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Organoleptic, physicochemical and microbiological properties of caviar at retail trade

Organoleptische, physikalisch-chemische und mikrobiologische Eigenschaften von Kaviar aus dem Einzelhandel

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Summary

To investigate the organoleptic, physicochemical and microbiological quality of caviar, fifty caviar samples from various fresh- and seawater fish species were obtained from retail markets and tested. Only 36 % of the samples were without organoleptic deviations. 46 % of the samples showed cell counts of mesophilic aerobic bacteria of >2 $\log_{10}$ CFU/g. Of these, the majority (n = 12) showed cell counts of 3–4 $\log_{10}$ CFU/g. Two samples exhibited high cell counts of >6 $\log_{10}$ CFU/g. Lactic acid bacteria were found in 20 % of the samples, reaching up to 5.8 $\log_{10}$ CFU/g. Yeasts were detected in 10 % of the samples and moulds were present in one sample. For coliforms, 4 % of the samples showed growth with counts of 2.3 $\log_{10}$ CFU/g and 3.4 $\log_{10}$ CFU/g respectively. However, *E. coli* was not detectable. One sample contained 3.43 $\log_{10}$ CFU/g coagulase positive staphylococci and 5 $\log_{10}$ CFU/g pseudomonads. *Mycobacterium* aubagnense, a potential zoonotic pathogen, was detected within four samples. Neither lower pH, lower water activity, higher salt concentrations nor pasteurization influenced the microbiological quality of these products significantly.

Taken together, microbiological quality of most caviar samples was acceptable. Nonetheless, single samples showed high microbiological loads. Many caviar samples exhibited organoleptic deviations. To limit microbiological growth and subsequent changes in organoleptic properties, a continuous cooling of these products is necessary. The importance of potentially pathogenic *Mycobacterium* spp. (incl. *Mycobacterium aubagnense*) in caviar must be evaluated in future studies.

Keywords: caviar, microbiological quality, organoleptic properties, chemical properties

Zusammenfassung

Zur Untersuchung der organoleptischen, physikalisch-chemischen und mikrobiologischen Qualität von Kaviar wurden 50 Kaviarproben von verschiedenen Süß- und Salzwasserfischarten aus dem Einzelhandel untersucht. Nur 36 % der Proben waren ohne organoleptische Abweichungen. 46 % der Proben wiesen mesophile aerobe Gesamtkeimzahlen von >2 $\log_{10}$ KBE/g auf. Von diesen zeigte die Mehrzahl (n = 12) Keimzahlen von 3–4 $\log_{10}$ KbE/g. Zwei Proben wiesen dabei eine hohe Keimzahl von >6 $\log_{10}$ KBE. Milchsäurebakterien wurden in 20 % der Proben nachgewiesen (bis zu 5,8 $\log_{10}$ KbE/g). Hefen wurden in 10 % der Proben detektiert, und Schimmelpilze fanden sich in einer Probe. Coliforme Keime waren in 4 % der Proben nachweisbar mit Keimzahlen von 2,3 $\log_{10}$ KBE/g und 3,4 $\log_{10}$ KBE/g. *E. coli* war jedoch nicht nachweisbar. Eine Probe enthielt 3,43 $\log_{10}$ KBE/g koagulasepositive Staphylokokken und 5 $\log_{10}$ KBE/g Pseudomonaden. *Mycobacterium aubagnense,* ein potentieller Zoonoseerreger, wurde in vier Proben nachgewiesen. Weder niedrige pH-Werte, geringe Wasseraktivitäten, höhere Salzkonzentrationen noch eine Pasteurisierung beeinflussten die mikrobiologische Qualität dieser Produkte signifikant.

Insgesamt war die mikrobiologische Qualität der meisten Kaviarproben akzeptabel. Dennoch zeigten einzelne Proben hohe mikrobiologische Belastungen. Viele Kaviarproben wiesen dazu organoleptische Abweichungen auf. Um eine Vermehrung der vorhandenen Mikroorganismen in Kaviarprodukten zu begrenzen und daraus folgende Änderungen der organoleptischen Eigenschaften zu vermeiden, ist eine kontinuierliche Kühlung solcher Produkte notwendig. Die Bedeutung von potentiell pathogenen *Mycobacterium* spp. (inkl. *Mycobacterium aubagnense*) in Kaviar muss in weiteren Studien evaluiert werden.

Schlüsselwörter: Kaviar, mikrobiologische Qualität, organoleptische Eigenschaften, chemische Eigenschaften

Introduction

Caviar in general is a product made from non-ovulated fish eggs. Sturgeon caviar, often denominate as "real" or "classic" caviar is a product prepared from fish eggs of sturgeon fishes belonging to the *Acipenseridae* family. Three different types of "real caviar" are sold: Beluga, made from European sturgeon *Huso huso,* Osetra mainly from Russian and Persian sturgeons, *Acipenser (A.) gueldenstaedtii* and *A. persicus,* respectively and Sevruga, stellate sturgeon, *A. stellatus.* Further important sturgeon species for caviar production are sterlets, *A. ruthenus,* or the Siberian sturgeon, *A. baeri.*

Caviar is a product derived from roe of fresh or salted fish. It can be produced from roe of the sturgeon family, roe of *Salmonidae* or other fish species. Within the last decade, caviar production from non-sturgeon fish species, like salmon, trout or lumpfish, is increasing.

After harvest, the roe is removed from fish, separated from the connective tissue, sieved with sieves of specific pore sizes, depending on species, and/or salted with differing percentages, usually resulting in a final concentration of 3 to 4 % salt.

Maintaining the cold chain and elevated salt concentration within caviar products are regarded as the crucial factors to preserve the microbiological quality (Shin and Rasco, 2007). In addition, preservatives like potassium sorbate (E202), benzoic acid (E210), boric acid (E284), borax (E285) or sodium benzoate (E211) can be added or pasteurization could be carried out (usually conducted at temperatures below 60 °C) to preserve the microbiological quality and extend shelf life (Bledsoe et al., 2003). According to German food law, boric acid (E284) or sodium tetraborate (borax) (E285) can be used for conservation purposes in sturgeon caviar only, with a maximum amount of 4g/kg (calculated as boric acid) (ZZulV).

The microbial status of caviar basically depends on the microbiological quality of the fish used for caviar production, the surrounding water quality, and personal hygiene during the preparation of the roe and the methods used for preservation (Bledsoe et al., 2003).

It was shown that an initial presence of esp. psychrotropic bacteria might cause a multiplication of these microorganisms to high concentrations even in high salted caviar, representing a potential risk for food safety and human health (Shin et al., 2010). Many, especially water or fish borne microorganisms could contaminate caviar, such as lactobacilli, *Bacillus cereus,* enterococci, aeromonads, *Listeria* spp., *Vibrio* spp. as well as yeasts and moulds. Many of those microorganisms have already been traced in caviar production units (Bagge-Ravn et al., 2003; Gram and Huss, 2000). Therefore the presence of such bacteria in caviar is very likely and a permanent control of bacterial loads should be performed.

Since little data are available about the quality of caviar, notably of products derived from alternative fish species (Jelodar and Safari, 2006), the aim of this study was to investigate the organoleptic, microbiological and physicochemical quality of caviar products derived from different fish species.

Material and Methods

Samples

Caviar samples (n = 50) were obtained from retail markets within their best-before date and analysed for their organoleptic, microbiological and physicochemical properties. Caviar of different species was included (salmon, n = 11; lumpfish, n = 7; trout, n = 7; capelin, n = 6; cod, n = 4; pike, n = 3; herring, n = 3; sturgeon, n = 2; bowfin, n = 2; whitefish, n = 1; mullet, n = 1; charr, n = 1; sea urchin, n = 1; pikeperch, n = 1). Details of the samples are shown in Tab. 1. When investigating multiple caviar samples of the same fish species, different brands or lots were chosen. 36 % (18/50) of the samples were heat treated (pasteurized).

Physicochemical parameters

The pH value (pH meter CG 841, Schott, Mainz, Germany) and water activity (CX-2, AquaLab 4, Decadon Devices, Pullmann, US) were measured for every sample. Tested chemical parameters included the measurement of sodium chloride (titration method) and boric acid concentration (Boron test, Spectroquant, Merck, Darmstadt, Germany on a Lambda-2 spectrometer, Perkin Elmer, Waltham, US) (lower detection limit for boric acid 0.11 %). According to German food law a maximal concentration of 4 g boric acid per kg is allowed for sturgeon caviar only (ZZulV).

Organoleptic analysis

Descriptive sensory analysis was performed according to Lehmann (2009) to detect and describe the qualitative sensory profile of caviar samples. A trained panel of three testers was used to evaluate the colour, texture, flavour and overall appearance.

Microbiological investigation

For microbiological investigation, 15 g samples were aseptically removed, suspended in 135 ml of 0.9 % NaCl solution and blended for 60 sec (Stomacher, Interscience, Saint-Nom-la-Bretèche, France). Enumeration of specific microorganisms was conducted using the drop plating technique for total mesophilic aerobic bacteria (Plate count agar), lactic acid bacteria (MRS agar), lactobacilli (Sorbic acid agar), pseudomonads (Glutamate starch phenol red agar), coagulase positive staphylococci (Baird Parker agar), coliforms (VRBD agar), yeasts and moulds (Rose Bengal chloramphenicol agar). For detection of sulfite reducing anaerobic bacteria, 1 mL of the initial suspension was covered with TSC agar. For *E. coli* enumeration, 1 mL of the initial dilution was put on a filter on Glutaminate agar which was transferred on ECD agar after 4 h of resuscitation at 37°C (all media Oxoid, Basingstoke, UK).

To test for *Mycobacteria,* the protocol of Radomski et al. (2010) was used with minor modifications. Briefly, after homogenisation, the samples were sieved using a cell strainer with 70 µM pores (BD, Heidelberg, Germany). To the suspension, 0.1 % cetylpyridinium chloride solution (AppliChem, Darmstadt, Germany) was added to reach a final concentration of 0.05 %, mixed for 30 min in a tumbler and neutralized in 20 mL Sorensen buffer (Merck, Darmstadt, Germany). Subsequently, the samples were centrifuged for 60 min at 4 °C with 2500 x g and the pellet solved in 1 mL sterile 0.9 % NaCl solution. 100 µL were streaked on Middlebrook 7H10-PANTA agar and Lowenstein-Jensen agar (both heipha Diagnostika, Eppelheim, Germany). Plates were incubated for six weeks in darkness at 28 °C. DNA was extracted from presumptive colonies by Chelex method (Bio-Rad, Munich, Germany). For genus and species verification the GenoType Mycobacterium CM/AS Kit (Hain Lifescience, Nehren, Germany) was used according to manufacturer's protocol. In addition, parts of 16S rRNA and *rpo*B genes of the identified *Mycobacterium* spp. were sequenced according to Adekambi et al. (2006), Hassan et al. (2008) and Mollet et al. (1997) (GATC, Koblenz, Germany).

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Statistical analysis

To investigate the influence of different physiochemical parameters on the microbiological quality, samples were grouped according to specific parameters (samples with pH: ≤5.7 vs. >5.7; samples with water activity: ≤0.95 vs. >0.95; samples with sodium chloride concentration: ≤3.9 vs. >3.9). Pasteurized and non-pasteurized samples were compared. Differences of median values of bacterial cell counts between the corresponding groups were tested using Mann-Whitney U test (GraphPad Prism v6, GraphPad, La Jolla, US). Only bacterial cell counts above detection limits were included in the analysis.

Results and Discussion

Organoleptic properties

Among all samples investigated, only 36 % (18/50) were without organoleptic deviations. 22 samples (44 %) exhibited a distinct fishy smell or taste. Single samples additionally showed bitter (n = 9), sour (n = 3), mouldy (n = 2) and rancid (n = 7) tastes. The texture of 58 % (29/50) showed no deviations, whereas single samples showed slimy (n = 9), dripping (n = 6), slimy/dripping (n=3) and slimy/ropy (n = 2) properties. No significant changes in colour were observed.

Physicochemical parameters

Details of the physicochemical parameters are shown in Tab. 1. Caviar samples showed pH values between 4 and 6.5 (pH average 5.7). Five samples with added sorbic acid (E200), benzoic acid (E210) or citric acid (E330) showed the lowest pH values. The water activity ranged from 0.86 to 0.99 (water activity average 0.95) and sodium chloride concentration ranged from 1.4% to 6.6% (average 3.9%). Two caviar products (codfish) showed water activity values below 0.9 owing to the high sodium chloride contents (> 5 %). Boric acid was not detectable in the samples.

Microbiological quality

Results of the microbiological analysis are summarized in Fig. 1. 46% (23/50) of the samples showed a total mesophilic aerobic plate count (APC) of > 2 $\log_{10}$ CFU/g. Of these, the majority (n=12) showed cell counts of 3–4 $\log_{10}$ CFU/g. Two samples exhibited high cell counts of >6 $\log_{10}$ CFU/g. These two samples (sea urchin and pike caviar) demonstrated rancid tastes in the organoleptic tests. APC is regarded as a general marker of the microbiological quality but does not correlate with the presence of pathogenic microorganisms (Altug and Bayrak, 2003). Lactic acid bacteria (LAB) were found in 20 % (10/50) of the samples. Cell counts for LAB reached up to 5.8 $\log_{10}$ CFU/g. Samples with high counts of LAB were sour or slimy, when tested organoleptically. Samples with high APC showed corresponding high levels of LAB. That result is in accordance to a study by Himelbloom and Crapo (1998). Lactobacilli were only detected in low concentrations in 4 % (2/50) of the samples. Yeasts were detectable in 10 % (5/50) of samples and moulds were present in one sample (2 %). For coliforms, 4 % (2/50) of the samples showed growth with counts of 2.3 $\log_{10}$ CFU/g and 3.4 $\log_{10}$ CFU/g, respectively. Similar levels of coliforms were reported by Altug and Bayrak (2003) in caviar from Russia and Iran. However, *E. coli* was not detectable in our samples. A trout caviar sample contained 3.43 $\log_{10}$ CFU/g coagulase positive staphylococci and 5 $\log_{10}$ CFU/g pseudomonads. The slimy appearance in the organoleptic tests corresponds to the latter results. Sulfite reducing anaerobic spore formers were not detected in any sample tested.

Mycobacterium aubagnense was identified from four caviar samples by the GenoType Mycobacterium kit and verified by partial 16S rRNA gene and *rpo*B sequencing. *Mycobacteria* spp. have already been associated with caviar fishes. Ghaemi et al. (2006) detected different *Mycobacterium* spp. in gills of fishes used for caviar production. In addition, one sample contained *Ralstonia pickettii.* Both, *Mycobacterium aubagnense* and *Ralstonia pikettii* have been recently described as potentially pathogenic to humans (Adekambi, 2009; Brunner et al., 1995; Ghaemi et al., 2006).

To investigate the influence of pH, sodium chloride concentration, water activity and pasteurization on the microbiological quality, samples were grouped and cell counts for individual bacterial groups were compared. Results demonstrated that none of the parameters influenced the microbiological quality significantly (Fig. 2). Nonetheless, there was a tendency for APC and LAB to be lower in pasteurized products (Fig. 2D). Single authors, like Shin and Rasco (2007), detected a lower APC level under higher salt concentrations.

In conclusion, even though the microbiological quality of most caviar samples was acceptable, single samples showed high microbiological loads and strong organoleptic deviations, correspondingly.

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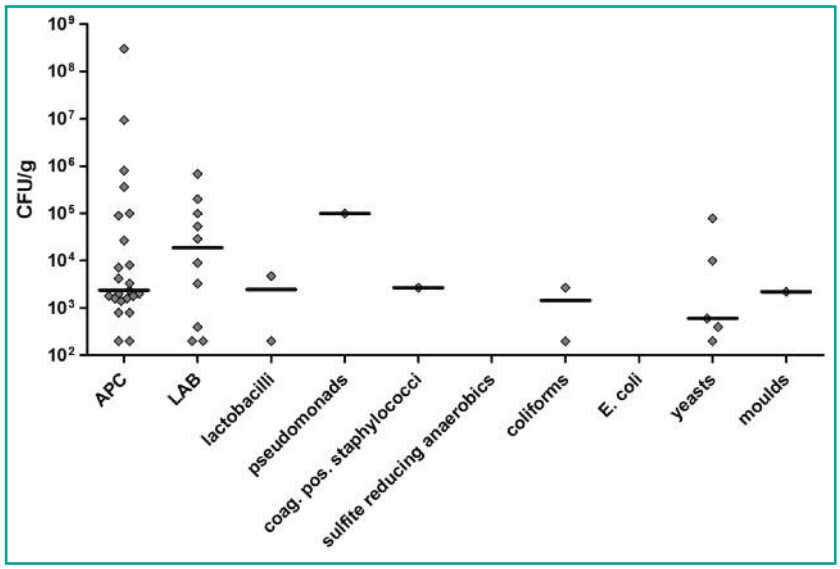

FIGURE 1: *Microbiological properties of the caviar samples (median CFU/g, APC-aerobic plate count, LAB-lactic acid bacteria).*

TABLE 1: *Properties of caviar samples investigated.*

Species	Colour	Pro-cessing[1]	Preser-vatives[2]	pH	a_w	NaCl (%)
American paddlefish *(Polyodon spathula)*	olive	–	–	5.8	0.95	3.5
Atlantic herring *(Clupea harengus)*	white	–	E200, E210, E330	4.5	0.91	5.6
Atlantic herring *(Clupea harengus)*	black	p	E330	4.0	0.97	2.8
Atlantic herring *(Clupea harengus)*	black	p	E330	4.0	0.98	2.7
Bowfin *(Amia calva)*	black	–	–	5.2	0.94	5.2
Bowfin *(Amia calva)*	black	–	–	5.2	0.95	4.4
Capelin *(Mallotus villosus)*	dark grey	–	E211, E330	5.1	0.96	5.7
Capelin *(Mallotus villosus)*	bright orange	p	E202, E211, E330	6.0	0.95	4.9
Capelin *(Mallotus villosus)*	bright orange	p	E202, E211, E330	6.0	0.95	5.0
Capelin *(Mallotus villosus)*	bright green	p	E202, E211, E330	5.5	0.96	4.0
Capelin *(Mallotus villosus)*	bright green	p	–	5.3	0.96	5.2
Capelin *(Mallotus villosus)*	black	–	E211, E330	5.4	0.96	5.7
Charr *(Salvelinus)*	orange	–	–	6.3	0.97	3.1
Cod *(Gadus)*	yellowish	–	E211, E300	5.2	0.86	5.7
Cod *(Gadus)*	yellowish	–	E211, E300, E330	5.6	0.86	5.0
Cod *(Gadus)*	yellowish	–	–	6.3	0.98	1.4
Cod *(Gadus)*	beige	–	–	5.9	0.98	1.4
Green sea urchin *(Strongylocentrotus)*	brown orange	–	–	6.1	0.98	2.8
Lumpfish *(Cyclopterus lumpus)*	anthracite	–	E211, E330	5.1	0.95	6.1
Lumpfish *(Cyclopterus lumpus)*	orange	p	E210	5.9	0.95	6.6
Lumpfish *(Cyclopterus lumpus)*	black	–	E211, E260	4.9	0.95	5.9
Lumpfish *(Cyclopterus lumpus)*	dark grey	–	E211	5.9	0.97	4.1
Lumpfish *(Cyclopterus lumpus)*	anthracite	–	E211, E260	4.9	0.96	5.5
Lumpfish *(Cyclopterus lumpus)*	anthracite	p	E202, E211, E330	5.5	0.96	4.8
Lumpfish *(Cyclopterus lumpus)*	orange	–	E211, E330	5.3	0.95	5.3
Mullet *(Mugil* spp.*)*	brown orange	s	–	5.6	0.86	n.d.
Pike *(Esox lucius)*	amber	–	–	5.7	0.97	4.0
Pike *(Esox lucius)*	bright yellow	p	–	5.7	0.97	3.7
Pike *(Esox lucius)*	amber	p	–	5.5	0.97	3.6
Pike-perch *(Stizostedion)*	orange	–	–	6.1	0.98	1.5
Salmon *(Oncorhynchus)*	orange	–	–	6.0	0.94	3.6
Salmon *(Oncorhynchus)*	orange	p	–	6.1	0.95	3.1
Salmon *(Oncorhynchus)*	orange	–	E200	5.2	0.95	2.9
Salmon *(Oncorhynchus)*	bright orange	–	–	6.0	0.96	3.3
Salmon *(Oncorhynchus gorbuscha)*	orange	p	–	5.9	0.96	2.9
Salmon *(Oncorhynchus)*	orange	–	–	6.0	0.94	4.5
Salmon *(Oncorhynchus)*	orange	–	E200	6.0	0.97	2.5
Salmon *(Oncorhynchus)*	red orange	–	E200	5.6	0.96	2.8
Salmon *(Oncorhynchus)*	orange	p	–	5.8	0.94	3.7
Salmon *(Oncorhynchus)*	luminous orange	–	E200	5.7	0.97	2.4
Salmon *(Oncorhynchus gorbuscha)*	orange	p	–	5.8	0.96	3.1
Siberian sturgeon *(Acipenser baerii)*	anthracite	–	E285	5.7	0.95	4.1
Trout *(Oncorhynchus mykiss)*	orange	–	E296, E330, E334	6.3	0.96	3.7
Trout *(Oncorhynchus mykiss)*	orange	–	–	6.4	0.99	0.4
Trout *(Oncorhynchus mykiss)*	bright orange	–	E330	6.2	0.97	3.3
Trout *(Oncorhynchus mykiss)*	orange	p	–	6.0	0.97	3.6
Trout *(Oncorhynchus mykiss)*	bright orange	p	–	6.3	0.96	4.3
Trout *(Oncorhynchus mykiss)*	orange	p	–	6.3	0.96	3.6
Trout *(Oncorhynchus mykiss)*	pink	–	E200	5.1	0.96	3.3
Whitefish *(Coregonus)*	bright yellow	s	–	5.9	0.96	3.9

([1]: processing abbreviations: p-pasteurized, s-smoked; [2]: preservatives added according to label; n.d. not determined)

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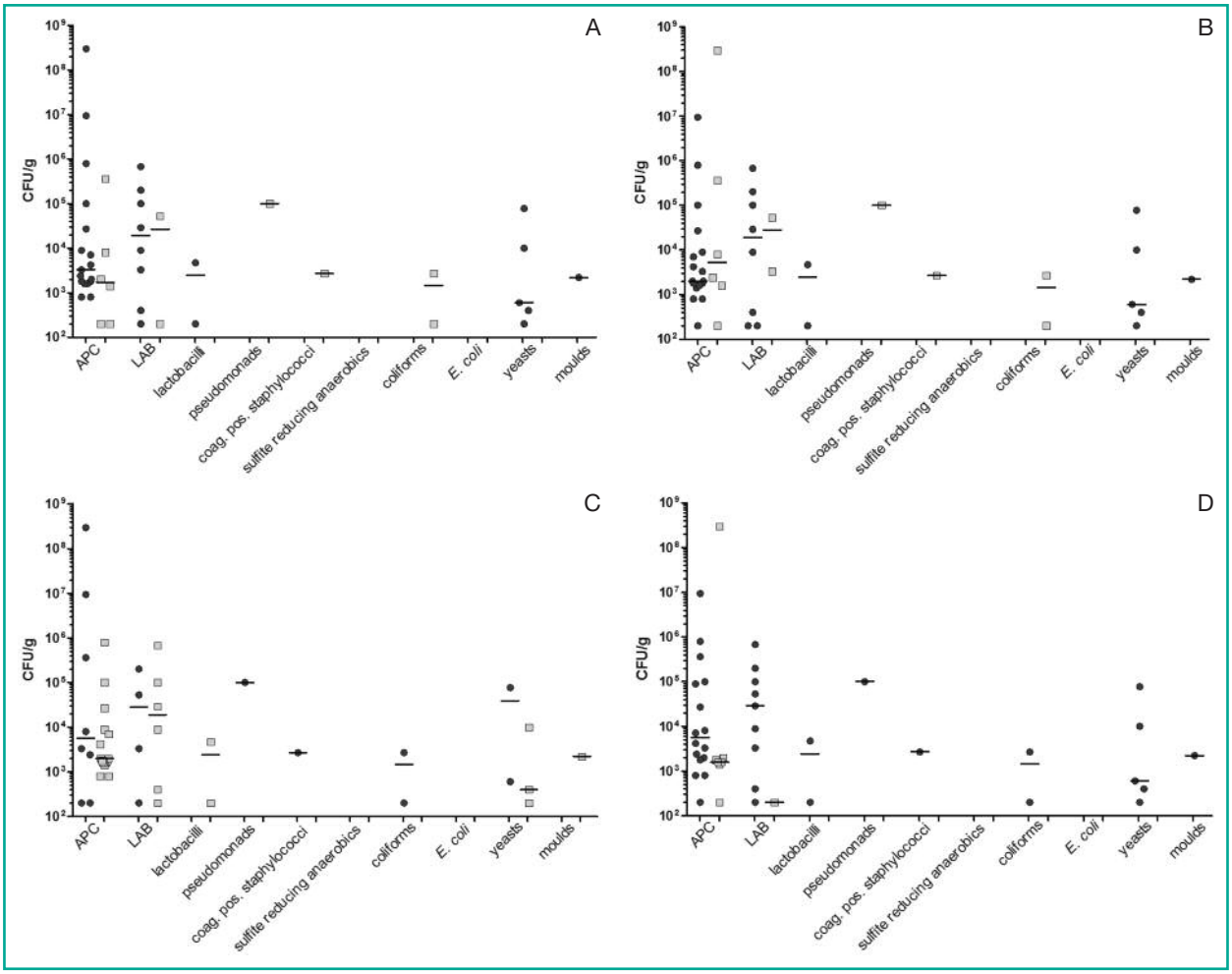

FIGURE 2: *Influence of pH, water activity, sodium chloride concentration and pasteurization on the microbiological properties of caviar (A: pH: black circles-pH ≤5.7, grey rectangles-pH >5.7; B: water activity: black circles-water activity ≤0.95, grey rectangles-water activity >0.95; C: sodium chloride concentration: black circles-sodium chloride concentration ≤3.9, grey rectangles-sodium chloride concentration >3.9; D: pasteurization: black circles-not pasteurized, grey rectangles-pasteurized) (APC-aerobic plate count, LAB-lactic acid bacteria).*

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