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YEAST OCCURRENCE IN HERRING PRODUCTS AND PROCESSING ENVIRONMENT AND THEIR BIOCHEMICAL PECULIARITIES

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The aim of this work was to ascertain yeast contamination of raw herring, the processed products and manufacturing environment, and to evaluate biochemical peculiarities of dominant species of the isolated yeasts, which could have influenced the product quality. A total of 36 yeast strains were isolated from herring products, raw material and their production environment during the manufacturing process. These yeasts were identified as 8 species belonging to *Saccharomyces*, *Candida*, *Pichia*, *Rhodotorula*, *Debaryomyces*, *Yarrowia* yeast genera. *Saccharomyces cerevisiae* and *Candida glabrata* yeasts dominated in herring products. *Candida blankii*, *Debaryomyces hansenii* var. *hansenii*, *Yarrowia lipolytica*, and *C. glaebosa* yeasts were isolated from raw herring. The studies on contamination of the production room air, equipment, dishes, packing and other surfaces showed that the majority of samples were infected by *Saccharomyces cerevisiae* and *Candida glabrata* yeasts that were also found in the processed herring production. Research on enzymatic activity showed that yeasts *Pichia membranifaciens* and *Debaryomyces hansenii* var. *hansenii* exhibited proteolytic activity, while *Yarrowia lipolytica* – lipolytic activity. Based on the preliminary study results of assimilation and fermentation of carbon sources as well as proteolytic and lipolytic activities, this could have had the negative influence upon the product quality.

INTRODUCTION

Microorganisms present in food products cause biochemical processes, which often determine product quality, and change their nutritious properties and flavor. Some saprophytic microorganisms can be the reason of food product deterioration. Due to reproduction of pathogenic microorganisms in raw material or food products, the food can become unsuitable for consumption. Therefore, permanent microbiological control of raw material and products is required. It is essential to control microbiological processes in food products, to ascertain factors promoting the cause of these processes and to determine microorganisms able to change the product quality and pose health risk to consumers.

Fish and its products can spoil especially rapidly. Psychrophilic microorganisms can grow in raw fish when the raw material is stored at 0–4°C temperature. Live fish can also be infected with microorganisms. Microorganisms are mostly found in mucous membrane between exuviae, on branchiae surface and in the alimentary tract. From 10^2 to 10^7 colony forming units (cfu) of microorganisms per 1 cm^2 of fish exuviae and branchiae surfaces can be recorded. In the alimentary tract, there were 10^4–10^8 cfu/g of microorganisms [Svičiulis *et al*., 1999]. Amounts of microbial propagules in a fish organism depend on clearness of a water basin, seasons and many other environmental factors. Usually higher amounts of microorganisms are detected in fish caught in March–April and July–November. Many authors indicate that the main agents of microbiological taints of fish and their products are bacteria of the genera *Listeria* and *Pseudomonas* and representatives of the *Enterobacteriaceae* family [Balasubramanian, 1992; McMeekin *et al*., 1992; Gram & Huss, 1996; Gram & Dalgaard, 2002; Bledsoe, 2003; Farben & Peterkin, 2000; Papadopoulou *et al*., 2007; Khan *et al*., 2007]. Yeasts of the *Candida, Rhodotorula* and *Saccharomyces* genera of various species are found, too. The presence of various species of fungi belonging to genera *Aspergillus*, *Eurotium, Penicillium, Fusarium, Cladosporium* was also revealed on the samples of smoke-dried or dried salted fish in various countries. The amount of fungi was 3.0×10^2 – 4.8×10^4 cfu/g [Wheeler *et al*., 1996; Spencer & Spencer, 1997; Jonsyn & Lahai, 2006; Adebayo-Tayo *et al*., 2008].

In the course of processing microorganisms from freshly caught fish easily spread, invade mechanically damaged fish tissues and deteriorate them causing rotting and other spoilage processes. In order to prevent rapid spoilage of fish, various technologies are applied: quick freezing, gradual freezing and other methods. The culinary properties of fish change the least when fresh fish and processed raw material are frozen quickly. In such cases microorganisms on fish are being killed or go into anabiosis – a resting state – and mass degradation processes are subdued, as well as spoilage processes are slowed down or even completely stopped. Nevertheless, as environmental conditions change, *e.g.* the temperature slightly increases, the degradation processes in raw material can start again because fluids, leaked out of fish due to autolysis of fish cells, are very suitable medium for the reproduc-

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tion and growth of microorganisms. Therefore, storage time of defrost fish should be reduced to the technically possible minimum [Levin & Witkowski, 1991; Hansen *et al*., 1996; Narkevičius, 2004].

The aim of this work was to ascertain yeast contamination of raw herring, the processed products and manufacturing environment, and to evaluate biochemical peculiarities of dominant species of the isolated yeasts, which could have influenced the product quality.

MATERIALS AND METHODS

The samples were collected at the fish processing enterprise (Lithuania) where production taints of microbiological origin arose. The expiry dates having been unexpired, the taste, odor and appearance taints of producible fish products were observed.

The samples for microbiological analysis were taken from one processing technological process starting from raw fish from Iceland and Norway and ending with ready products. The products studied were: herring fillets, herring fillets in oil, smoke-flavored herring fillets, lightly salted herring fillets and lightly salted herring fillets in oil. Furthermore the air of production rooms, various surfaces and worker overalls were analysed. The samples were collected at a production time. Altogether 25 samples were studied.

Herring raw material and products samples were taken by cutting off 3 pieces of 100 g from different fish sections and put into sterile containers. The fish pieces (10 g) were chopped, stirred for 10 min and dilutions were made. Next, 1 mL of outwashs was spread on solid medium for yeast isolation. Yeast amount is presented as colony forming units per gramme (cfu/g).

The sampler of microorganisms ("Krotov 818", Russia) was used for collection of airborne yeasts. Particles together with the air were sucked through a tiny slit and directed at the surface of a solid medium in a Petri dish where they adhered. While collecting, a Petri dish with the medium rotated at 60–100 rpm velocity. This ensures equal distribution of particles onto the medium surface. The flow rate was 25 dm^3/min, and sampling time – 2 min. Every sample was taken in 3 replications [Nevalainen *et al*., 1993]. Yeast amount is presented as colony forming units per cubic meter (cfu/m^3).

Herring brine samples were taken into sterile vessels. For inoculation the water suspensions of the investigated microorganisms were prepared. Herring brine samples were spread on media in 1-mL doses. Yeast amount is presented as colony forming units per 1 mL (cfu/mL).

Swabs from equipment, work clothes and other surfaces were taken from 100 cm^2 workspaces with sterile cotton swabs and put into sterile tubes. The swabs were washed in 10 mL of sterile physiological solution and diluted if necessary. Next, 1 mL of outwashs was spread on solid medium for yeast isolation. Yeast amount is presented as colony forming units per 1 cm^2 (cfu/cm^2).

Yeasts were isolated onto Yeast extract-dextrose-chloramphenicol-agar of the following composition (g/L): yeast extract – 5.0; dextrose ($C_6H_{12}O_6$) – 20.0; chloramphenicol ($C_{11}H_{12}C_{l2}N_2O_5$) – 0.1; agar – 20.0; and water – 1 L. Yeast were grown for 4–5 days in an incubator at +25±1°C temperature. Experiments were run in 3 replications. Yeast counts were assessed according to the international ISO 7954 [1987] standard.

Identification of yeasts was performed according to macro- and micromorphological properties of the isolated cultures, their biochemical and physiological peculiarities following the manuals for yeast identification [Kreger-van Rij, 1984; Kurtzman & Fell, 1998].

Physiological characteristics were analysed by performing carbohydrate fermentation and assimilation tests. Fermentation of carbohydrates was tested in Durham tubes with D-glucose, D-galactose, maltose, sucrose, lactose, raffinose and trehalose. Tubes were incubated at +25±2°C and observed every few days during four weeks.

The carbohydrate assimilation was tested using Yeast Nitrogen Base (Difco, USA) with 33 carbon compounds: hexoses (D-glucose, D-galactose, L-sorbose), pentoses (D-xylose, L-rhamnose, L-arabinose, D-arabinose, D-ribose), disaccharides (sucrose, maltose, trehalose, melibiose, lactose, cellobiose), trisaccharides (raffinose, melezitose), polysaccharides (inulin, soluble starch), glycosides (α-Methyl-D-glucoside, arbutin, salicin), alcohols (ethanol, glycerol, erythritol, ribitol, galactitol, D-mannitol, D-glucitol, m-inositol), organic acids (succinate, citrate, DL-lactate, D-gluconate).

The assimilation of nitrogen compounds was performed on Yeast Carbon Base (Difco, USA) with potassium nitrate and potassium nitrite. Yeast growth was examined after 5 days.

To detect lipolytic activity of the yeasts, following media were used (%): tributyrate – 1; KH_2PO_4 – 0.25; NaH_2PO_4 – 0.25; $(NH_4)_2\ SO_4$ – 0.1; $MgSO_4$ x $7H_2O$ – 0.05; yeast extract – 0.05; agar – 1.5; Victoria Blue – 0.01; (pH 7-8). Yeast lipolytic activity was measured applying the method of Watanabe *et al*. [1977].

In order to detect yeast proteolytic activity, 2% agar with 1.5% of sterile milk was used. A yeast culture was inoculated using the streak method. Inoculates were cultivated for 5 days at +25±1°C temperature. Visually clear zones formed around a streak were treated as a positive result.

Yeast detection frequency (A) was calculated according to formula: A= b/c × 100%, where b – number of analyses, when yeast species was detected; c – total amount of analyses.

The obtained data were processed using Microsoft Excel XP (mean, standard deviation).

RESULTS AND DISCUSSION

The investigation showed (Table 1) that the number of yeasts in the herring products made up from 1.3 to 12×10^3 cfu/g. The highest number of yeasts was isolated from smoke-flavoured herring fillets (12.0×10^3 cfu/g). Research of yeast species composition showed that *Saccharomyces cerevisiae* Meyen ex E. C. Hansen and *Candida glabrata* (Anderson) S. A. Meyer & Yarrow (Yarrow and Meyer) species dominated in the tested herring products and their detection frequency (A) reached even 80%. Yeasts of the *Debaryomyces hansenii* (Zopf) Lodder & Kreger-van Rij var. *hansenii, Yarrowia lipolytica* (Wickerham, Kurtzman & Herman) van der Walt & von

TABLE 1. Cell number and diversity of yeast species in herring products and raw material.

Isolation source	Cell number $\bar{x}\pm SD$ (cfu/g $\times 10^3$)	Isolated yeast species
Herring products		
Heavily salted herring fillets	0	Not detected
Lightly salted herring fillets	1.3±0.1	*Saccharomyces cerevisiae, Candida glabrata, Pichia membranifaciens*
Herring fillets in oil	2.1±0.2	*Candida glabrata, Saccharomyces cerevisiae*
Smoke-flavoured herring fillets	12.0±0.6	*Saccharomyces cerevisiae, Candida glabrata, Rhodotorula mulcilaginosa*
Lightly salted herring fillets in oil	8.5±0.5	*Debaryomyces hansenii* var. *hansenii, Yarrowia lipolytica, Saccharomyces cerevisiae, Candida glabrata*
Raw material		
Herring fillets brought in summer from Norway	0.2±0.02	*Candida blankii*
Herring fillets brought in autumn from Norway	0.1±0.01	*Debaryomyces hansenii* var. *hansenii*, *Yarrowia lipolytica*
Herring fillets brought in summer from Island	0.2±0.02	*Candida glaebosa*

Arx, *Pichia membranifaciens* (E. C. Hansen) E. C. Hansen and *Rhodotorula mulcilaginosa* (Jörgensen) F. C. Harrison species were rarely isolated (A=20%), from these products.

Aiming to determine the reasons of the product contamination with yeasts, raw material and brine were investigated (Table 1). The raw material was also contaminated with yeasts. The yeast species composition in different raw material batches varied. *Candida blankii* H. R. Buckley & van Uden yeasts dominated in samples of raw material brought from Norway in the summer, while *Debaryomyces hansenii* var. *hansenii* and *Yarrowia lipolytica* prevailed on the same raw material delivered in the autumn. *Candida glaebosa* Komagata & Nakase yeasts dominated in raw material from Island. It was estimated that though the detection frequency of *Debaryomyces hansenii* var. *hansenii* and *Yarrowia lipolytica* species yeasts was 20% in herring raw material, they were detected in ready products. Yeasts amount was considerably lower in herring raw material than in ready products and reached from 0.1 to 0.2×10^3 cfu/g.

The research results showed that on the surface of herring brine *Candida glabrata* yeasts dominated. This species of yeasts was not isolated from herring raw material but in final production its detection frequency reached even 80%. A presumption could be made, therefore, that yeasts could get from production environment air or the mechanisms and other surfaces present in the manufacturing premises.

There is very little data about yeast incidence in herring raw material and products. Our results coincide with the results of other authors showing that raw fish and processed fish products can be contaminated with yeasts of different genera. Yeast species frequently isolated from fish and shellfish reflect species prevalent in water from which they are taken. Commonly the red-pigmented yeasts (*Rhodotorula glutinis, Rh. mulcilaginosa*) dominate as well as other basidiomycetous yeasts (*Cryptococcus albidus, Trichosporon cutaneum*) and ascomycetous yeasts (*Candida zeylanoides, C. tropicalis, Yarowia lipolytica*) [Kobatake *et al*., 2008; Papadopoulou *et al*., 2007]. Das *et al*. [2007] showed that the count of yeasts in raw fish depended on fish species and varied between 1.0×10^3 cfu/g in Batashi (*Clupisoma atherinoides*) and 7.0×10^1 cfu/g in Rui (*Labeo rohita*).

The investigation revealed that the number of yeasts in the indoor air of the manufacturing premises was low; in the range from 0 to 1.3×10^2 cfu/m^3 (Table 2). *Candida glabrata* and *Saccharomyces cerevisiae* species yeasts dominated in the air of the manufacturing premises. The detection frequency of these yeasts was 57.1% and 42.8%, respectively. *Debaryomyces hansenii* var. *hansenii* and *Rhodotorula mulcilaginosa* species yeasts were isolated rarely from the air of manufacturing premises and their detection frequency was 14.3%. Yeasts were not detected in both control (the outdoor air) and the ventilation air.

The results of yeast contamination on equipment in the manufacturing premises, tools, working clothes and other surfaces showed (Table 3) that majority of the samples were contaminated with yeasts *Saccharomyces cerevisiae* and *Candida glabrata* that were also found during other stages of processing and in the ready products. The detection frequency of *Saccharomyces cerevisiae* species yeasts reached even 85.7% and that of *Candida glabrata* – 57.1%. Yeasts amount on the equipment and other surfaces reached from 0 to 50.0 cfu/cm^2. The highest contamination with yeast was detected on the used package – 50.0 cfu/cm^2. High numbers of yeasts (20.0 cfu/cm^2) were found in samples from working clothes.

The analysis of species composition of yeasts showed that *Saccharomyces cerevisiae* and *Candida glabrata* prevailed in samples taken from various surfaces. Yeasts of these spe-

TABLE 2. Cell number and species composition of yeasts isolated from the indoor air of the manufacturing premises.

Sampling site	Cell number $\bar{x}\pm SD$ (cfu/m$^3\times10^2$)	Isolated yeast species
Preparation room	1.3±0.2	*Debaryomyces hansenii* var. *hansenii*
Raw herring processing room	0.2±0.1	*Candida glabrata*
Ripening chamber No 1	0.2±0.1	*Saccharomyces cerevisiae*
Ripening chamber No 2	0.8±0.2	*Candida glabrata*
Packing room	1.3±0.1	*Saccharomyces cerevisiae, Candida glabrata, Rhodotorula mulcilaginosa*
Container washing room	0.6±0.09	*Saccharomyces cerevisiae, Candida glabrata*
Ventilation air	0	Not detected
Outdoor air (control)	0	Not detected

TABLE 3. Cell number and species composition of yeasts isolated from equipment and other devices.

Sampling site	Cell number $\bar{x} \pm SD$ (cfu/cm^2)	Isolated yeast species
Raw herring defrosting chamber	20.0±2.0	*Saccharomyces cerevisiae, Candida glabrata*
A bath after disinfections	10.0±1.0	*Saccharomyces cerevisiae*
Washing-up brush	10.0±1.0	*Saccharomyces cerevisiae*
Floor broom	9.0±0.5	*Saccharomyces cerevisiae, Rhodotorula mulcilaginosa, Candida glabrata*
Laminate before and after heating	0	Not detected
Working clothes	20.0±2.0	*Saccharomyces cerevisiae, Candida glabrata*
Containers after use	50.0±3.0	*Saccharomyces cerevisiae, Candida glabrata*

cies were widespread in both the indoor air of manufacturing premises and products. On the ground of preliminary results of the conducted research it is possible to state that the manufacturing environment influenced more the incidences of yeasts in herring products than raw material from which they were produced.

Despite that food processing enterprises usually have the hazard analysis and critical control point system, nevertheless, such measures not always ensures the complete safety of food products in respect of microbial contamination. Microorganisms can occur on raw material from the outside – water, air, equipment, workers, and from these sources eventually microorganisms can get on food products and cause spoilage [Willinghan *et al*., 1996; Langsrud *et al*., 2003].

Biochemical peculiarities of yeasts isolated from the herring products and during their processing did not show a wide spectrum of fermentation of carbon sources. It was assessed that yeasts *Saccharomyces cerevisiae* and *Pichia membranifaciens* fermented glucose intensively, while glucose fermentation by *Candida glabrata, Debaryomyces hansenii* var. *hansenii* and *Candida blankii* yeasts was weak. Only *Saccharomyces cerevisiae* fermented galactose, sucrose and maltose. It should be mentioned that the tested yeasts did not fermented lactose. Yeasts *Saccharomyces cerevisiae* exhibited the widest fermentation spectrum of carbon sources. Meanwhile, *Yarrowia lipolytica, Candida glaebosa* and *Rhodotorula mulcilaginosa* did not ferment the tested carbon sources.

Investigation of carbon source assimilation showed that if yeasts ferment a certain carbon source, they are also able to assimilate it. Many of the tested yeasts intensively assimilated galactose, sucrose, maltose, citric and succinic acids. *Debaryomyces hansenii* var. *hansenii* and *Candida blankii* were able to assimilate starch, whereas only *Candida blankii* assimilated inositol. It was noticed that the widest spectrum of carbon source assimilation was characteristic of *Candida blankii* and *Debaryomyces hansenii* var. *hansenii* yeasts.

Investigations on the assimilation of nitrogen sources revealed that the tested yeasts did not assimilate nitrates.

TABLE 4. Frequency of strains within yeast species isolated from raw herring and herring products able to produce extracellular proteolytic (ExP) and lipolytic (ExL) enzymes.

Yeast species	ExP	ExL
Saccharomyces cerevisiae	0/4[a]	0/4
Pichia membranifaciens	1/1	0/1
Candida glabrata	0/5	5/5
Debaryomyces hansenii var. hansenii	2/2	0/2
Yarrowia lipolytica	0/2	2/2
Candida blankii	0/1	0/1
Candida glaebosa	0/1	0/1
Rhodotorula mulcilaginosa	0/1	0/1

[a] number of positive reaction of strains over the total number of strains examined.

Numerous works of researches from different countries indicate that yeast show a wide spectrum of assimilative and fermentative abilities of carbon sources. Yeasts can grow on fish products under conditions of high NaCl concentration and various pH [Gram & Huss, 1996; Betts *et al*., 1999; Paludan-Muller *et al*., 2002].

Research on enzymatic activity showed (Table 4) that yeasts *Pichia membranifaciens* and *Debaryomyces hansenii* var. *hansenii* exhibited proteolytic activity, *Candida glabrata* and *Yarrowia lipolytica* – lipolytic activity. The obtained results confirm the opinion that *Yarowia lipolytica* possesses high lipolytic and proteolytic activities. Proteolytic psychrotrophic yeasts are widely distributed in raw seafood and are able to grow under low temperature (from 0 to 5°C), therefore, they can exert a negative influence on the quality of fish products [Kobatake *et al*., 2008].

The preliminary results of research showed that yeasts, alike other microorganisms, can infect the herring products and cause the taints of microbiological origin. On purpose to estimate the reasons of yeast infection it is necessary to ascertain yeast incidence in raw material and on various technological process stages in which the product or its components interact mostly with manufacturing environment.

CONCLUSIONS

A total of 36 yeast strains were isolated from herring products, raw material and their production environment during the manufacturing process. These yeasts were identified as 8 species belonging to *Saccharomyces*, *Candida*, *Pichia*, *Rhodotorula*, *Debaryomyces* and *Yarrowia* yeast genera.

It was assessed that *Saccharomyces cerevisiae* and *Candida glabrata* yeasts prevailed in herring products. *Candida blankii*, *Debaryomyces hansenii* var. *hansenii*, *Yarrowia lipolytica*, and *C. glaebosa* yeasts were isolated from raw herring. Investigation of yeast contamination of the air in the premises as well as containers and other surfaces showed that most samples were contaminated with *Saccharomyces cerevisiae* and *Candida glabrata* that were also found in the processed products. Consequently it is possible to state that the infection of manufacturing environment influenced more the herring product infection by

yeasts than raw material from which they were produced.

Biochemical peculiarities of the isolated yeasts revealed that they had the wide spectrum of assimilation and fermentation of carbon sources as well as proteolytic and lipolytic activities, and this could have had the negative influence on product quality.

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