Nutritional quality, safety and sensory properties of smoked tench (*Tinca tinca*) pâté from Trasimeno Lake, Italy

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Abstract

The present study evaluated the safety, nutritional and sensory properties of fish pâtés made from smoked tench (*Tinca tinca*) pulp. Two formulations of tench pâté were produced, one of which included olive oil and the other contained mascarpone cheese. The prepared pâté jars were thermally treated according to the method applied in the canning industry. The inactivation of *Clostridium botulinum* during thermal treatment was determined through a challenge test study, demonstrating the complete depletion of targeted bacteria. Tench pâtés showed high nutritional properties, especially the formulation with olive oil, which was characterised by lower contents of cholesterol and saturated fatty acids and a high level of monounsaturated fatty acids. The two experimental formulations possessed a specific sensory profile, due to the ingredients incorporated into the products. Consumers' evaluation established differences in the acceptance of the two products: the formulation with added olive oil was more appreciated by consumers, who displayed a higher purchase intent for this product. The study suggests that minced smoked tench is not only a promising ingredient for pâté production but that it also represents a value-added product able to contribute to freshwater food consumption.

Introduction

Fish in the human diet is considered as an important source of nutrients of high biological value with a range of health benefits (Özogul *et al*., 2007; Branciari *et al*., 2017a). Many freshwater fish species have not yet been utilised effectively because of various reasons, including bony flesh, characteristic flavour and odour, small size, unacceptable textural properties and other factors. However, the utilisation of such fish species would be desirable for their valuable nutritional properties (Steffens, 1997; Vasconi *et al*., 2015). A strategy to increase the consumption of freshwater fish is by pre-processing the fish (Venugopal *et al*., 1995). This approach is in line with consumers' demand towards safe, user-friendly and high in nutritional value products and with the strong attitude in consuming locally-sourced products, both typical and innovative (de Quadros *et al*., 2015; Branciari *et al*., 2016a; Branciari *et al*., 2017a). Smoking of fish is an ancient preservation method, and it has economic importance for the seafood market (Özogul *et al.,* 2010). In the Umbria region (Italy), smoked tench (*Tinca tinca*) an autochthonous fish species of Trasimeno Lake is an artisanal fish product (Natali, 2002) prepared by a small fishery consortium along Trasimeno Lake. This economically important fish product has emerged in numerous markets. Due to the increasing consumers' demand for fish foods with prolonged shelf-life, the consortium started to process the smoked tench in ready-to-eat (RTE) pâté. As a cooked RTE product, pâté has greatly appreciated sensory characteristics and is popular in the gastronomy of many countries. Pâtés are traditionally prepared with goose "foie-gras" or pork liver. Nevertheless, fish-based pâtés are present in today's market mainly due to their nutritional benefits, offering a great variety of products with unique sensory characteristics (Lobo *et al*., 2015; Minozzo *et al*., 2004). The objective of this study was to use smoked tench from Trasimeno Lake, for the preparation of two pâtés' formulations and to assess their safety, nutritional value, sensory properties and consumers acceptability.

Materials and Methods

Fish meat pastes preparation

Smoked tench (*T. tinca*) was obtained, as reported by Branciari *et al*. (2016a). Briefly, tench was prepared by gutting, salting in brine (22% salt) at 10 °C for 2 h, washing to remove the brine, hanging from a support at 0–4°C for 16–24 h and, finally, smoking in a convection smoking oven (inner final temperature around 72°C) for 6 h. The smoked tench pulp was minced and trimmed in order to prepare tench pâté in two different experimental formulations: one with olive oil (O-TP) and the other one with mascarpone cheese (M-TP). In the O-TP recipe, the minced fish meat was mixed with olive oil (41.1%), parsley (1.65%) and lemon juice (1.65%). In M-TP, the minced fish meat was mixed with mascarpone cheese (42.55%), cream (12.77%), parsley (1.28%) and lemon juice (0.85%). One hundred and fifty grams of the mix pâté was placed in a jar (212-mL volume) and transferred to the autoclave (Frigojollinox Stery 50, Frigojollinox, Campobasso, Italy). Three batches of 90 jars were produced for the experiment. In order to ensure the product's safety, the steriliser was set in accordance to the standard thermal treatment parameters used in the canning industry: 121.1°C for 12 s (Diao *et al*., 2014; Margosch *et al*., 2006).

Evaluation of *Clostridium botulinum* inactivation during thermal treatment

In order to define the time–temperature profile, the internal temperature of the samples was recorded by eight miniature, self-contained, computer downloadable elec-

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tronic data loggers (Thermo Button 22T, Proges-Plus, Willems, France) inserted into eight separate fish pâté jars (four O-TP and four M-TP). The above-mentioned jars were placed randomly at different points inside the steriliser, namely, near the centre, and close to the top, bottom and sides of the steriliser (Figure 1) as reported by other authors (Giannakourou *et al*., 2005; Jedermann *et al*., 2009). The procedure was performed for each batch, for the evaluation of three sterilisation cycles. Temperatures recorded by data loggers were downloaded using the Thermotrack software (Plug and Track, Proges-Plus).

For calculating the thermal inactivation (death) kinetics, a D_{120}-value of 0.48 min, and a *z*-value of 10.2°C were applied, as reported for *C. botulinum* proteolytic types ABF, by van Asselt and Zwietering (2006). Data from data loggers were analysed by the software of the Food Safety and Inspection Service of the United States Department of Agriculture (www.fsis.usda.gov) to evaluate the worst-case scenario for the process.

Microbiological challenge tests play an important role in the validation of processes that are intended to deliver some degree of lethality against a target organism and in the verification of compliance with the predetermined performance standard (US FDA, 2001). For canned food, it is appropriate to conduct inoculated studies as part of the protocol for process validation (US FDA, 2001). Hence, to validate the fish pastes production process and ensure the safety of the products, the efficacy of thermal treatment on *C. botulinum* was evaluated by a specific challenge test detailed in the Guidelines for Safe Practices for Food Processes published by the US Food and Drug Administration (US FDA, 2001). The experimental design involved the production of three batches for each of the two fish pâté recipes. Each batch included three experimental groups of six samples each. The first experimental group (CONTAM) consisted of fish pâté experimentally inoculated with spores of *C. botulinum* (approximately 10^5 spores/g). These samples units were used to assess the efficacy of thermal treatment on *C. botulinum*. For the second group (CTR), the suspension used in the CONTAM group was replaced by the same volume of sterile saline solution. In the CTR group, physical and chemical analyses were conducted to verify that the addition of a defined amount of liquid to the original formulation does not affect the product. The third experimental group (BLANK) was produced by strictly following the producer's recipe, to compare the products submitted to challenge testing with the products that are routinely manufactured by the producer and to evaluate the physical–chemical characteristics of fish pâté. These BLANK samples were also used to exclude contamination by *C. botulinum* occurring naturally in this type of product.

A non-toxigenic strain of *C. botulinum* (*C. botulinum*-like type B, CL14NT), provided by the Italian Centro Nazionale di Riferimento per il Botulismo, was used in the challenge test. The strain was revitalised in tryptone–peptone–glucose–yeast broth (Biokar Diagnostics, Pantin Cedex, France) incubated at 30±1°C for 4 days. The bacterial suspension was then stored at 4±1°C under aerobic conditions for 10 days until 95% sporulation was observed by microscopic examination. In order to eliminate all the vegetative cells, the spore suspension was heated at 75°C for 15 min (Dutra *et al.*, 2016). Subsequently, the suspension was centrifuged (1800 rpm, 4°C for 1 h) and the pellet re-suspended in 10 mL of sterile saline water. For spore enumeration by the standard UNI EN ISO 7218:2007 method, serial decimal dilutions in 0.1% peptone solution of the suspension were performed. Each dilution was then inoculated (0.1 mL) onto egg yolk agar (EYA) culture media plates (Biokar Diagnostics) and incubated at 37±1°C for 48±3 h under anaerobic conditions (detection limit 10 CFU/g). The spore mixture was stored at 4±1°C until used, as reported by Reddy *et al*. (2010). The contamination of pâté was performed in an experimental plant. For this, the inoculums were added to the fish paste mass (1.5 kg), homogenised and then poured into glass jars. *Clostridium botulinum* was enumerated on BLANK samples (3 jars) before the heat treatment and on CONTAM sample units before (3 jars) and after (3 jars) the thermal treatment, by growth on EYA, as mentioned above.

In BLANK and CTR samples, the water activity (a_w), pH and NaCl analysis were achieved on three samples units for each batch. The pH measurements were carried out using a puncture electrode probe connected to a portable pH meter (Crison pH25, Crison Instruments, S.A., Barcelona, Spain). The salt content was determined by adopting the Volhard method (AOAC, 2000), and the a_w was measured in according to Pecorelli *et al*., (2018) using an AquaLab Series 3 TE device (Decagon Devices, Inc., Pullman, WA, USA).

Determination of nutritional declaration

In order to define the nutritional declaration of fish pâtés, O-TP, M-TP and smoked tench, the moisture, ash and total nitrogen (N) were measured (AOAC 2000), and the fatty acid profile (Branciari *et al*., 2014), fat and sugar contents (Ranucci *et al*., 2018), were determined, as detailed in the indicated references. Ten samples were analysed in triplicate. The protein content was calculated by multiplying the total N by a factor of 6.25. Total cholesterol of each

Table 1. Physical-chemical characteristics of BLANK and CTR samples of both experimental recipes (O-TP and M-TP)(mean ± standard deviation).

		Batch 1	Batch 2	Batch 3	Mean value
BLANK O-TP	a_w	0.968 ± 0.001	0.971 ± 0.001	0.978 ± 0.003	0.972 ± 0.005
	pH	5.72 ± 0.02	5.73 ± 0.02	5.79 ± 0.02	5.75 ± 0.03
	NaCl (%)	0.32 ± 0.03	0.37 ± 0.02	0.31 ± 0.03	0.33 ± 0.03
CTR O-TP	aw	0.969 ± 0.001	0.970 ± 0.001	0.977 ± 0.001	0.972 ± 0.004
	pH	5.73 ± 0.01	5.72 ± 0.01	5.81 ± 0.01	5.75 ± 0.04
	NaCl (%)	0.30 ± 0.05	0.37 ± 0.03	0.32 ± 0.02	0.33 ± 0.04
BLANK M-TP	a_w	0. 984 ± 0.003	0.982 ± 0.001	0.984 ± 0.001	0.983 ± 0.002
	pH	5.62 ± 0.02	5.62 ± 0.03	5.66 ± 0.03	5.63 ± 0.03
	NaCl (%)	0.18 ± 0.03	0.20 ± 0.03	0.21 ± 0.03	0.20 ± 0.03
CTR M-TP	a_w	0.984 ± 0.001	0.982 ± 0.001	0.981 ± 0.002	0.983 ± 0.002
	pH	5.64 ± 0.04	5.67 ± 0.03	5.63 ± 0.01	5.65 ± 0.03
	NaCl (%)	0.19 ± 0.04	0.21 ± 0.02	0.22 ± 0.04	0.21 ± 0.03

pâté was assayed using a commercial kit (Cat. number 10139050035: Biopharm, Darmstadt, Germany) and absorbance reading at 405 nm (Ultrospec 2011 Pro spectrophotometer, Amersham Pharmacia Biotech, Milan, Italy).

Sensory analysis of pâté

A descriptive sensory analysis was performed on O-TP, M-TP and a commercial tuna pâté (CP) using an eight-member panel, which was trained and selected based on the ISO 8586-1:1993 and ISO 8586-2:2008 criteria. The attributes were generated in pre-testing sessions, and the descriptive analysis of the pâté was replicated three times (Branciari *et al*., 2014). For quantification of the intensity of each attribute, the judges evaluated the sample in a monadic sequential manner, scoring the attributes on an unstructured 100-point scale, where 0 is the minimum intensity, and 100 is the maximum intensity, for each parameter. Fifteen attributes were assessed, according to ISO 13299:2003, comparing the two experimental pâtés (O-TP and M-TP) with CP. The assessors tasted samples coded with three-digit random numbers and served at room temperature.

Consumer analysis

Consumer tests were performed at the Fisheries Consortium of the Lake Trasimeno. Before testing, all consumers completed a questionnaire that included information regarding their age, sex, and the frequency of consumption of freshwater fish products (regular consumers were considered those who reported consuming fish at least once every 2 weeks). In total, 110 regular fish consumers (aged 20–60 years, 60 females and 50 males) were used for consumer evaluation. The pâté was served, using bread as a carrier. Practicing sessions were conducted before the test to allow consumers to become familiar with the use of a hedonic scale and to explain the meaning of the examined attributes (Branciari *et al*., 2016b). Samples were monadically served on white plastic plates identified by random three-digit codes. Consumers were asked to rate sensory attributes using a 9-point hedonic scale (from 1, "dislike extremely" to 9, "like extremely") for overall appearance, odour, taste, overall texture and overall liking. Purchase intent were evaluated using the binomial (yes/no) scale (Branciari *et al*., 2017b).

Results and Discussion

Among the time–temperature profiles belonging to both recipes and analysed using the software provided by the USDA, the most unfavourable was considered and is shown in Figure 2. The software reported that the non-isothermal profile applied to tench pâtés is equivalent to an isothermal treatment at 120°C for 6.869 min (Figure 3), thereby generating a 14.31 log reduction for *C. botulinum*. The results confirm that the heating treatment complies with the 12D concept ("botulinum cook") widely employed in the canned food industries, according to which, a 12-log cycle reduction in the numbers of *C. botulinum* is considered safe for ambient stored canned food

Table 2. Nutritional declaration of smoked tench pâtés, O-TP and M-TP.

	Smoked tench*	O-TP	M-TP
Energy value (kcal)	133	447	277
Energy value (kJ)	556	1869	1160
Carbohydrates (%)	0.38	0.18	0.53
Total sugars (%)	0.34	0.13	0.08
Fat (%)	4.69	44.15	25.90
Saturated fatty acids (%)	13.67	27.33	64.78
Protein (%)	22.71	12.17	10.53
Moisture (%)	69.96	41.85	59.76
Cholesterol (mg/100 g)	90.00	61.83	110.64
Ash (%)	2.67	2.04	3.28
Salt (%)	0.56	0.33	0.21

*Smoked tench stored at 4°C under vacuum.

Table 3. Sensory profile of tench pâté with olive oil (O-TP) and tench pâté with mascarpone cheese (M-TP) compared with commercial pâté (CP).

Attributes	O-TP	M-TP	CP	F	P	P<0.05	P<0.01	P<0.001
Fresh vegetable odour	70.59c	27.55a	14.10b	156.48	0.00	7.16	9.93	13.81
Smoked odour	32.49a	58.31b	40.22a	27.13	0.00	7.72	10.71	14.89
Butter odour	28.70a	73.36c	51.59b	56.58	0.00	9.01	12.50	17.38
Salt	54.41a	55.18a	76.96b	18.70	0.00	8.98	12.46	17.33
Sweet	36.06a	67.38b	29.18a	41.54	0.00	9.58	13.30	18.50
Bitter	20.16a	16.58a	59.02b	34.12	0.00	12.22	16.97	23.59
Acid	20.77a	53.77b	39.00ab	31.42	0.00	8.94	12.41	17.26
Graininess	72.08c	63.62b	8.43a	333.20	0.00	5.74	7.97	11.09
Cohesiveness	39.83a	48.92a	67.66b	19.82	0.00	9.67	13.42	18.66
Fresh vegetable flavour	71.93c	33.63b	18.79a	117.74	0.00	7.66	10.64	14.79
Smoked flavour	43.06a	60.98b	41.96a	6.51	0.01	12.70	17.62	24.51
Butter flavour	29.93a	71.31c	43.65b	39.16	0.00	10.22	14.18	19.72
Mouth coating	76.74	69.52	78.09	1.34	0.29	12.05	16.73	23.26
Persistence of bitter taste	40.75b	10.77a	63.77c	68.40	0.00	9.74	13.51	18.79
Persistence of salty taste	36.05a	26.45a	68.96b	61.11	0.00	8.65	12.01	16.70

a, b, c: within each attribute, different lowercase letters indicate differences between pâté recipes

Table 4. Mean scores assigned by consumers for sensory acceptability and purchase intent of fish pâtés.

Attribute	O-TP	M-TP	CP	P-value
Overall appearance	7.41 ± 1.19b	7.51 ± 1.11b	6.82 ± 0.95a	<0.001
Odour	7.49 ±1.04b	7.28 ± 1.23b	6.41 ± 1.27a	<0.001
Taste	7.90 ± 1.20c	7.19 ± 1.54b	6.15 ± 1.52a	<0.001
Texture	7.72 ± 1.01	7.39 ± 1.22	7.32 ± 0.97	0.070
Overall liking	7.77 ± 1.20c	7.22 ± 1.50b	6.29 ± 1.37a	<0.001
Positive ("yes") purchase intent (%)		78.26%	69.57%	55.07%

Mean ± standard deviation, based on 68 consumers and a 9-point hedonic scale (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely). Means within the same row followed by different letters are significantly different (P < 0.05).

products (Lindström *et al*., 2006; Silva and Gibbs, 2010).

The enumeration of *C. botulinum* performed on BLANK samples before the thermal treatment showed the absence of natural contamination of the product (*C. botulinum* <10 CFU/g). The contamination level of CONTAM samples before the thermal treatment was approximately 5 log of *C. botulinum* in each pâté recipe (5.43 ± 0.31 log CFU/g in O-TP; 5.63 ± 0.37 log CFU/g in M-TP). After the thermal treatment, the *C. botulinum* spores were inactivated to below the detection limit (<10 CFU/g). The reported results confirm that the employed production process complied with the pre-determined performance standard (12D concept). In particular, the applied thermal treatment delivers an appropriate degree of lethality against the target organism, and it is suited to ensure the safety of the products. The heat-treated product was approved for human consumption and sensory evaluation, as it did not show evidence of leakage or swelling, a defect of heat-treated fish paste (Lobo *et al*., 2015).

The physicochemical characteristics of the BLANK and CTR samples are reported in Table 1, for O-TP and M-TP, respectively. No difference in pH, a_w or NaCl was detected among the batches, between the two different sample units (BLANK and CTR). Knowledge of the physical–chemical properties is necessary for the correct execution of the challenge test in order to compare the products submitted to challenge testing with those routinely manufactured by the processor (Berefourt *et al*., 2011).

Tench pâté is a product of considerable nutritional value, mainly due to its high protein content. Although lower than that of tench pulp (22.71%), the protein level of tench pâté is considerably high when compared with the data for other fish pastes (Lobo *et al*., 2015; Minozzo *et al*., 2004). It was not surprising to find the two pâtés possessed limited carbohydrate contents, as it is known that fish have low amounts of carbohydrate (Tocher, 2003). The smoked tench contained about 5% lipid, and the addition of the oil and mascarpone to the experimental recipes increased the level, as shown in Table 2. Differences in lipid profile were recorded among the two formulations, as the M-TP contained a lower percentage of fat, but a higher level of saturated fatty acids (SFA), due to the presence of mascarpone in the formulations. Even though O-TP is characterised by a higher level of fat than M-TP, this is constituted mainly by monounsaturated fatty acids (MUFA) (77.55%) because the presence of olive oil in the recipe limited the increase of SFA, as olive oil is high in MUFA, with a considerable amount of oleic acid (C18:1Δ^{9c}) (Visioli and Galli, 1998). This aspect is of utmost importance, considering that a diet rich in MUFA containing oleic acid from virgin olive oil has been found to have beneficial effects in the prevention of cardiovascular disease (Visioli and Galli, 1998; Schwingshackl and Hoffmann, 2014). The oleic acid is responsible for the reduction of plasmatic cholesterol levels and the improvement of the low-density lipoprotein/high-density lipoprotein ratio.

The European Food Safety Authority (EFSA, 2011) recommends the replacement of SFA with an equal amount of MUFA in order to reduce blood low-density lipoprotein and cholesterol levels. Cholesterol content varied among the two formulations. The literature states that this value can vary in freshwater fish, especially tench, depending on the age and harvest season (Kopicova and Vavreinova, 2007). The

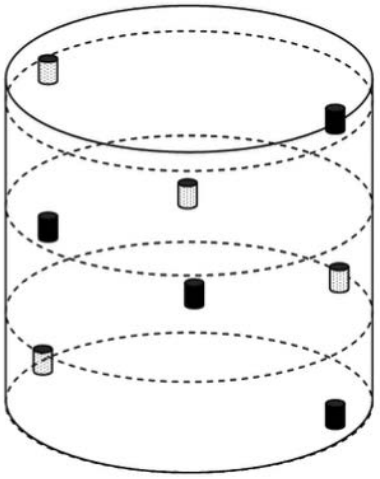

Figure 1. Measuring points of the data loggers inside the steriliser (the shaded and black loggers correspond to different fish pâté recipes; the dotted lines defines the steriliser's layers).

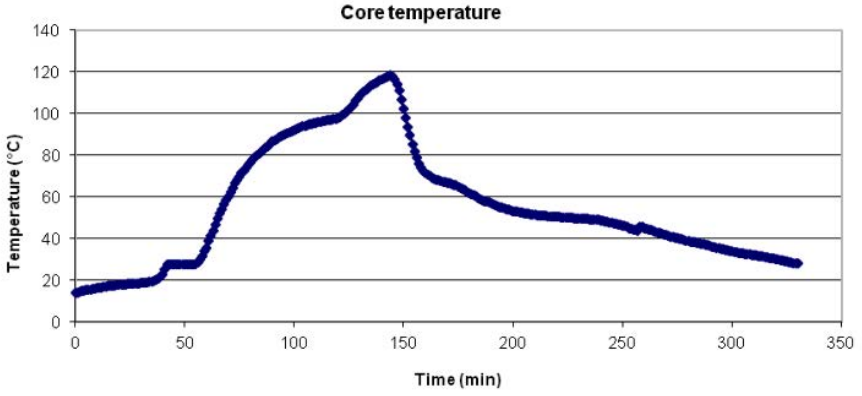

Figure 2. Most unfavourable thermal profile logged during the thermal treatment.

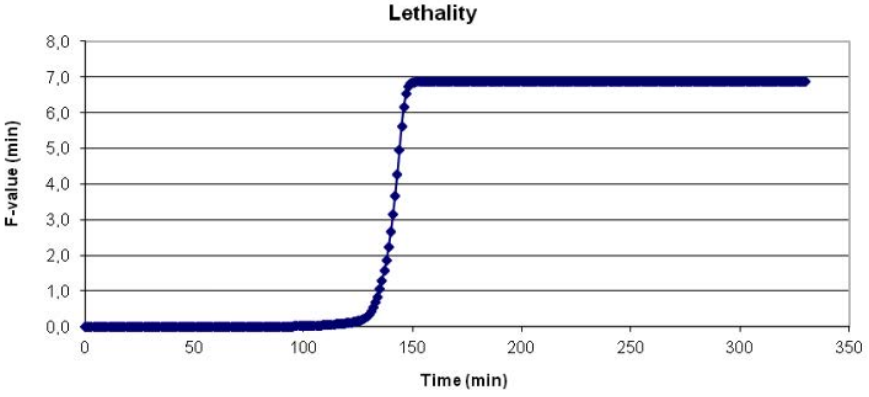

Figure 3. Inactivation *of Clostridium botulinum* according to the thermal treatment time.

amount of cholesterol was lower in O-TP than M-TP, reflecting the amount in smoked tench and the presence of olive oil in the pâtés. On the contrary, the addition of mascarpone to the M-TP recipe enhanced the cholesterol level in M-TP. While a moderate level of salt (NaCl) was present in all samples, these formulations are in line with current consumers habits and the food industry's approach to promote health and prevent diseases, by limiting certain nutrients in the diet (Kloss *et al*., 2015).

The descriptive analysis revealed higher intensity of the fresh vegetable attribute in O-TP. The flavour associated with freshwater fish is usually mild, and most fish have a common sweet and plant-like aroma (Turchini *et al*., 2004). This sensory attribute was exalted in O-TP, in which the addition of olive oil and parsley contributed to the vegetable-like flavour (Table 3). This intense aromatic note of oil and parsley diminished the perception of aromatic molecules associated with the smoking process of the tench, resulting in a lower intensity of the smoked attribute in O-TP than M-TP (considering the same degree of smoking of the starting material). The more intense lactic/butter and smoked attributes in M-TP than O-TP characterised the aromatic profile of the M-TP product, which also presented more intense sweet and sour tastes, attributable to the taste profile of mascarpone and cream. The CP had heightened and more persistent bitter and salty flavours and the consistency was characterised by a higher adhesiveness and a lower graininess than the two experimental formulations (O-TP and M-TP). The greater saltiness derived from the relatively higher salt content in CP.

In addition, Tench flesh is characterized by a considerable amount of bone therefore a grainy perception could be a consequence of milder mincing process than CPs (Shaviklo *et al*., 2010). Furthermore, chopped parsley and the physical state of the carrier (olive oil) may contribute to the grainy perception of M-TP and even more of O-TP.

The consumer acceptance data (Table 4) highlighted differences in the examined attributes and consumer acceptability among the groups. O-TP received the highest scores for taste and overall liking while CP received the lowest score for overall appearance, odour, taste and overall liking. Differences in the acceptance of the appearance were found, probably because the experimental tench pâtés showed a more artisanal appearance, usually more appreciated by consumers that usually eat this product (Di Monaco *et al*., 2009).

Species, such as tench, could present a muddy/hearty odour and flavour, owing to their ecological habits. This off-flavour is mainly caused by two isoprene compounds, geosmin and 2-methylisoborneol, usually found in pond bottom soil, which is the major feed intake of this species (Papp *et al*., 2007; Varga *et al*., 2015). Nevertheless, the process and the ingredients in both recipes removed the possibility of the presence of this off-flavour, and, consequently, both formulated pâtés were more appreciated than CP. Despite the graininess in the formulated pâtés perceived by the panel and confirmed by the consumers, no difference in the texture acceptability among the three formulations was recorded, probably because the two carriers (olive oil and mascarpone) made the perception of the grain more pleasant.

For the evaluation of purchase intent, consumers would prefer O-TP (affirmative purchase intent of 78.3%) over M-TP (46.3%) and CP (44.4%). The overall liking and taste ($P < 0.05$) were significantly important in determining the higher purchase intent of O-TP and M-TP versus CP, especially O-TP.

The two tench pâté recipes prepared in this study proved to be safe products for human consumption, with significant nutritional values and sensory acceptance, especially O-TP. Therefore, considering the current consumers' preferences directed toward fast-food consumption, the developed pâtés can be regarded as a time-saving strategy, more easily incorporable into busy lifestyles compared with homemade food (van der Horst *et al*., 2011). The study findings also reveal that there is great potential in the consumption of tench pâté as an alternative freshwater food product of high value.

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