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Microbiological safety criteria for products from unconventional raw materials: raw bear fat

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Abstract:
Fat of game animals is a prospective raw material for bioactive additives. Before such a product enters the market, food science has to make sure it is safe for consumption.

This research featured subcutaneous adipose tissue of brown bears tested with standard methods for microbiological safety indicators. The microbial properties were studied on liquid and solid nutrient media. Staphylococcus was profiled using a VITEK 2 Compact biochemical automatic analyzer and Gram-positive cards (Bio-Mérieux, France). The analysis followed the Technical Regulations of the Customs Union TR TS 021/2011 On Food Safety (December 09, 2011).

The microbial count for mesophilic aerobic and facultative anaerobic microorganisms was 1.5×10^3 CFU/g. The fat samples revealed no molds, yeasts, or *Escherichia coli* bacteria. Liquid and solid nutrient media made it possible to describe the qualitative profile and cultural properties of the bear fat microflora against pork fat, which served as control. The automatic system identified Gram-positive, coagulase-negative, and oxidase-positive *Staphylococcus lentus* and *Staphylococcus sciuri*. In line with the modern classification, they belong to the new genus of *Mammaliicoccus*, namely *Mammaliicoccus sciuri*.

Subcutaneous adipose tissue of brown bears needs to undergo a microbiological safety test before consumption. Bear fat requires additional research in order to become a safe raw material for food products and bioactive additives.

Keywords: Brown bear fat, subcutaneous adipose tissue, microbiological indicators, *Mammaliicoccus lentus*, *Mammaliicoccus sciuri*

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INTRODUCTION

The Strategy for Improving the Quality of Food Products in the Russian Federation through 2030 introduces several ways to develop an independent national food quality management system. This document stresses that the technical regulations for certain food products need a system of food quality indicators. New quality indicators will make it possible to test new foods with unconventional plant and animal raw materials, as well as to develop new biologically active additives and functional foods [1–5].

Adipose tissue of game animals is a potential source of bioactive additives [6]. In traditional medicine, bear (*Ursus arctos* L.) fat is an excellent anti-burn ointment. It also treats various diseases of the upper respiratory tract and heals skin damage of various severity. Bear fat is applied to relieve back and joints pain symptoms. The Mongols consume it raw; the Yakuts use twice-melted bear fat to treat tuberculosis [7]. Bear fat also is a popular folk remedy against gastrointestinal diseases and atherosclerosis [8, 9]. Customers buy it mostly online, and no technical regulations have been developed for it so far. As a result, the market is full of bear fat products of unknown safety status.

Raw fat is most often obtained from hibernating game animals, e.g., bears, badgers, marmots, etc. It is

divided into subcutaneous and visceral. Most animal fat deposits are subcutaneous and are located right under the skin. Visceral adipose tissue is scarce and envelops the internal organs of well-fed adult specimen.

Meat and fat of farm and game animals can transmit zoonotic infections to people. Table 1 classifies them according to the carrier.

Game animals, being asynanthropic, are potential carriers of zoonotic diseases that can be transmitted to humans via infected meat and offal or via infected domestic and synanthropic animals. Table 2 describes the most common zoonoses of asynanthropic game animals.

Science knows more than a thousand pathogens of infectious diseases, 60% of which are of a zoonotic nature, i.e., people get them from animals. About 70% of such cases are connected with wild animals [10].

People usually get infected by inhaling contaminated aerosol secretions of rodents, e.g., feces, saliva, and urine. Meat and offal obtained from infected animals are also contagious. To be used as food, game meat and offal are to undergo a veterinary and sanitary examination, according to the Law on Veterinary Medicine of the Russian Federation (N 4979-1, May 14, 1993).

Meat and meat products contain saprophytic, sanitary-indicative, opportunistic, and pathogenic microorganisms. Microbial contamination occurs in endogenous and exogenous ways. Endogenous contamination takes place when the animal is still alive. Posthumous endogenous infection is usually associated with damaged intestines or ungutted carcasses. Exogenous contamination happens when microorganisms penetrate from the environment during butchering, storage, transportation, and processing. Retrocession from the gastrointestinal tract, i.e., endogenous infection of deep tissues in a living animal, occurs as a result of starvation, physical strain, diseases, and injuries. Microbial contamination *in vivo* is connected not only with the digestive system, but also with those systems that contact with the external environment, i.e., genitourinary, respiratory, and integumentary. Microbiotic studies of game animals are important precisely because the microflora of slaughter products depends on the composition of microorganisms the animal had when it was alive.

Bear microbiota is a popular subject of foreign studies. Bear intestinal microbiomes differ from those of other omnivores because bears have no caecum. The caecum restricts the rate at which nutrients pass through the intestinal tract. Apparently, it serves as a reservoir for microbial populations that replenish the microbiome diversity depending on the diet and health. Therefore, bears intestinal microbiomes are vulnerable to systemic changes in diet, health, or other factors. Gillman *et al.* believe that fecal samples provide insight into the intestinal microbiota of black bears, as well as other carnivores and omnivores with simple intestinal morphology [11].

Table 1 Zoonotic infections

Animals	Carrier	Infection
Synanthropic	Rats, pigeons, etc.	Anthrax, brucellosis, leptospirosis, ornithosis, etc.
Domesticated	Cattle, pigs, chicken, etc.	
Asynanthropic	Hares, bears, badgers, etc.	

Table 2 Zoonotic diseases transmitted by asynanthropic game animals

Asynanthropic animals	Zoonotic disease	Pathogen
Hares (*Lepus europaeus* L., *Lepus timidus* L.)	Trichinosis	*Trichinella spiralis*
Bears (*Ursus arctos* L.)	Rabies	*Rabies lyssavirus*
Beavers (*Castor fiber* L.)	Paratyphoid	*Salmonella*
Badgers (*Meles meles* L.)	Listeriosis	*Listeria monocytogenes*
Marmots (*Marmota* L.)	Tularemia	*Francisella tularensis*

Glad *et al.* studied the intestinal microbiome of polar bears and profiled microorganisms that belonged to the phylum of *Firmicutes* [12]. They identified 160 sequences as *Clostridiales* and found a new, unclassified sequence of *Firmicutes*. Most of the sequences (70%) belonged to *Clostridium*. The aerobic heterotrophic cell count on chocolate agar ranged from 5.0×10^4 to 1.0×10^6 CFU/mL for rectal swabs and from 4.0×10^3 to 1.0×10^5 CFU/g for feces samples.

Franz *et al.* studied intestinal microbiomes from two polar bear populations and identified eight most common classes of bacteria: *Clostridia*, *Gammaproteobacteria*, *Actinobacteria*, *Coriobacteriia*, *Nogativicutes*, *Bacilli*, *Bacteroidia*, *Fusobacteria*, *Campylobacteria*, and *Saccharimonadia* [13]. The microbiomes were different and reflected the habitat, diet, sex, and age of the animals. The authors decided that *Bacilli* were especially important for restoring intestinal health and maintaining intestinal homeostasis.

Schwab *et al.* studied fecal microbiota from ten grizzly bears [14]. The samples that belonged to wild grizzly bears contained more eubacteria than those obtained from captive bears. *Enterococci* and *Enterobacteria* were numerous in all samples. Pathogenic *Clostridum perfringens* group I had a positive correlation with protein intake and a negative correlation with dietary fiber content. Although considered healthy, the wild bears that lived on a normal protein-based diet were more likely to carry *C. perfringens* than those wild bears that relied mostly on plant-based food. Three samples even contained *Clostridium sordellii*, which can cause toxic shock syndrome in humans. Thus, the count of pathogenic *Escherichia coli* depended neither on the diet nor on the habitat.

Watson *et al.* and Trujillo *et al.* reported that subpopulations of brown bears living in different national parks shared five types of bacteria: *Firmicutes*, *Proteobacteria*, *Epsilonbacteraeota*, *Bacteroidetes*, and *Actinobacteria* [15, 16]. The authors identified 16 major genera.

Therefore, bear intestinal microbiomes depend on the habitat, climate, age, sex, food diversity, and hunting strategy. Raw materials from game animals are to be checked for pathogenic and opportunistic pathogenic microflora before being processed into food products or biologically active additives.

Raw bear fat has a high lipid content, but it may also contain lipophilic microorganisms capable of synthesizing enzymes that hydrolyze lipids. Many bacteria, fungi, yeasts, and actinomycetes produce lipases that hydrolyze lipids at the water-fat phase boundary. Lipase-producing bacteria usually belong to *Acinetobacter*, *Bacillus*, *Burkholderia*, *Pseudomonas*, *Staphylococcus*, *Microbacterium*, *Lactobacillus*, *Stenotrophomonas*, *Arthrobacter*, *Serratia*, *Aeromonas*, *Thermosyntropha*, *Achromobater*, *Chromobacterium*, *Burkholderia*, *Streptomyces*, etc. [17–20].

Cooling, freezing, and thermal processing protect adipose tissue from bacterial contamination. Freezing and subsequent defrosting change the microbial quantitative and qualitative composition. Refrigerated storage gradually kills mesophilic microorganisms; however, some psychrophilic microorganisms remain viable for a long time.

Psychrotrophs proliferate on livestock products. Food-spoiling psychrotrophs are known to affect commercial foodstuffs. Zhang *et al.* identified microorganisms of 38 genera and 20 families, including Gram-negative bacteria [21]. Saprophytic *Pseudomonas* and especially *Pseudomonas fragi* had the highest count, followed by *Psychrobacter*, *Brochothrix*, *Serratia*, and *Stenotrophomonas*. Li *et al.* also reported other pathogenic and toxic microorganisms, such as *Salmonella*, *Staphylococcus aureus*, and *C. perfringens* [17]. Moschonas *et al.* detected psychrophilic and psychrotrophic anaerobic microflora in commercial Irish beef abattoir environments and vacuum-packed beef [22]. They tested 431 isolates and profiled 25 microbial species, with the most frequently recovered species being *Clostridium gasigenes*, followed by *Clostridium estertheticum* and *Clostridium algidixylanolyticum*. These species often cause spoilage in chilled lamb and vacuum-packed beef, which poses a significant commercial threat to the meat industry.

Pathogenic bacteria survive various methods of freezing and defrosting. Choi *et al.* studied the effect of freezing and defrosting on the microbiological quality and changes in the microstructure of chicken breasts inoculated with *Listeria monocytogenes* and *Campylobacter jejuni* [23]. They detected no differences in the count of *L. monocytogenes* under different freezing conditions. However, air freezing (–20°C) reduced the total aerobic bacterial count and *C. jejuni* in particular, compared to other freezing methods. Defrosting by hot/cold air flow, water immersion, and high pressure at 4 and 25°C caused no significant differences in the count of *L. monocytogenes*.

Metzger *et al.* reported that foodborne pathogens survived freezing in cheese [24]. They produced three samples of semi-soft cheese with milk inoculated with two pathogen mixes of *L. monocytogenes/S. aureus* and *E. coli/Salmonella typhimurium*. Storage at –20°C for 2, 7, or 30 days resulted in little to no reduction in *L. monocytogenes*. However, 90 days at –20°C reduced the count of *L. monocytogenes* significantly, while the count of *S. aureus* remained constant over the 90-day storage in the freezer. *E. coli* and *S. typhimurium* rapidly decreased at –20°C. The defrosting conditions were 4°C for 14 h and 20°C for 4 h, but these factors had no effect on the viability of microorganisms.

Apparently, game meat and offal have to undergo a number of microbiological tests before consumption.

The research objective was to determine the microbiological safety profile and assess the qualitative composition of the microflora of bear adipose tissue.

The list of tasks included:
– reviewing scientific publications;
– developing a scheme for microbiological safety studies;
– analyzing microbiological safety criteria;
– isolating pure microbial cultures;
– profiling the isolated microflora; and
– comparing the obtained results with available publications.

The identified microbiological safety indicators can be used in technical regulations on quality and safety requirements for foods and dietary supplements based on bear fat.

STUDY OBJECTS AND METHODS

The research featured subcutaneous fat of *Ursus arctos* L. The samples belonged to a bear shot by a licensed hunter in the Kemerovo Region in 2021. The adipose tissue was separated from the carcass within 2 h after the slaughter. The butchering took place at –15 ± 5°C. For veterinary and sanitary examination, the samples were frozen at –18 ± 2°C and delivered to the laboratory within three days.

The Technical Regulations of the Customs Union TR TS 021/2011 On Food Safety contain no requirements for the microbiological safety of wild animal raw fat. Thus, we used the requirements for pork fat as control. The pork fat was purchased from the market and frozen under similar conditions.

After 12 h of freezing, the samples were defrosted at 20 ± 2°C in open air for 4 h, crushed in a cutter to a particle size of 3–5 mm, and packaged.

The sampling followed State Standard 31904-2012. We diluted 10 g of each sample in 90 cm^3 of saline in the ratios of 1:10, $1:10^2$, $1:10^3$, and $1:10^4$ by volume. Then, we dropped 1 cm^3 of the substance with sterile pipettes in sterile Petri dishes and into test tubes with the Kessler medium. The procedure followed the microbiological control scheme illustrated in Fig. 1.

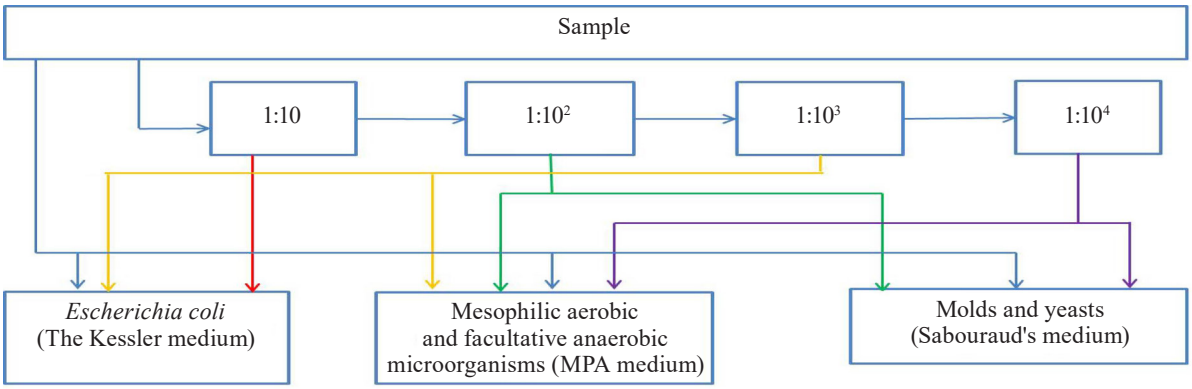


Figure 1 Microbiological test scheme

Table 3 Microbiological count of pork and bear raw fat

Indicator	Pork fat	Bear fat	TR CU 021/2011
Mesophilic aerobic and facultative anaerobic microorganisms, CFU/g	4.5×10^4	1.5×10^3	$\leq 5.0\times10^4$
Escherichia coli (coliforms), per 0.001 g	n.d.	n.d.	Unavailable for 0.001 g
Staphylococcus aureus, per 0,1 g	n.d.	n.d.	n.d.
Mold, CFU/g	n.d.	n.d.	n.d.
Yeasts, CFU/g	n.d.	n.d.	n.d.

n.d. – not detected

We profiled mesophilic aerobic and facultative anaerobic microorganisms in line with State Standard 10444.15-94. To study the QMAFAnM indicator, we used sterile meat peptone agar. After the nutrient medium solidified, Petri dishes were placed in a thermostat for cultivation at 37°C. After 48 h of cultivation, we counted the colonies and tested the indicators for the compliance with the requirements.

The procedure for *Escherichia coli* followed State Standard 31747-2012. We put the diluted product into a test tube with the sterile Kessler medium and a float and stored it in a thermostat at 37°C for 24 h. A bubble in the float indicated the presence of *E. coli*. The samples were tested for compliance with the Technical Regulations.

The mold and yeast tests corresponded with State Standard 10444.12-2013. We poured Sabouraud's sterile nutrient medium into Petri dishes. After it solidified, we put the Petri dishes in a thermostat for cultivation at 25°C and counted the colonies after 72 h of cultivation.

The *Staphylococcus aureus* test was in line with State Standard 31746-2012.

The cultural property test involved liquid and solid nutrient media. *Staphylococcus* bacteria were profiled using a VITEK 2 Compact biochemical analyzer (Bio-Mérieux, France) and a VITEK 2 Gram-positive identification card. This automated system provides 24-h microbial profiling and antimicrobial susceptibility testing. The software compares the test responses with the standard responses for each organism or group of organisms. A score and percentage probability indicate how the observed responses match the typical responses for each organism with a 99% probability.

RESULTS AND DISCUSSION

The Technical Regulations of the Customs Union provide no safety indicators for raw bear fat, so we used the requirements for pork fat as indicative indicators. Although they do not standardize the content of mold fungi and yeast in pork fat, we studied these indicators to assess the qualitative composition of the microflora, as well as the chance that these microorganisms might contaminate fat.

The pork fat and the bear fat complied with the TR CU 021/2011 in terms of microbiological safety criteria for pork fat. Table 3 shows the indicators of defrosted fat samples.

Pork fat and raw bear fat have a favorable chemical composition for microbial growth. The lipid part is 92% for pork fat and 72% for bear fat; they also contain 2–17% of proteins and 4–5% of moisture, respectively. Microorganisms can use these components as nutrients.

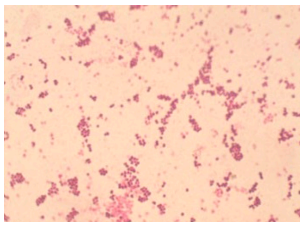
The total bacterial contamination was consistent with the data published by Maduka *et al.*, who linked the higher bacterial count in pork fat with its physical properties, i.e., mucous nature and high fat content [25].

To determine QMAFAnM for the samples grown on the media, we established the taxonomic affiliation of microorganisms based on cultural, morphological, and tinctorial properties. The microflora of raw bear fat was represented by *Bacilli*. The microorganisms could be natural microflora or contamination during butchering, transportation, and storage. Other studies on the intestinal microbiota of brown, black, and polar bears also reported *Bacilli* [11, 13, 15]. Their representatives are known to produce lipase [19].

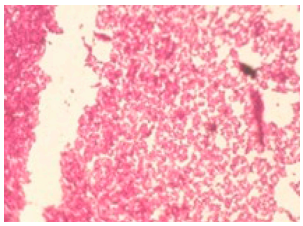
Colony 1

Colony 2

Figure 2 Colonies in bear fat samples



Colony 1



Colony 2

Figure 3 Microscopy of Gram-stained preparations in bear fat samples

Table 4 Identification results

Species	Probability, %,	Atypical results	
Staphylococcus lentus	96	Tyrosinarylamidase TyrA	+
		Alpha-Glucosidase AGLU	+
Staphylococcus sciuri	97	Alpha-Mannosidase AMAN	+

The research revealed some cocci that could be opportunistic pathogens for humans. Figures 2 and 3 show the colonies and the microscopy of bacterial preparations isolated from bear raw fat.

Colony 1 had a round shape with a diameter of 7 mm; it was white, flat, smooth, shiny, and opaque. Homogeneous in structure, it had a thick consistency, with serrated edges.

Colony 2 was round and slightly larger in diameter (11 mm). It was yellow, wavy, shiny, and opaque, with a thick and homogeneous consistency.

The microscopic image of the Gram-stained preparations made it possible to assess the shape and location of the microorganisms as cocci clustered like grape bunches. The cultural and morphological properties suggested that the microorganisms belonged to the genus of *Staphylococcus*.

Table 4 shows the identification of *Staphylococcus* isolated from the raw bear fat samples using the VITEK 2 Compact Bio-Mérieux automated system.

The isolated microorganisms were identified as Gram-positive, coagulase-negative, and oxidase-positive staphylococci consisting of clustered cocci.

Chervyakova *et al.* proposed to include the following parameters into the list of authenticity markers: utilization of *β*-galactosidase and *α*-glucosidase, resistance to polymyxin B and novobiocin, ability to alkalinize lactate and N-acetyl-d-glucosamine [26]. These indicators could provide more accurate intraspecific profiling of *Staphylococcus* bacteria. Tables 5 and 6 give a detailed biochemical information on how the isolated microorganisms utilize particular components.

The experimental data suggested that *Staphylococcus lentus* and *Staphylococcus sciuri* belonged to the new genus of *Mammaliicoccus*, of which *Mammaliicoccus sciuri* is the type species. *Staphylococcus*

Table 5 Biochemistry of *Staphylococcus lentus*

2	AMY	+	4	PIPLC	–	5	dXYL	–	8	ADH1	+	9	BGAL	+	11	AGLU	+
13	APPA	–	14	CDEX	–	15	AspA	–	16	BGAR	–	17	AMAN	–	19	PHOS	+
20	LeuA	–	23	ProA	–	24	BGURr	–	25	AGAL	–	26	PyrA	+	27	BGUR	–
28	AlaA	–	29	TyrA	+	30	dSOR	+	31	URE	–	32	POLYB	–	37	dGAL	–
38	dRIB	+	39	iLATk	+	42	LAC	–	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	–	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	–
57	dRAF	–	58	O129R	–	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	–
64	OPTO	+															

Table 6 Biochemistry of *Staphylococcus sciuri*

2	AMY	–	4	PIPLC	–	5	dXYL	–	8	ADH1	–	9	BGAL	–	11	AGLU	–
13	APPA	–	14	CDEX	–	15	AspA	–	16	BGAR	–	17	AMAN	+	19	PHOS	+
20	LeuA	–	23	ProA	–	24	BGURr	–	25	AGAL	–	26	PyrA	–	27	BGUR	–
28	AlaA	–	29	TyrA	–	30	dSOR	–	31	URE	–	32	POLYB	–	37	dGAL	–
38	dRIB	–	39	iLATk	–	42	LAC	–	44	NAG	–	45	dMAL	–	46	BACI	+
47	NOVO	–	50	NC6.5	–	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	–
57	dRAF	–	58	O129R	–	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	–
64	OPTO	+															

fleurettii was also assigned to the new genus of *Staphylococcus stepanovicii* and *Staphylococcus vitilinus* [27].

M. sciuri and *Mammaliicoccus lentus* live on the skin and mucous membranes of many domestic, farm, and wild animals, as well as in foods of animal origin [28–33]. They occur in soil, sand, water, and marsh grass [34]. Adkins *et al.* found *M. sciuri* in milk and bedding on free-stall dairy farms [33]. *M. sciuri* were isolated from sick goats, cows with mastitis, dogs with dermatitis, cats with sepsis, and minks with urinary infections [35–37]. *M. sciuri* were also isolated from healthy and diseased humans [29].

CONCLUSION

The microbiological safety of raw bear fat complied with the requirements for pork fat listed in Technical Regulation of the Customs Union TR CU 021/2011 On Food Safety. The VITEK 2 Compact Bio-Mérieux automated system detected *Mammaliicoccus lentus* and *Mammaliicoccus sciuri* in the samples grown on the QMAFAnM test medium. These microorganisms were reported as pathogens in animals.

The microbial profiling of bear adipose tissues indicated that foods and biologically active substances based on raw bear fat require safety tests, and their quality indicators have to be introduced into the existing regulatory documents.

CONTRIBUTION

E.A. Vechtomova and I.V. Dolgolyuk supervised the research, analyzed the data, interpreted the results, and wrote the article. M.M. Orlova and A.V. Zaushintsena reviewed scientific publications and performed the research.

CONFLICT OF INTEREST

The authors declared no conflict of interests regarding the publication of this article.

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