{ABCDEab}	*3.3 ± 0.73*^{Cab}	***3.4 ± 1.16***^{DEab}
C10	4.9 ± 0.93^{BCa}	5.0 ± 0.39^{Cb}	3.4 ± 0.73^{ABa}	*2.8 ± 0.38*^{ABa}	*3.1 ± 0.30*^{ABa}	*2.1 ± 0.65*^{Aa}	***2.8 ± 1.80***^{ABCab}
CH	4.9 ± 0.75^{ABa}	4.6 ± 0.84^{ABab}	4.8 ± 0.59^{ABa}	3.4 ± 0.68^{Aa}	4.4 ± 0.57^{ABb}	4.6 ± 1.19^{ABb}	*5.8 ± 1.03*^{Bb}
Spermine							
Control	2.9 ± 0.11^{Ca}	*2.3 ± 0.15*^{ABa}	*3.2 ± 0.45*^{CDabc}	***2.0 ± 0.25***^{Aa}	***4.7 ± 1.35***^{ABCDEa}	***3.6 ± 1.17***^{ABCDEab}	***4.2 ± 1.00***^{ABCDEa}
C8	2.9 ± 1.28^{ABCDab}	3.8 ± 0.31^{Cb}	2.6 ± 0.20^{Babc}	1.8 ± 0.24^{Aa}	5.6 ± 0.44^{Da}	*4.8 ± 0.15*^{Dab}	***5.5 ± 0.56***^{Dab}
C10	3.8 ± 0.43^{Bab}	3.9 ± 0.73^{ABCabc}	2.3 ± 0.31^{Aa}	*2.3 ± 0.29*^{Aa}	*5.1 ± 0.18*^{Ca}	*3.5 ± 0.51*^{Ba}	***3.4 ± 1.01***^{ABCa}
CH	3.9 ± 0.33^{Bb}	3.9 ± 0.71^{ABCDabc}	3.1 ± 0.23^{Ac}	3.3 ± 0.17^{ABCb}	6.2 ± 0.85^{Ea}	5.7 ± 0.35^{DEb}	*7.0 ± 0.79*^{Eb}
Histamine							
Control	ND	ND	ND	***5.3 ± 0.72***Aa	***2.6 ± 2.44***^{Aa}	***7.3 ± 5.53***^{Aa}	***18.5 ± 27.3***^{Aa}
C8	ND	ND	ND	1.5 ± 2.58Aa	ND	*1.7 ± 2.90*^{Aa}	***2.0 ± 1.60***^{Aa}
C10	ND	ND	ND	ND	ND	*2.9 ± 2.49*^{Aa}	***3.1 ± 4.88***^{Aa}
CH	ND	ND	ND	ND	ND	ND	ND

ND – not detected; means indicated by different capital letters in the same row differ significantly (P < 0.05); means indicated by different lowercase letters in the same column differ significantly (P < 0.05); values in italics indicate sensory level 2; values in bold italics indicate sensory level 3

https://doi.org/10.17221/310/2019-CJFS

already detected on the 14th or 21st day (for C10 and C8, resp.). PUT, CAD and TYM are amines that best correspond to the decomposition processes in fish meat. With these amines, at a content of above 10 mg kg^{-1}, we can assume that the decomposition processes have already started and a further decline in quality is unavoidable under unchanged conditions (Wang et al. 2016). A rapid increase in the content of these three amines above this level was observed especially in control samples on the 14th day of storage. This was linked with a significant deterioration in the sensory properties of samples. Elevated levels of PUT, CAD and TYM were also found in MAG samples on the 14th day, indicating the onset of degradation processes, in spite of the fact that these samples were still of acceptable sensory quality. When comparing the samples treated with additives, on the 14th day of storage chitosan-treated samples showed the best appearance in terms of both chemical and sensory aspects. They contained less than 10 mg kg^{-1} of each of the three amines and no sensory worsening was recorded.

By comparison with chitosan, the addition of MAG was slightly less effective. When comparing both MAGs, C8 appeared to be more effective. Even on the last day of storage, the positive effect of C8 was still evident, although samples (similarly to control samples) showed sensory level 3. Nevertheless, the difference in efficacy between C8 and C10 was not very pronounced. Although PUT and CAD are very similar diamines, their content in samples was different. PUT was present in all our samples, but CAD only in some of them. This is so because PUT is a substance present in all living cells (Sarkadi 2019). Chitosan-treated samples did not contain any CAD, especially at the beginning of storage. In fish tissues, CAD is present in much lower quantities compared to other polyamines (Cai et al. 2015). CAD is released from lysine by the action of bacterial decarboxylases. We did not find any CAD in fresh trout meat in our experiments. If the stored samples were of good quality, they did not contain this amine either.

TYM is a substance with different chemical structure compared to polyamines. However, the dynamics of its formation was similar to that of PUT or CAD (Nie et al. 2014). Contrary to the possible formation of HIM, TYM was found in practically all samples.

HIM was found only in few samples that were of very bad quality. The formation of TYM was very well linked with the sensory properties, considering the border limit of 5–10 mg kg^{-1}, which was proposed earlier (Křížek et al. 2012; Moreira et al. 2018). In control samples, this limit was exceeded on the 14th day (sensory score 3). In many cases, the TYM content and the sensory signs of decay increase continuously with storage time. TYM contents are sometimes preceding the sensory signs (Křížek et al. 2017). In this study, this progression is especially evident in C8 samples, but also in C10 and chitosan-treated samples. Samples of the best quality treated with chitosan and C8 contained very low amounts of TYM. At this point the dynamics of PUT and CAD showed similar characteristics.

Spermidine, spermine and histamine. TYM is a substance with different chemical structure compared to polyamines. However, the dynamics of its formation was similar to that of PUT or CAD (Nie et al. 2014). Contrary to the possible formation of HIM, TYM was found in practically all samples.

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With the exception of one sample (C8), HIM was not found in trout flesh of the best sensory quality (1). In freshwater fish, elevated HIM contents are likely to appear only in samples of deep decay (Prester 2011). HIM does not seem to be a substance that would adequately signal or predict the beginning of the degradation process. On the other hand, in CH samples HIM was not detected, at strong variance with the control samples that contained the highest amounts of HIM in our experiment. Control samples showed a steady increase of HIM with the worsening of quality. Thus, samples C8 and C10 were somewhere between the control samples and chitosan samples in the HIM content. However, none of our samples exceeded the toxicologically significant histamine content (HIM > 100 mg kg^{-1}) (Hungerford 2010). Organoleptic signs of quality reduction preceded its massive accumulation in the flesh.

https://doi.org/10.17221/310/2019-CJFS

CONCLUSION

The concentrations of BAs in packed trout flesh can be effectively reduced by the addition of either of the two monoacylglycerols and especially by chitosan. For samples that showed good sensory properties, HIM and TYM levels above 2 and 12 mg kg^{-1}, resp., were not recorded. Relatively sensitive quality markers were PUT and CAD. Their concentrations did not exceed 30 mg kg^{-1} for good quality samples. As reported by the producers, at the temperature used in our experiments vacuum-packed trout meat can be stored for approximately 5 days. The addition of chitosan can substantially extend this period – by more than four times, to 21 days. Monoacylglycerols C8 and C10 were less effective. Of both types, C8 was the more potent, prolonging the storage time to about 14–17 days. In spite of the fact that MAG C10 was the least effective, its influence was still evident, prolonging the storage time to about 10 days, which is about twice longer duration compared to the control samples and to the recommendations of fish processors. All the additives used are very easily applicable and do not substantially affect the organoleptic properties of the meat.

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Received: October 29, 2019
Accepted: May 25, 2020