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KERATINIZATION OF THE LINGUAL EPITHELIUM OF THE RACCOON DOG (*NYCTEREUTES PROCYONOIDES*)

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Abstract. Under the light microscope, the epithelium of filiform papillae consists of an anterior and a posterior side. On the anterior side of filiform papillae the epithelium exhibited characteristics of soft keratinization, while on the posterior side – there were characteristics of hard keratinization. Conical papillae were covered by nonkeratinized epithelium. The keratinized layer was observed only on their ends. The filiform and conical papillae were separated by the interpapillary epithelium, which does not keratinize. The epithelium of the filiform and conical papillae were composed of three layers, i.e. basal, intermediate, and superficial layer.

Key words: tongue, epithelium, keratinization

INTRODUCTION

Epithelium of filiform papillae of mammals is composed of regularly ordered columns of cells: the anterior cell columns and the posterior cell columns. The interpapillary epithelium is located in the area among filiform papillae [Cane and Spearman 1969, Farbman 1970]. Hume and Potten [1976] suggested more complex composition for the epithelium of filiform papillae.

Many studies of the mechanical papillae of the tongue have shown that keratinization patterns in the anterior side differ from that of the posterior side [Farbman 1970, Singh et al. 1980, Iwasaki et al. 1999a].

In this study the fine structure and arrangement of the filiform and conical papillae of the raccoon dog was examined by light microscop (LM).

The main purpose of the present study was to increase our knowledge of keratinization of filiform and conical papillae epithelium.

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MATERIAL AND METHODS

Examinations were conducted on 11 tongues of adult raccoon dogs, *Nyctereutes procyonoides*, of both sexes. The animals came from fur animal farm.

Light microscopy samples were fixed in 10% neutral-buffered formalin and Bouin's solution, dehydrated in a series of alcohols with increasing concentrations (50–96%), embedded in paraplast and sliced using a microtome (Leica RM 2055) into sections with thickness ranging from 3 to 5 μm. Tissue samples were sliced in three planes, i.e. sagittal, transverse and dorsal. The following staining methods were applied in the study: Masson-Goldner and hematoxylin-eosin (HE). Microscopic observations were conducted using a Jenaval (Carl Zeiss Jena) microscope.

RESULTS

Two types of mechanical papillae were found on the tongue in the raccoon dog, i.e. filiform papillae and conical papillae. The most numerous filiform papillae were distributed on the dorsum of the apex and the body as well as the margins of the tongue. Filiform papillae were arranged densely. Between these papillae were distributed fungiform papillae.

Conical papillae were located behind vallate papillae on the body and root of the tongue. Their density decreases caudally.

Filiform papillae were formed by the basal part, on which several processes were found. Their projections were extended towards the pharynx. The number of processes ranges from 11 to 14 in each filiform papilla. The longest process was located in the posterior part of the papilla (Figs. 1, 3). In sagittal section, the filiform papillae exhibited a convex anterior surface and a concave posterior surface. The connective tissue papillae were penetrated into the center of filiform papillae (Fig. 1).

Filiform papillae were covered by keratinized stratified squamous epithelium. The epithelium of the filiform papillae consists of an anterior and a posterior epithelial cell column. Filiform papillae were covered on the anterior side by epithelium, which showed signs of soft keratinization. The epithelial cells from the basal layer were cuboidal. The intermediary cell layers had a stratum granulosum with keratohialin granules. The superficial cell layers contained remnants of nuclei (Fig. 2).

On the posterior side of filiform papillae, epithelium with signs of hard keratinization was observed. The basal layer was composed of cuboidal cells. The epithelium on the posterior side of filiform papillae did not contain keratohialin granules. The superficial layers were formed the stratum corneum (Fig. 2).

Conical papillae have sharply ended apexes extending towards the pharynx. Apexes of conical papillae were covered by keratinized epithelium with sings of hard keratinization. The other parts of conical papillae were covered by nonkeratinized stratified squamous epithelium (Figs. 3 and 4).

Between filiform papillae observed interpapillary epithelium. The epithelium of the interpapillary area was not keratinized (Figs. 1, 3).

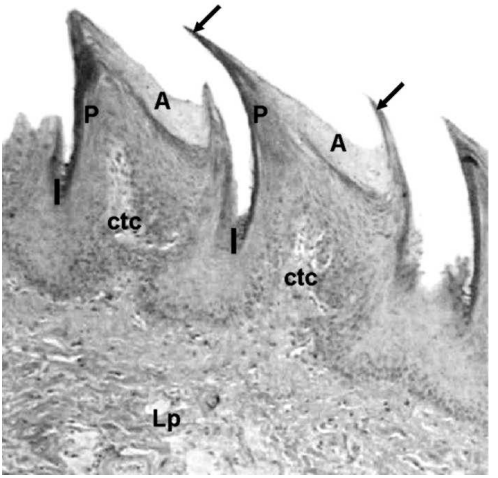

Fig. 1. A sagittal section through the filiform papillae: A – the epithelium on the anterior side of the papilla, P – the epithelium of the posterior side of the papilla, arrows – processes of the papilla, I – the interpapillary epithelium, ctc – connective tissue core of the papilla, Lp – lamina propria of the mucosae (LM, x 6.3)

Ryc. 1. Przekrój strzałkowy przez brodawki nitkowate: A – nabłonek na przedniej stronie brodawki, P – nabłonek na tylnej stronie brodawki, strzałki – wyrostki brodawki, I – nabłonek międzybrodawkowy, ctc – zrąb łącznotkankowy brodawki, Lp – blaszka właściwa błony śluzowej (LM, x 6.3)

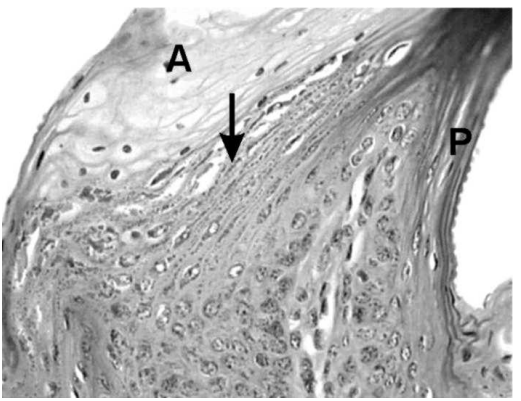

Fig. 2. A sagittal section through the filiform palilla: A – the epithelium on the anterior side of the papilla, P – the epithelium of the posterior side of the papillla, arrow – intermediate layer with keratohialin granules (LM, 2.5)

Ryc. 2. Przekrój strzałkowy przez brodawkę nitkowatą: A – nabłonek na przedniej stronie brodawki, P – nabłonek na tylnej stronie brodawki, strzałka – warstwa pośrednia z ziarnami keratohialiny (LM, x 25)

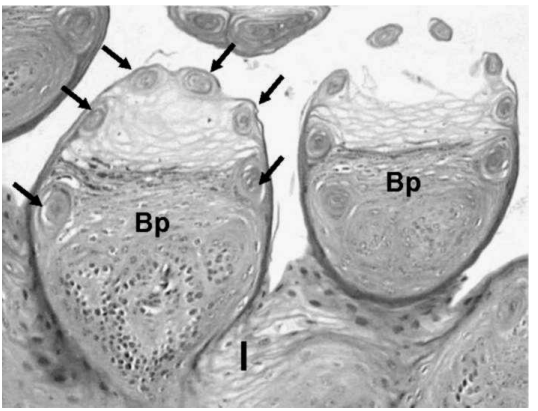

Fig. 3. A dorsal section through the filiform papillae: Bp – basal part of the papilla, arrows indicate processes of the papilla, I – the interpapillary epithelium (LM, x 6.3)
Ryc. 3. Przekrój grzbietowy przez brodawki nitkowate: Bp – część podstawna brodawki, strzałki wskazują wyrostki brodawki, I – nabłonek międzybrodawkowy (LM x 6.3)

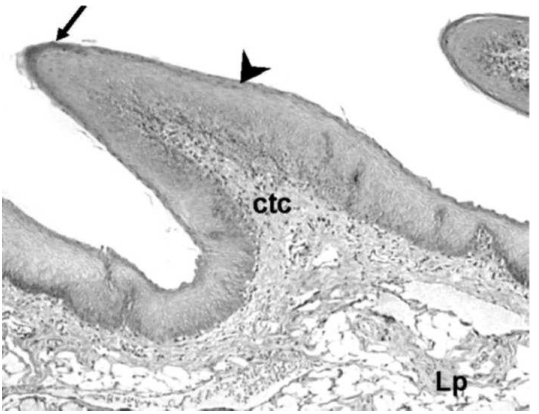

Fig. 4. A sagittal section through the conical papilla: arrow – keratinized epithelium, arrowhead – nonkeratinized epithelium, ctc – connective tissue core of the papilla, Lp – lamina propria of the mucosae (LM, 2.6)
Ryc. 4. Przekrój strzałkowy przez brodawkę stożkowatą: strzałka – nabłonek zrogowaciały, grot – nabłonek niezrogowaciały, ctc – zrąb łącznotkankowy brodawki, Lp – blaszka właściwa błony śluzowej (LM, 2.6)

DISCUSSION

The epithelium of the oral cavity is stratified squamous epithelium. It is divided into orthokeratinized, parakeratinized and nonkeratinized epithelium. In places particularly threatened with damage, i.e. on gingivas [McHugh 1964, Philipsen et al. 1982] and the hard palate [Gibbins 1962, Hayward et al. 1973, Philipsen et al. 1982], orthokeratinized epithelium was observed. Parakeratinized epithelium is characterized by the presence of

pycnotic and condensed nuclei and other cellular organelles in the superficial layer [Stern 1980]. Parakeratinization differs from the orthokeratinization by a less developed or absenct granular layer and a keratinized layer in which cells in an incomplete keratinization stage are found [Ostrowski 1995]. Nonkeratinized epithelium of the oral cavity was observed on the soft palate, cheeks and the fundus of the oral cavity [Ostrowski 1995]. However, in the rat parakeratinization was shown in the buccal epithelium [McMillan 1979, Philipsen et al. 1982].

Orthokeratinization is divided into soft keratinization and hard keratinization. Soft keratinization was observed in the epithelium. It is characterized by the presence of a keratinized layer on the surface of the epithelium. Cells of this layer are dead and transformed into squamous corneous plates. The interior of cells is filled with keratin fibrils. It is the so-called soft keratin. It contains few sulfur atoms in its molecules. Grains of keratohyalin are found between keratin fibrils [Allen i Potten 1975, Matoltsy 1976, Ostrowski 1995]. Two types of keratohyalin granules are distinguished. One type is formed on the basis of tonofibrils, which are transformed and the other originates from ribosomes [Fukuyama and Epstein 1967, Suzuki et al. 1973]. Hard keratinization was shown in the nail plate [Forslind 1970, Hashimoto 1971] and the hair cortex [Ito and Hashimoto 1982]. This type of keratinization is characterized by the presence of densely packed keratin fibrils, the so-called hard keratin, which is not accompanied by keratohyalin granules [Ostrowski 1995].

Soft keratinization of the epithelium on the anterior side and hard keratinization on the posterior side of filiform papillae as in the raccoon dog were also shown in the rat [Farbman 1970, Baratz and Farbman 1975, Iwasaki et al. 1999b], guinea pig [Iwasaki and Kobayashi 1988], mouse [Iwasaki et al. 1999a], dog [Iwasaki and Miyata 1989] and in the cat [Iwasaki 1990].

Shimada et al [1990] showed that in the American alligator filiform papillae were covered both on the anterior and posterior parts by the epithelium, which exhibited characteristics of hard keratinization. The epithelium did not have a granulous layer. Filiform papillae found on the body of the tongue on both sides of the lingual torus in the rabbit exhibit the same structure. The epithelium did not have a granulous layer [Kulawik-unpublished data].

Interpapillary epithelium on the tongue does not keratinize in the raccoon dog. No keratohyalin granules were shown. Similar results of observations were presented in the musk shrew [Iwasaki and Miyata 1985]. For comparison in the guinea pig, rat and the mouse in the interpapillary epithelium numerous keratohylin granules were found [Farbman 1970, Iwasaki and Kobayashi 1988].

CONCLUSIONS

1. Filiform papillae on the tongue in the raccoon dog are separated by the interpapillary epithelium, which does not keratinize.
2. On the anterior side of filiform papillae there is epithelium with characteristics of soft keratinization, while on the posterior side these papillae are covered by epithelium with characteristics of hard keratinization.
3. Conical papillae are coverd by epithelium with characteristics of hard keratinization on their apex.

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ROGOWACENIE NABŁONKA JĘZYKOWEGO JENOTA (*NYCTEREUTES PROCYONOIDES*)

Streszczenie. W mikroskopie świetlnym nabłonek brodawek nitkowatych składał się z nabłonka przedniej i tylnej strony. Na przedniej stronie brodawek nitkowatych nabłonek wykazywał cechy miękkiego rogowacenia, natomiast na tylnej stronie – cechy twardego rogowacenia. Brodawki stożkowate pokrywał nabłonek nierogowaciejący. Warstwę zrogowaciałą obserwowano jedynie na ich końcach. Brodawki nitkowate i stożkowate oddzielone były przez nabłonek międzybrodawkowy, nierogowaciejący. Nabłonek brodawek nitkowatych i stożkowatych składał się z trzech warstw, tj. warstwy podstawnej, pośredniej i powierzchniowej.

Słowa kluczowe: język, nabłonek, rogowacenie

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ANALYSIS OF QUALITY PROPERTIES IN RAW MEAT AND FATS FROM FATTENERS BREEDING IN WIELKOPOLSKA

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Abstract. The objective of the presented research project was to analyze pork quality factors with regard to various breeders of slaughter animals. Experiments were carried out on swine half carcasses, collected from fatteners delivered to slaughter house by three breeders from Wielkopolska province. Two types of muscles: *m. longissimus lumborum* and *m. semimembranosus* and two types of adipose tissue: subcutaneous (back fat) and internal (leaf fat) were analyzed. In meat samples the content of the following chemical components was analyzed: dry matter, total protein, fat and collagen content as well as physicochemical properties, such as: colour, acidity and drip loss. Besides, the effects of heat treatment on the sensory value of the raw meat, colour, exudates and tenderness were studied. Assessment of raw fats was carried out on the basis of measurement chemical components, selected physicochemical properties and content of fatty acids.
Raw materials under investigation characterized by significant diversity between delivers of slaughter animals, however the quality of analyzed material was sufficient.

Key words: raw meat, raw fat, quality, fatteners

INTRODUCTION

Meat and meat products are basic components in diets of developed countries. The amount of meat consumed in different countries varies enormously with social, economic and political influences, religious beliefs and geographical differences [Millward 1999, Jimenez-Colomero et al. 2001]. In Europe pork is the most often consumed kind of meat. Polish consumer eats about 40 kg of this meat in a year [GUS 2006]. Consumption of pork is still increasing, so manufacturers of slaughter animals have to be subjected to the requirements of consumers. The growing demand for high quality of swine meat and products is stimulating development in raw meat production. Creation of quality traits of pork starts with animal breeding and ends with the culinary preparation of meat by consumer. It is know that majority of pork quality traits are related to

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fattener breed, line or genotype [Florkowski et al. 2006]. Carcasses from polish breeds have a good quality, but insufficient meatiness. In recent years was carried out many researches on increasing pigs meatiness using high-yielding breeds in cossbreeding. Unfortunately alongside with increase of quantity of meat tissue there were observed decrease of meat quality [Rybarczyk et al. 2002, Wojciechowski et al. 2000].

The aim of the study was to analyze some quality factors with regard to various breeders of slaughter animals.

MATERIAL AND METHODS

The study was conducted on swine half carcasses, collected from fatteners delivered to slaughter house by three breeders from Wielkopolska province. The fatteners under investigation with meatiness amounted to 57% were born from sows of Polish Landrace (P.L.) breed matted with a cross-breed boar (pietrain x duroc). The researches were carried out on two types of muscles: *musculus semimembranous* (*m. s.*) and *musculus longissimus lumborum* (*m. l. l.*) and two types of adipose tissue: subcutaneous (back fat) and internal (leaf fat).

In meat samples the content of the following chemical components was analyzed: dry matter (PN-ISO 662:2000); protein content according to Kjeldahl's method, using Kieltec^{TM} 2300; free fat (PN-ISO 1444:2000); connective tissue by determining hydroxyproline content (PN-ISO 3496:2000). The estimation of the physicochemical properties of muscles included a measurement of pH according to PN-ISO 2917:2001 and drip loss following the OECD method [Honikiel 1998]. In the samples of muscles after heat treatment in water medium (0.8 % solution of NaCl, 1:2 proportion meat to water, time of heat treatment: about 30 minutes until final temperature in the sample centre reached 75°C) was estimated cooking loss and tenderness as texture measurement using the Zwick/Reoll type Z010 machine. Sensory evaluation of cooked meat was carried out by 6 panelists according to 5-point Tilgner's scale of acceptance [Baryłko-Pikielna 1975]. The overall acceptability, colour, flavor, taste and tenderness were selected as the sensory descriptors.

Assessment of raw fats was carried out on the basis of measurement chemical components, selected physicochemical properties and content of fatty acids. In comminuted raw fats was analyzed dry matter content according to Polish standards of PN-ISO 662:2000 also free fat content (PN-ISO 1444:2000) and protein content by Kjeldahl's method. Also was estimated melting point (PN-EN ISO 6321:2004) and fatty numbers: saponification and iodine value (PN-EN ISO 3657:2004, PN-EN ISO 3961:2006). Fatty acid contents was identified by GC/MS analyses (PN-EN ISO 5508:1996) of their methyl esters produced according to (PN-EN ISO 5509).

The raw material under investigation were collected from three fatteners (both carcass sides) from each of the four series. The data were analyzed statistically (one-way analysis of variance), using STATISTICA 8.0 software. Significant differences between the mean values were determined using Duncan's test (α=0.05).

RESULTS AND DISCUSSION

The results obtained for basic chemical components of raw meat under investigation (Tab. 1) showed that the dry matter content was different in case of both types of muscles from all three breeders. The highest content of dry matter and the lowest content of water, was observed in samples of *m.l.l.* and *m.s.* in carcasses from breeder I (26,89 and 27,34%, respectively). The lowest quantity was recorded in muscles from carcasses delivered by breeder III (25,73 and 25,58%, respectively). A majority of authors confirmed that the dry matter content of *m.l.l.* should be within the range from 25 to 30%, although in *m.s.* from 25 to 32%. The dry matter content depends on animal species, sex, age, breed, raising conditions and feeding [Buczma 1999, Litwińczuk et al. 2002]. The results of these analyses were similar with the earlier observation of Rybarczyk et al. [2002] in crosses with pietrain, duroc and Polish Landrace breeds.

Table 1. Chemical components of raw meat
Tabela 1. Skład chemiczny surowca mięsnego

Parameter [%] Parametr	Type of muscles – Rodzaj mięśni					
	m. longissimus lumborum			*m. semimembranous*		
	I	II	III	I	II	III
Dry matter Sucha masa	26,89 ^{B}	26,73^{AB}	25,73^{A}	27,34^{B}	26,21^{AB}	25,58^{A}
Protein Białko	23,04^{A}	23,32^{A}	23,02^{A}	21,73^{A}	22,02^{B}	22,21^{B}
Fat Tłuszcz	2,91^{A}	3,09^{A}	2,96^{A}	3,85^{A}	3,80^{A}	3,73^{A}
Collagen Kolagen	0,100^{A}	0,109^{A}	0,109^{A}	0,171^{A}	0.,171^{A}	0,172^{A}

A, B – different letters in the same row for the same parameter means differences statistically significant ($\alpha \leq 0,05$)
A, B – różne litery w wierszu przy tym samym parametrze oznaczają znaczące statystycznie różni ceprzy $\alpha \leq 0,05$

The protein content in samples of *m.l.l.* was estimated on similar level for carcasses from all three breeders. Buczma [1999], Litwińczuk et. al. [2002] indicated slight less content of this parameter. The results recorded for *m.s.* samples were significantly different. From carcass delivered by breeder II and III received muscles of the highest protein content (22,02 and 22,21%, respectively), which was consistent with researches of mentioned before authors.

The measurements of collagen showed that breeder was not a factor differentiating the content of this protein in raw meat. Samples of *m.l.l.* contained on average 0,11% of collagen, in samples of m.s. indicated 0,17% this protein. The content of connective protein depends on intravital factors and in swine meat tissue should be within 0.1–0.2% [Purslow 2005, Sadowska 1992].

Also, no significant differences in intramuscular fat content were recorded in meat samples under investigation. Fattener's muscles *m.l.l* from all three breeders consisted 3% of fat, however *m.s.* about 0,8% more. Intramuscular fat content affects the technological, nutritional and sensory value of meat and meat products. The results of numerous authors indicate that its optimum content for those values should be about 1.5–3% in *m.l.l.* and 2.2–4% in *m.s.* [Litwińczuk et al. 2002, Tereszkiewicz et al. 1998, Wood et al. 1996, Daszkiewicz et al. 2005].

Table 2 shows the differences in values of acidity. Statistical analyze proved significant differences in value of this parameter for *m.l.l.* samples. Muscles of carcasses from breeder III had higher pH value than from breeder I (5,71 and 5,59, respectively). Acidity value for second type of muscles was similar for carcasses from all three breeders. The meat from fatteners under investigation characterized good quality [Wojciechowski et al. 2002], what had confirmation in high water holding capacity. The results of this analysis for both types of muscles were consistent with those reported by Barton-Gade et al. [1993], but there was higher variety of this parameter in samples of *m.l.l.*

Table 2. Physico-chemical traits of meat
Tabela 2. Właściwości fizykochemiczne surowca mięsnego

Parameter Parametr	Type of muscles – Rodzaj mięśni					
	m. longissimus lumborum			*m. semimembranous*		
	I	II	III	I	II	III
pH	$5,59^{A}$	$5,65^{B}$	$5,71^{C}$	$5,69^{A}$	$5,68^{A}$	$5,70^{A}$
WHC [%]	$2,78^{B}$	$2,48^{A}$	$2,83^{B}$	$2,64^{A}$	$2,38^{A}$	$2,65^{A}$
After heat treatment – Po obróbce termicznej						
Shear force [N/cm^2] Siła cięcia	$58,91^{A}$	$60,30^{AB}$	$60,56^{B}$	$51,26^{A}$	$50,36^{A}$	$51,72^{A}$
Drip loss [%] Wyciek	$25,57^{A}$	$27,92^{B}$	$29,19^{B}$	$28,52^{A}$	$28,31^{A}$	$28,40^{A}$

A, B – different letters in the same row for the same parameter means differences statistically significant ($\alpha \leq 0,05$)
A, B – różne litery w wierszu przy tym samym parametrze oznaczają znaczące statystycznie różnice przy $\alpha \leq 0,05$

Also in a case of second type of muscles were observed similar results after heat treatment. The higher shear force was noted in samples of *m.l.l.* than in *m.s.* Tenderness estimated by this parameter was lower in *m.l.l.* from carcass delivered by breeder I and the highest in muscles from breeder III. Those muscles characterized by lower value of cooking loss.

The results of sensory evaluation (Tab. 3) of muscles showed that overall acceptability, colour and taste were higher pointed for samples *m.l.l.* of breeder III. However in case of the second type of muscles the highest colour value was estimated in muscles of carcass delivered by breeder I and III, besides samples of m.s. collected from the last breeder were the least tasty. Flavor and tenderness of muscles under investigation were noted on similar level.

On technological utility of swine carcasses apart from content of raw meat have a strong impact on the quantity and quality of raw fat.

The content of dry matter (Tab. 4) estimated in raw fat was similar for all samples of subcutaneous fat on average level 91–92%. The content of this component was not much higher in samples of internal fat. The highest amount of dry matter was recorded in leaf fat of fatteners from breeder III, the least in raw fat from breeder II (93,61% and 92,21%, respectively).

Also in internal fat were observed significant differences in protein content compared to back fat. Samples of subcutaneous fat contained average 2,8% of protein. Results obtained in internal fat were lower (2,2% respectively) and the highest protein level was observed in back fat from breeder III.

Table 3. Results of sensory assessment of meat samples after heat treatment
Tabela 3. Wyniki oceny sensorycznej mięśni poddanych obróbce termicznej

Traits (pts) Cechy (pkt.)	Type of muscles – Rodzaj mięśni					
	m. longissimus lumborum			*m. semimembranous*		
	I	II	III	I	II	III
Overall acceptability: Ocena ogólna:	$3,84^{A}$	$4,02^{A}$	$4,25^{B}$	$3,96^{A}$	$3,73^{A}$	$3,78^{A}$
colour barwa	$3,76^{A}$	$3,80^{A}$	$4,12^{B}$	$3,93^{B}$	$3,63^{A}$	$4,01^{B}$
taste smak	$3,75^{A}$	$3,75^{A}$	$4,06^{B}$	$3,96^{B}$	$3,76^{AB}$	$3,63^{A}$
flavour zapach	$3,72^{A}$	$3,68^{A}$	$3,56^{A}$	$3,73^{A}$	$3,70^{A}$	$3,76^{A}$
tenderness kruchość	$4,0^{A}$	$3,79^{A}$	$3,80^{A}$	$3,53^{A}$	$3,75^{A}$	$3,73^{A}$

A, B – different letters in the same row for the same parameter means differences statistically significant ($\alpha \leq 0,05$)
A, B – różne litery w wierszu przy tym samym parametrze oznaczają znaczące statystycznie różnice przy $\alpha \leq 0,05$

Table 4. Chemical composition of fat
Tabela 4. Skład chemiczny surowca tłuszczowego

Parameter Parametr Group Grupa	Dry matter Sucha masa	Protein Białko	Fat Tłuszcz	H_2O
	[%]	[%]	[%]	[%]
back-fat – słonina				
I	$90,96^{A}$	$2,89^{A}$	$88,07^{A}$	9,04
II	$90,89^{A}$	$2,75^{A}$	$88,15^{A}$	9,11
III	$91,98^{A}$	$2,76^{A}$	$89,22^{A}$	8,02
leaf-fat – sadło				
I	$92,41^{AB}$	$2,12^{A}$	$90,29^{A}$	7,59
II	$92,21^{A}$	$1,96^{A}$	$90,24^{A}$	7,79
III	$93,61^{B}$	$2,51^{B}$	$91,10^{A}$	6,39

A, B – different letters in the same column for the same parameter means differences statistically significant ($\alpha \leq 0,05$)
A, B – różne litery w kolumnie przy tym samym parametrze oznaczają znaczące statystycznie różnice przy $\alpha \leq 0,05$

Statistical analyze showed that there was no significant differences in fat content in both raw fats delivered by all three breeders. Samples of back fat contained average 88,5% of fat and samples of second tape of raw fat included average 90,5% of fat.

The observed level of basic chemical composition confirm with reports of Grela and Winiarska [1999], only the protein content in leaf fat samples was minimally higher than in researches of mentioned authors. Higher amount of protein improves nutritive value of raw fats, but also can negative influence on utility value by increasing shelf life of raw materials.

Table 5 showed results for measurement melting point and fats numbers. Lower melting point was noted in samples of internal fat than in subcutaneous fat. The dependence of this parameter from fat localization in carcass was confirmed in results of researches of other authors [Rak , Morzyk, 2002, Wielbo et. al. 2004]. Recorded results for melting temperatures proved that their were significant deference in raw fat between breeders, what can be connected with feeding systems. The highest malting point was observed in back fat fatteners from breeder I (34,13°C), the lowest in the case of fatteners from breeder III (32,39°C). Average temperature in internal fat under investigation amounted 38,07°C, besides the highest was measured in leaf fat carcasses from breeder III.

Table 5. Melting temperature and fatty numbers
Tabela 5. Temperatura topnienia i liczby tłuszczowe

Parameter / Parametr Group / Grupa	Melting point Temperatura topnienia [°C]	LJ iodine value liczba jodowa [mg/g]	LZ saphonification value liczba zmydlenia [mg/g]
back-fat – słonina			
I	34,13 ^{B}	56,58 ^{A}	183,71 ^{A}
II	33,18 ^{AB}	64,95 ^{B}	193,36 ^{B}
III	32,39 ^{A}	68,60 ^{B}	189,18 ^{B}
leaf fat – sadło			
I	37,71 ^{A}	46,66 ^{A}	184,80 ^{A}
II	36,91 ^{A}	53,74 ^{B}	182,70 ^{A}
III	39,60 ^{B}	60,08 ^{C}	183,19 ^{A}

A, B – different letters in the same column for the same parameter means differences statistically significant ($\alpha \leq 0,05$)
A, B – różne litery w kolumnie przy tym samym parametrze oznaczają znaczące statystycznie różnice przy $\alpha \leq 0,05$

The estimation of iodine value was recorded on average level 64,3 in a case of back fat and 53,5 in leaf fat. The harder texture of internal fat results in lower iodine value. Both types of raw fat from fatteners of breeder I characterized by the lowest iodine value (56,58 and 46,66, respectively). The highest value of analyzed parameter was noted in back fat of carcasses delivered by breeder II and III and in case of leaf fat by breeder III.

Statistical analysis showed that there was no differences in saponification value for internal fats under investigation. The average value was estimated on level 182,70–184,80. In case of back fat this fat number was measured in range 183,71–193,36.

Fatty acid contents in raw fats was identified by GC/MS analyses. The highest content of polyunsaturated fatty acid was observed in back fat delivered by breeder I and II (59,76% and 59,8%, respectively). Subcutaneus fat of fatteners from breeder I also characterized by high amount of oleic acid (48,2%). In leaf fat the highest content of unsaturated fatty acids was recorded in experimental material from breeder II (53,85%), this fat was reached in linolenic acid at the same time.

Table 6. Fatty acids content of lipid fraction [%]
Tabela 6. Udział poszczególnych kwasów tłuszczowych

Fatty acids Kwasy tłuszczowe	Back-fat – Słonina			Leaf fat – Sadło		
	group I grupa I	group II grupa II	group III grupa III	group I grupa I	group II grupa II	group III grupa III
C 10:0	0,032 ^{A}	0,04 ^{C}	0,035 ^{B}	0,04 ^{B}	0,032 ^{A}	0,048 ^{C}
C 12:0	0,051 ^{C}	0,046 ^{B}	0,038 ^{A}	0,062 ^{A}	0,069 ^{B}	0,064 ^{A}
C 14:0	1,107 ^{C}	0,893 ^{B}	0,748 ^{A}	1,285 ^{B}	1,324 ^{C}	1,118 ^{A}
C 16:0	25,854 ^{C}	25,312 ^{B}	24,369 ^{A}	30,44 ^{B}	26,407 ^{A}	30,729 ^{C}
C 17:0	0,273 ^{B}	0,294 ^{C}	0,25 ^{A}	0,346 ^{C}	0,327 ^{B}	0,275 ^{A}
C 18:0	12,817 ^{C}	11,624 ^{A}	11,827 ^{B}	19,949 ^{C}	17,163 ^{B}	16,928 ^{A}
C 20:0	0,194 ^{B}	0,239 ^{C}	0,188 ^{A}	0,245 ^{B}	0,165 ^{A}	0,206 ^{AB}
Total SFA Ogółem	40,329 ^{C}	38,449 ^{B}	37,455 ^{A}	52,366 ^{C}	45,488 ^{A}	49,369 ^{B}
C 16:1	2,265 ^{C}	2,144 ^{A}	2,168 ^{B}	1,298 ^{A}	1,757 ^{B}	1,902 ^{B}
C 18:1	48,207 ^{C}	45,861 ^{B}	45,149 ^{A}	36,454 ^{A}	40,191 ^{B}	42,255 ^{C}
C 18:2	7,915 ^{A}	10,253 ^{B}	10,044 ^{B}	8,251 ^{B}	10,540 ^{C}	6,200 ^{A}
C 18:3	0,427 ^{A}	0,65 ^{B}	0,673 ^{C}	0,501 ^{C}	0,451 ^{B}	0,385 ^{A}
C 20:1	0,704 ^{B}	0,616 ^{A}	0,709 ^{B}	0,685 ^{C}	0,484 ^{B}	0,399 ^{A}
C 20:2	0,140 ^{A}	0,344 ^{B}	0,333 ^{B}	0,205 ^{A}	0,324 ^{B}	0,333 ^{B}
C 20:4	0,104 ^{A}	0,144 ^{B}	0,106 ^{A}	0,118 ^{A}	0,103 ^{A}	0,113 ^{A}
Total UFA Ogółem	59,763 ^{B}	59,803 ^{B}	59,391 ^{A}	47,512 ^{A}	53,850 ^{C}	51,587 ^{B}

A, B, C – different letters in the same row for the same parameter means differences statistically significant ($\alpha \leq 0,05$)
A, B, C – różne litery w wierszu przy tym samym parametrze oznaczają znaczące statystycznie różnice przy $\alpha \leq 0,05$

Results of the study showed that the highest content of C 18:2 and C 18:3 acids was estimated in back fat delivered by breeder II and III and in leaf fat delivered by breeder I and II. Internal fat was more saturated (45,5–52,4%) than back fat, with high amount of stearic and palmitic acids. High content of saturated acids influenced on firmed textured of internal fat. The technological and healthy quality is determine by proportion of un- and saturated fatty acids .The profile of fatty acids depends on feeding, age, weight of animal, sex, hormones, origin also breed and anatomic localization of fat [Ellis, McKeith 1999, Lo Fiego et al. 2005, Nürnberg 1998].

CONCLUSIONS

In recent years manufacturers of slaughter animals are subjected to increasing meatiness of native breeds. Crossing with high-meatiness breeds like: pietrain and duroc can improve quantity and quality of raw meat. Conducted researches proved the rightness of those type investigations on animal husbandry.

Raw meat and fats under investigation characterized by significant diversity between delivers of slaughter animals, what can be connected with rearing systems. However the quality of analyzed material was sufficient.

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OCENA JAKOŚCI SUROWCÓW MIĘSNYCH I TŁUSZCZOWYCH POCHODZĄCYCH OD TUCZNIKÓW Z REJONU WIELKOPOLSKI

Streszczenie. Przeprowadzone badania miały na celu ocenę wybranych wyróżników jakości surowca wieprzowego pochodzącego od różnych hodowców. Materiał doświadczalny stanowiły półtusze, dostarczone przez trzech producentów żywca z rejonu Wielkopolski. Oceniano dwa rodzaje mięśni: najdłuższy lędźwi i mięsień półbłoniasty oraz dwa rodzaje tkanki tłuszczowej: podskórną (słoninę) i wewnętrzną (sadło). Analiza tkanki mięsnej obejmowała podstawowy skład chemiczny, tj. zawartość suchej masy, białka, tłuszczu oraz kolagenu, a także oznaczono takie parametry fizykochemiczne jak: barwa, kwasowość, wyciek swobodny. Dodatkowo poddano analizie sensorycznej mięso po obróbce termicznej. W próbach mięsa ogrzewanego zmierzono również wyciek termiczny oraz barwę. Badania surowca tłuszczowego obejmowały podstawowy skład chemiczny, także wybrane właściwości fizykochemiczne oraz profil kwasów tłuszczowych. Badania pokazały, że dostarczany surowiec zarówno mięsny, jak i tłuszczowy cechował się dobrą jakością. Jednakże wykazano dużą zmienność badanych parametrów w zależności od producentów żywca.

Słowa kluczowe: surowiec mięsny, surowiec tłuszczowy, tuczniki

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DETERMINATION OF BIOELEMENTS CONTENT IN YEASTS USING SPECTROSCOPIC METHODS*

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Abstract. The metodology of a determination of some biometals content, i.e. chromium, cobalt, manganese, nickel and iron in *Saccharomyces cerevisiae* yeasts using UV-VIS spectrophotometer was worked out. An accuracy and precision of chosen analytical methods was determined. The comparison of results obtained with spectrometric ICP-AES method was conducted. Differences of mean values of results between those methods for analysed elements were +10.52, +8.11, -15.79, +1.33, +13.89, and +7.48 %, respectively. It was demonstrated that the UV-VIS spectrophotometric method may be successfully applied in analytical studies concerning a determination of microelements content in enriched yeasts biomass.

Key words: yeasts, UV-VIS, ICP-AES, chromium(III), cobalt(II), manganese(II), copper(II), nickel(II), iron(III)

1. INTRODUCTION

Yeasts, and especially baker's ones *Saccharomyces cerevisiae* are of a special significance in a supplementation of a diet. They are a source of valuable protein, vitamins (especially those of B group) and they have an ability to a stable bonding of bioelements scarce for human and animals in their cellular structures. Bonded cations are subject to bioaccumulation inside the cell or are fixed with an external cell wall (biosorption). The structure of cell wall contains phosphate, carboxyl, amine and hydrosulfide anions originating, inter alia, from mannoproteins that are capable to bond cations of metals.

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Hard ions are accumulated in cellular organelle to a higher degree, while soft ones are bonded by polarizable ligands of cell wall [Błażejczak et al. 2002, Pasternakiewicz 1997, Ułaszewski 1994].

The possibility of natural bonding of microelements by yeasts' cells is connected with a need of a precise monitoring of an amount of bioelements incorporated. That is especially important due to the fact, that an outpass of toxicity levels of chosen metals causes an inhibition of bodily functions of a cell and even its death. Some bioelements, i.e. chromium, cobalt, manganese, copper, nickel or iron are essential components of a diet and have a direct influence on a proper functioning of live organisms. They take part in numerous biochemical processes in organisms of human and animals, and most of them, as coenzymes, regulate the course of cellular metabolism.

When developing the technology of a production of biopreparations with microelements, there is a need to conduct numerous analytical assessments in order to determine an influence of various parameters on process indices of microelements bonding by biomass. An application of advanced instrumental analytical techniques like ICP-AES or ICP-MS is connected with high costs, since it requires, except the specialist equipment, adequately high competences of the staff. Thus, there is a necessity of a development of simple and cheap analytical methods, that would simplify procedures connected with an assessment of bioelements in biological material like yeasts or yeasts-derivatives products, enrichment in some biometals, not only during production processes but during experiments as well, that is also emphasized in numerous publications [Cava-Montesionos et al. 2005, Górecka et al. 1996, Leśniewska et al. 2002, Madejczyk and Baralkiewicz 2008, Baretto et al. 2007].

The aim of the study was a comparative assessment of the two spectroscopic methods (UV-VIS and ICP-AES) of a determination of the content of some elements like chromium, cobalt, manganese, copper, nickel and iron in *Saccharomyces cerevisiae* yeasts.

2. MATERIAL AND METHODS

Baker's yeasts of *Saccharomyces cerevisiae* species produced by Lesaffre Biocorporation Company (Wołczyn, Poland) were used in the present study.

2.1. Mineralization of samples

Mineralization of samples was conducted using a wet method according to a standard of Pharmaceutical Research-Production Company "Biochefa" – ZN-98/BIOCHEFA/-01, using a mineralizer of M-9 type (WSL Bytom, Poland) according to a PN-74C-04578/OC standard. The mineralization was done with concentrated nitric acids and perchloric acid as an oxidising agent (analytically pure, produced by „POCh" Ltd. Gliwice, Poland) mixed in a ration of 2:1. The special attention was paid not to allow for losses of analysed bioelements during the mineralization process, and not to introduce their additional amounts together with reagents used. Mineralization of samples containing bioelements, and so called blind samples were done at the same time.

2.2. Spectroscopic measurements

Measurements were conducted on UV-VIS "CECIL 3021" spectrophotometer of Instruments Company. Photometric accuracy of the spectrophotometer was on a level of ±0.005A. Comparative analysis were conducted on inductively coupled plasma atomic emission spectrometer ICP AES Liberty 220 of Varian Company.

2.3. Methodology of chosen bioelements content determination

2.3.1. Chromium (III)

Among of numerous methods of chromium determination, the one with the use of diphenylcarbazide, that is the most suitable for a determination of trace amounts of that bioelement in a mineralized sample, was chosen [Friese and Umland 1997, Marczenko and Balcerzak 1998, Minczewski et al. 1973]. The base of an assessment is a reaction of 1,5-diphenylcarbazide in an acid environment with Cr (VI) ions.

Cr^{3+} ions were oxidized to $Cr_2O_7^{2-}$ using $KMnO_4$ (about 0.02M; analytically pure produced by "POCh" Ltd. Gliwice) in elevated temperature in acid environment (solution acidity should correspond a concentration of 0.05-0.1 M H_2SO_4), or using $(NH_4)_2S_2O_8$ (analytically pure, produced by "POCh" Ltd. Gliwice) in the presence of silver ions. An excess of an oxidizing agent (MnO_4^-, MnO_2 ions) was reduced using 2.5% solution of sodium azide (analytically pure, produced by PPH "POCh" Ltd. Gliwice). Coloured reaction was conducted using 0.2% solution of 1,5-diphenylcarbazide (0.2g of a reagent was dissolved in 100 ml of acetone with an addition of 1 ml of H_2SO_4 (1+9); analytically pure, produced by "POCh" Ltd. Gliwice). An absorbance of created complex was measured with a wave length of λ = 545 nm, in the presence of distilled water as a reference. Standarization curve of an absorbance relation from Cr(III) concentration was described using an equation y = 0.3764x. Linearity range was demonstrated for concentrations on a level of 0.2–2.0 μg/ml (Fig. 1).

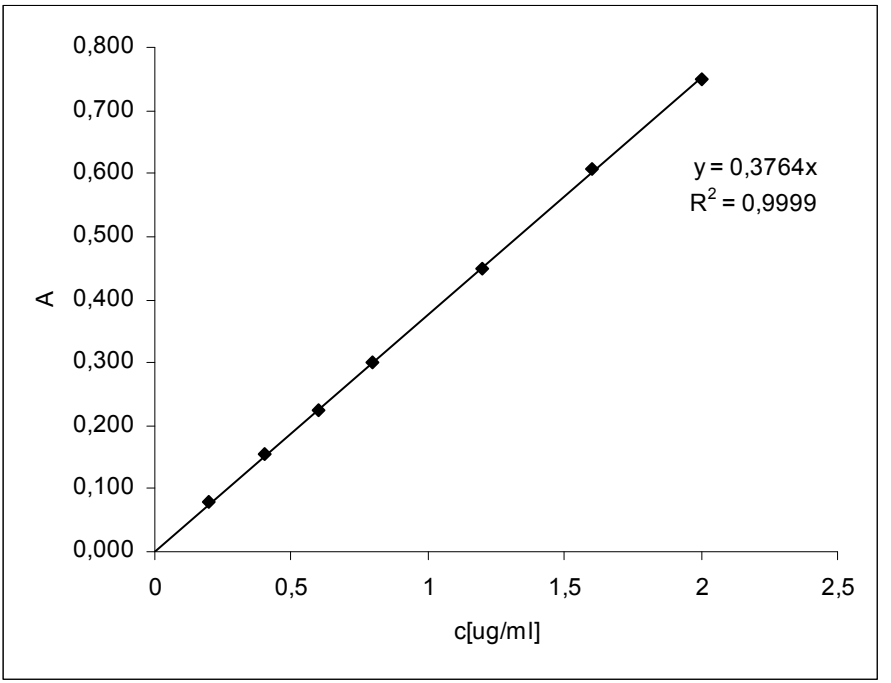

Fig. 1. Standarization curve for chromium determination using the method with diphenylcarbazide
Ryc. 1. Krzywa wzorcowa do oznaczania chromu metodą z użyciem difenylokarbazydy

2.3.2. Cobalt (II)

Nitroso-R-salt method is the most suitable for a determination of trace amounts of cobalt in yeasts. That compound is a specific reagent for cobalt. The presence of sulpho groups in a molecule of nitroso-R-salt causes that reagent and its complex with cobalt (red colour) dissolve in water, not in non-polar solvents [Marczenko and Balcerzak 1998, Minczewski et al. 1973].

Complexing reaction was conducted in an elevated temperature, in slightly acid environment (using 1 M HCl) previously neutralized with diluted ammonia. Nitroso--R-salt (0.1%; analytically pure, produced by "POCh" Ltd. Gliwice) was added to an obtained solution, and then sodium acetate (25%) was introduced in order to buffer, and the whole mixture was heated. Next, the solution was acidified with concentrated H_3PO_4 and addition of HCl (in 1+1 ratio), decomposing possibly present complexes of nitroso-R-salt with other metals (e.g. Cu, Ni, Fe, Mn). The solution was heated again, cooled down and an absorbance of formed complex was measured at a wave length of $\lambda = 415$ nm, in the presence of a blind sample as a reference. Standarization curve of an absorbance relation from cobalt concentration was described using an equation y = 0.4974x. Linearity range was demonstrated for concentrations on a level of 0.2–1.2 μg/ml (Fig. 2).

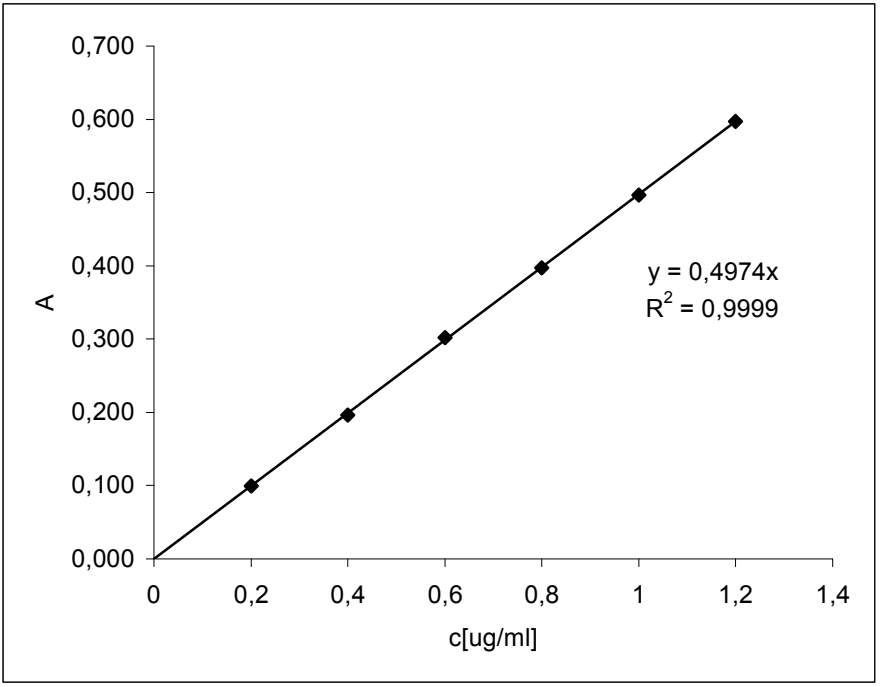

Fig. 2. Standarization curve for cobalt determination with nitroso-R-salt method
Ryc. 2. Krzywa wzorcowa do oznaczania kobaltu metodą nitro-soli

2.3.3. Manganese (II)

The most proper method for manganese assessment in baker's yeasts in permanganate method with an application of potassium periodate [Marczenko and Balcerzak 1998, Minczewski et al. 1973]. Its base is an oxidation of Mn^{2+} ions in acid environment using strong oxidizing agents to violet coloured MnO_4^- ions.

After the mineralization, the obtained product free of chlorides and other reducing agents, was acidified in a following order: concentrated H_2SO_4 (2 ml), concentrated HNO_3 (0.5 ml), and concentrated H_3PO_4 (1 ml). Next, about 0.15 g of solid KIO_4 was added (analytically pure, produced by "POCh" Ltd Gliwice). The solution was heated and maintained in a temperature of about 90°C for 10 minutes. Then it was cooled, and an absorbance with the wave length of λ = 528 nm was measured in the presence of distilled water as a reference. Standarization curve of an absorbance relation from manganese concentration was described using an equation y = 0.0423x. Linearity range was demonstrated for concentrations on a level of 2.0–10.0 µg/ml (Fig. 3).

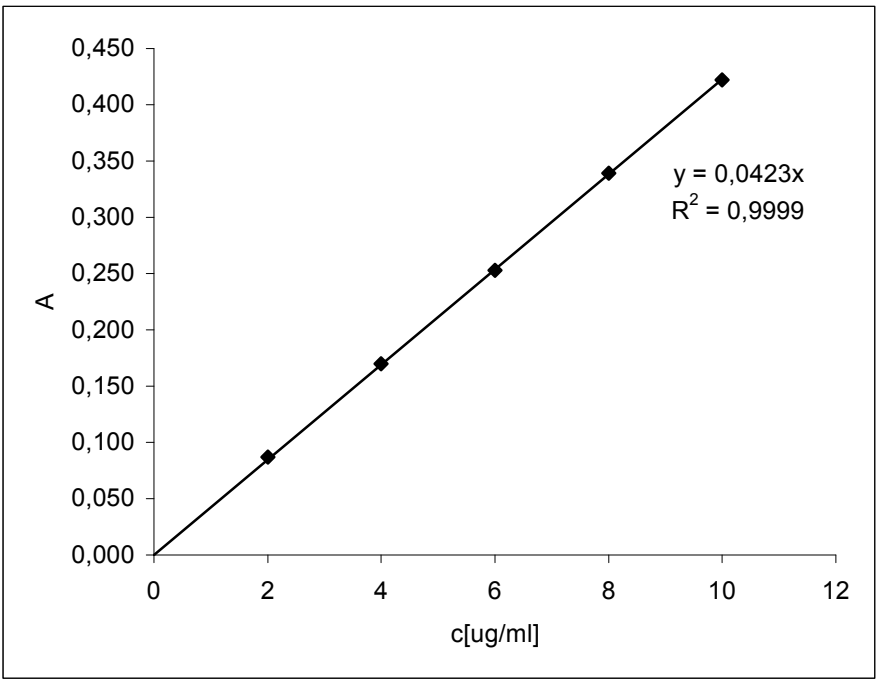

Fig. 3. Standarization curve for manganese determination with permanganate method
Ryc. 3. Krzywa wzorcowa do oznaczania manganu metodą nadmanganianową

2.3.4. Copper (II)

The proposed method is used for a determination of small amounts of copper using lead diethyldithiocarbamate $Pb(DDTK)_2$, that together with Cu(II) forms a yellow coloured $Cu(DDTK)_2$ complex in the range of pH = 8–9. Copper ions bond the reagent by two sulphur atoms, creating the rare chelate with quaternary rings [Marczenko and Balcerzak 1998, Minczewski et al. 1973, Masłowska and Kunaszewska 1976].

Buffering-masking mixture was added to the solution obtained after mineralization (13 g of $Na_3PO_4 \cdot 12H_2O$ – analytically pure, produced by "POCh" Ltd Gliwice, and 100 g of sodium citrate $Na_3C_6H_3O_7 \cdot 2H_2O$ – analytically pure, produced by "POCh" Ltd Gliwice, dissolved in 600 ml of distilled water in a flask of a volume of 1l, next 200 ml of concentrated $NH_3 \cdot H_2O$ was added – analytically pure, produced by "POCh" Ltd Gliwice, and the whole was completed with water). $Pb(DDTK)_2$ solution was added (0.1% in CCl_4; analytically pure, produced by Fluka Chemie GmbH) and the mixture was shaking. Bottom organic layer was percolated, completed with dissolvent, and an absorbance was measured with w wave length of λ = 440 nm, in the presence of a blank sample as a reference. Standarization curve of an absorbance relation from copper concentration was described using an equation y = 0.1863x. Linearity range was demonstrated for concentrations on a level of 0.8–2.8 µg/ml (Fig. 4).

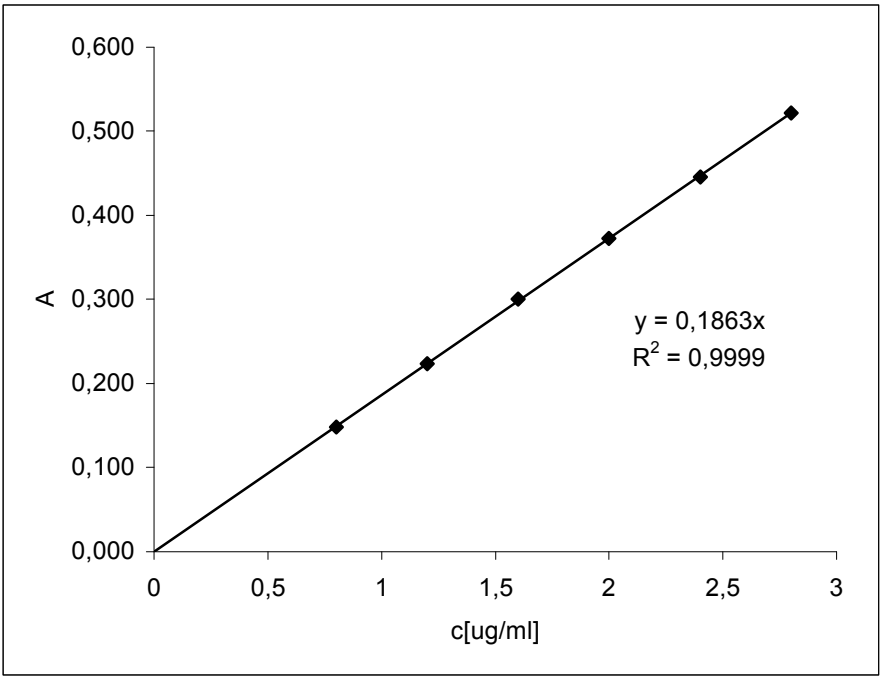

Fig. 4. Standarization curve for copper determination with an application of lead diethyldithiocarbamate

Ryc. 4. Krzywa wzorcowa do oznaczania miedzi z użyciem dityloditiokarbaminianu ołowiu

2.3.5. Nickel (II)

Nickel content was determined using a sensitive method with 2-(5Bromo-2--pyridylazo)-5-diethylaminophenol. That reagent is hardly soluble in water, but very well soluble in ethanol, dimethylfurane, acetone, dioxane and water mixtures of these solvents [Marczenko and Balcerzak 1998, Minczewski et al. 1973].

1 ml of acetate buffer (pH = 5.5) was added, continuously mixing, to an analysed sample in order to decrease an influence of other metals, then 8 ml of ethanol and 2 ml of 5-Br-PADAP solution (0.02% ethanol solution; analytically pure, produced by Fluka

Chemie GmbH). An absorbance was measured after 30 minutes with a wave length of λ = 558 nm, in the presence of a blank sample as a reference. Standarization curve of an absorbance relation from nickel concentration was described using an equation y = 2.2119x. Linearity range was demonstrated for concentrations on a level of 0.08–0.4 μg/ml (Fig. 5).

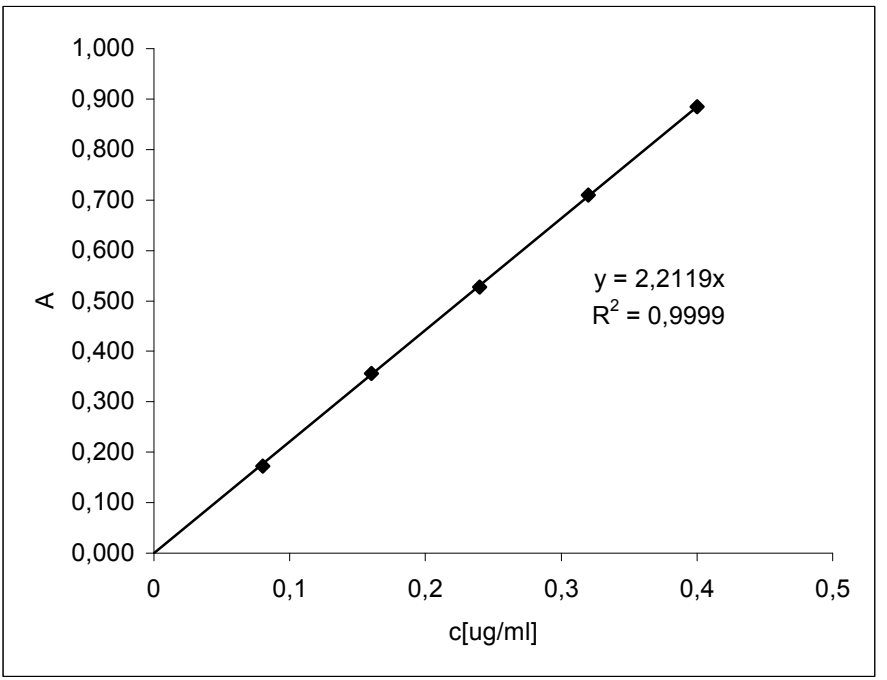

Fig. 5. Standarization curve for nickel determination with an application of 2-(5Bromo-2--pyridylazo)-5-diethylaminophenol

Ryc. 5. Krzywa wzorcowa do oznaczania niklu metodą z użyciem 2-(5Bromo-2-pirydyloazo)--5-dietyloaminofenolu

2.3.6. Iron (III)

The o-phenanthroline creates an orange-red electropositive complexes with Fe^{2+} irons in the range of pH = 2–9. Three molecules of o-phenanthroline accrue on one molecule of iron. In the case of complexing agent deficiency, yellow compounds are created, where the ratio of Fe^{2+} to o-phenanthroline is 1:1. Solutions of complexes are stable, and iron bond with o-phenanthroline is resistant to an oxidation [Marczenko and Balcerzak 1998, Minczewski et al. 1973].

The solution of hydroxylamine was added to an obtained sample (10%; analytically pure; produced by Fluka Chemie GmbH) in order to reduce Fe(III), next 10% solution of sodium citrate (analytically pure; produced by Fluka Chemie GmbH) up to pH = 3–4. At the end, 0.25% o-phenanthroline was added (hydrate of 1,10-phenanthroline was dissolved in 0.1 M HCl; analytically pure; produced by Sigma-Aldrich Chemie GmbH). An absorbance was measured after 5 minutes with a wave length of λ = 512 nm, in the

presence of distilled water as a reference. Standarization curve of an absorbance relation from iron concentration was described using an equation y = 0.2014x. Linearity range was demonstrated for concentrations on a level of 0.5–4.0 μg/ml (Fig. 6).

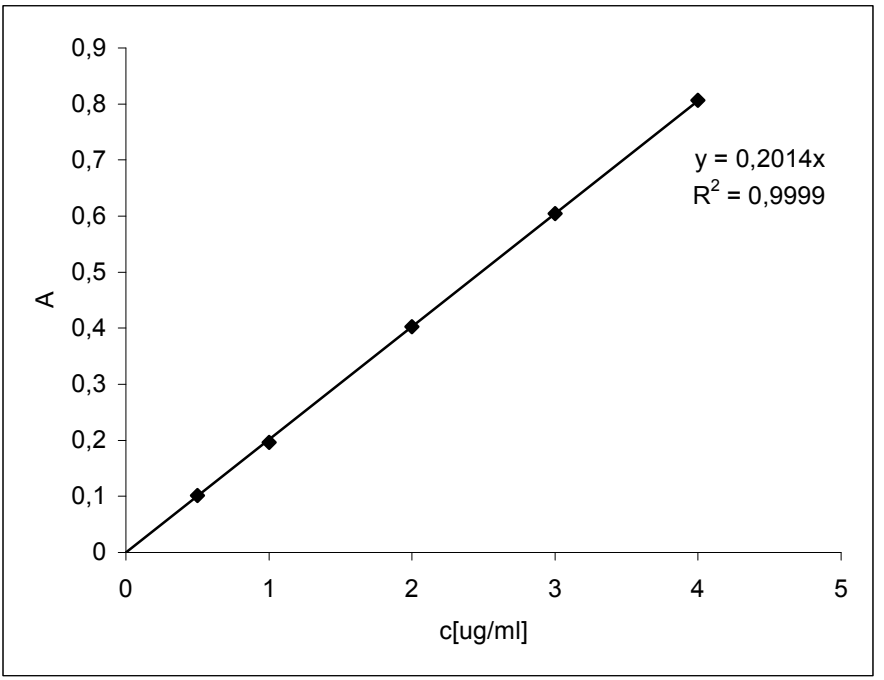

Fig. 6. Standarization curve for iron determination with an application of o-phenanthroline
Ryc. 6. Krzywa wzorcowa do oznaczania żelaza z użyciem o-fenantroliny

2.3.7. Statistical analysis

Uncertainty of measurements of results obtained using the two spectroscopic methods was calculated by statistical analysis of series of individual measurements using Statistica 8.0 software of StatSoft Company. The analysis of normality of variable distribution was conducted using Shapiro-Wilk's test. Differences between mean values was determined using t-Student's test for independent samples.

3. DISCUSSION OF RESULTS

Table 1 presents parameters determining an accuracy and precision of proposed analytical methods (UV-VIS).

Chosen spectrophotometric methods are characterised by universality, sensitivity and precision. Values of molar absorbance indices are higher than $1 \cdot 10^4$ [l/mol·cm], while sensitivity of methods is high, similarly like the precision (0.14–0.26%). An index of standardization curve correlation (R^2) was very high (0.9999), and all measured values of absorbance are within the limits of a range determined by a deviation of linear regression.

Table 1. Parameters determining the accuracy and precision of the proposed analytical method (UV-VIS)
Tabela 1. Parametry określające dokładność i precyzję zaproponowanej metody analitycznej

Analysed element Oznaczany pierwiastek	Molar absorption coefficient (ε) [l/mol·cm] Molowy współczynnik absorpcji (ε)	Sensitivity of the method [ng/ml] Czułość metody	Precision of the method [%] Precyzja metody	Coefficient of standardization curve correlation (R^2) Współczynnik korelacji krzywej wzorcowej	Deviation of linear regression Błąd regresji liniowej
Chromium Chrom	$4.3*10^4$	241.85	0.23	0.9999	$8.51*10^{-4}$
Cobalt Kobalt	$3.5*10^4$	33.67	0.21	0.9999	$10.65*10^{-4}$
Manganese Mangan	$2.4*10^3$	457.83	0.23	0.9999	$9.68*10^{-5}$
Copper Miedź	$1.4*10^4$	90.77	0.14	0.9999	$2.67*10^{-4}$
Nickel Nikiel	$1.26*10^5$	9.31	0.26	0.9999	$57.76*10^{-4}$
Iron Żelazo	$1.10*10^4$	100.63	0.22	0.9999	$4.43*10^{-4}$

From the economic point of view, an accessibility of chosen methods is significant. Spectrophotometer (UV-VIS) is considerably cheaper than an equipment needed for an assessment with other instrumental methods (ICP-AES), and is also easy to operate. Obtained results point that an expensive analytical method may be in this case replaced by easier and considerably cheaper technique.

Using the proposed UV-VIS method, the concentrations of bioelements may be determined in following ranges: Cr(III) – 0.2–2.0 µg/ml, Co(II) – 0.2–1.2 µg/ml, Mn(II) – 2.0–10.0 µg/ml, Cu(II) – 0.8–2.8 µg/ml, Ni(II) – 0.08–0.40 µg/ml, Fe(II) – 0.5–4.0 µg/ml. That ranges may be widened by a proper dilutions of analysed samples, where the multiplication factor should be taken into consideration while calculating the result.

Elaborated method was used to determine the content of chosen microelements in baker's yeasts, and following mean results as recalculated on 1 g of yeasts were obtained: Cr(III) – 152 µg/g; Ni (II) – 3.7 µg/g; Co(II) – 3.8 µg/g; Cu(II) – 15 µg/g; Mn(II) – 3.6 µg/g; Fe(II) – 294 µg/g.

The analysis on inductively coupled plasma atomic emission spectrometer ICP AES was conducted for a comparison. Following mean results as recalculated on 1 g of yeasts were obtained: Cr(III) – 168 µg/g; Ni (II) – 4.0 µg/g; Co(II) – 3.2 µg/g; Cu(II) – 14.8 µg/g; Mn(II) – 4.1 µg/g; Fe(II) – 316µg/g (Tab. 2). Differences are however considerable (from – 15.7 to +13.89%), but insignificant statistically.

It should be mentioned in the context of above values, that daily chromium (III) demand established by World Health Organisation (WHO) is 50–200 µg, for cobalt it is 5–8 µg, manganese 2.5–6 mg, copper 1.5–4.0 mg, nickel 25–35 µg, and iron 10–18 mg in an

adult man. A bit different data concerning the recommended daily consumption of Cr, Cu, Mn and Fe for people in various age are given by Elmadfa and Muskat [2003]. Only Cu, Fe and Mn are standardised in fodders for farm animals [Dobrzański et al. 2006].

Table 2. Results of comparative analytical studies of bioelements in yeasts in μ g/g (n=6)
Tabela 2. Wyniki porównawczych badań analitycznych biopierwiastków w drożdżach μ g/g (n=6)

Method Metoda	Cr	Ni	Co	Cu	Mn	Fe
UV–VIS	152 ± 9.3	3.7 ± 0.2	3.8 ± 0.3	15.0 ± 1.8	3.6 ±0.2	294 ±13.0
ICP-AES	168 ± 8.6	4.0 ± 0.3	3.2 ± 0.3	14.8 ±1.6	4.1 ± 0.4	316 ±10.4
Difference Różnica%	+10.52	+8.11	-15.79	+1.33	+13.89	+7.48

It should be thus stated in the summary, that the proposed spectrophotometric UV-VIS method may be applied for a determination of microelements concentration in enriches yeasts biomass, however some authors point certain analytical problems when using these methods [Shabatina et al. 2002, Zachariadis et al. 1998, Zhong et al. 2002]. It is interesting that UV-VIS method may be used also for determination of a number of yeasts cells in a given biomass [Bercu et al. 2006].

4. CONCLUSIONS

Quantitative determination of biometals in *Saccharomyces cerevisiae* yeasts was conducted using following methods: diphenylcarbazide one for chromium(III) determination, nitroso-R-salt for cobalt determination, permanganate one with an application of potassium periodate for manganese determination, method with lead diethyldithiocarbamate for copper determination, with 2-(5Bromo-2-pyridylazo)-5-diethylaminophenol for nickel determination, and o-phenanthroline for iron determination, using UV-VIS spectrophotometer.

The comparative analysis of the content of these biometals in yeasts was done on inductively coupled plasma atomic emission spectrometer ICP AES. The differences observed for particular elements were from – 15.7 to +13.89%, but were insignificant statistically.

The proposed methods of analyses using UV-VIS spectrophotometer are selective, precise, and also cheaper and easier to conduct as compared to ICP-AES spectrometer. They may be used for a determination of biometals content in yeasts or yeast-derivatives used in the production of diet supplements or feed premixes.

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OZNACZANIE ZAWARTOŚCI BIOPIERWIASTKÓW W DROŻDŻACH METODAMI SPEKTROSKOPOWYMI

Streszczenie. Opracowano metodykę oznaczania zawartości niektórych biometali, tj. chromu, kobaltu, manganu, miedzi, niklu i żelaza w drożdżach *Saccharomyces cerevisiae* przy użyciu spektrofotometru UV-VIS. Określono dokładność i precyzję wybranych metod analitycznych. Dokonano porównania otrzymanych wyników z metodą spektro-

metryczną ICP-AES. Różnice wartości średnich wyników między tymi metodami wyniosły dla tych pierwiastków odpowiednio: +10,52, +8,11, -15,79, +1,33, +13,89, i +7,48%. Stwierdzono, że metoda spektrofotometryczna UV-VIS może być z powodzeniem stosowana w badaniach analitycznych do oznaczania zawartości mikroelementów we wzbogaconej biomasie drożdży.

Słowa kluczowe: drożdże, UV-VIS, ICP-AES, chrom(III), kobalt(II), mangan(II), miedź(II), nikiel(II), żelazo(III)

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