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Preču un produktu kvalitātes pētījumi

Quality Research of Products and Goods

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KVALITĀTES PĒTĪJUMI

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PREFACE

During the past decade there has been an ever-growing increase in our knowledge of material and food product testing methods.

This book is a result of 1 year experience period since the last issue of ACTA UNIVERSITATIS LATVIENSIS NR 572 with research reports of industrial and agricultural merchandise of the Latvian University, Faculty of Economics has emerged. Our aims in this edition are to reflect research results with modern analytical methodology including measurements and examinations with spectrophotometric appliances in combination with modern nondestructive testing methods which are employed in farming products' analysis and in merchandise quality control as well as relative advantages in terms of methodology. Special attention was paid to ultrasound nondestructive testing methods. These precise methods cast a new light upon fundamental research achievements in basic research of oxidizable products which is especially important in analysing food-stuffs.

CONTENTS

Ideas for Developing Postgraduate Courses
With specialization in Production, Management and Marketing at the University of Latvia

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INTRODUCTION

With the enormous changes sweeping through the economies of the Baltic states new types of specialists with new skills are urgently required. In this article, I would like to propose some ideas, witch could be useful to:
- provide opportunities for recent graduates to improve their knowledge and skills in the new fields of management, production or marketing - transitional courses, which in time could become fully fledged university courses.
- utilize the pool of current academics and their knowledge, at the same time giving them an opportunity to become familiar with the modern market economy requirements, problems and alternatives for existing research and development facilities, these facilities will gradually be made more suitable for the modern developed market economies.

It is suggested that a course in seven areas of specialization be developed, with most, but not all subjects made compulsory, allowing the postgraduate students to select the balance of topics. Thus giving them opportunity to study subjects they would find most suitable in their future field of work. It is also suggested that emphasis in these courses be put on topics witch have practical application and are useful to students after completion of studies.

The ideas proposed below have been developed after discussion with L.U. reader, Ilmars Skards, for with I am indebted to him.

1.Quality Control: Management of Quality and Methodology.

The basic aim of this course would be to prepare the postgraduate student for an active role in the process of change in industry and agriculture, with

the main focus on the customers' role And to provide guidance in the ongoing development and support of the manufacturing processes.

In order to be able to produce products of quality, with would improve the level of products on the local market and even more importantly, ensure that the products produced for export meet the standard expected by the importers - buyers, proper quality control methods need to be applied.

There is an aspect witch is very important for the whole local population. It is essential to develop acceptable methods for controlling that the products offered (and others such as water supplied) on the domestic market meet certain health standards. This area is of national importance, as neglect in this area could lead to high expenditure in others, particularly the health services.

There are academics with knowledge in this field and this knowledge should be properly utilized. This course could offer practical training in various laboratories of the Latvian Academy of Science, with already uses research in developing the chemical and physical parameters of materials used in production and processing. This course would prepare the graduates for setting up and operating methods aiming to improve the quality of products offered on the market. Training in mass spectroscopy, atomic absorption spectrometry, X-ray florescence spectrometry, optical emission spectrometry and classic chemical techniques, might need to be modified for more practical application.

It is important to develop Latvian industry and agriculture for high quality exports and control of products offered on local markets, therefore it is essential to develop techniques for mass quality control and its application. This should include statistically valid sampling methods, thus an introduction to basic statistics may be helpful. A proper development in this field would allow Latvian export to regain the good reputation enjoyed prior WWII.

2.Proper evaluation of various materials used or sold by industry.

It would help, if a range of selected topics was available for students to specialize in the last term(s) of their studies (or a period considered suitable

for these specialized short courses). Considering the content of these courses. it might help it if consideration was given to the requirements of the Latvian government policies in rational utilization of natural resources, developing them for export and at the same time considering the effect on the local environment.

According to the information given to me, there are specialists who can immediately provide instruction covering analysis, research and, if necessary, modification involving the following materials:

a) wood, its protection, modification and by applying modern methods estimates of pulp quality, product quality and estimate radioactivity which is important in the export of pulp, and may still exist as a rasult of Chornobyl.

b) pulp production and the necessity to consider the effect of the various processes on the environment, considering the costs of protecting the environment.

c) quality checks of cellulose derivate and natural polymers for medical applications.

d) controlling chemistry and biotechnology of polysaccharides, which are often contaminated with life threatening ingredients.

e) quality control of the leather industry, not only of finished product, but also controlling the processing, the materials used and its effects on the surroundings. This may include she production technology, new materials and new and better products for the consumer. These are areas which overlap with chemistry, but during the period of transition might be very useful training for the graduates who plan to occupy management positions in enterprises (government and private) involved in these fields.

3. Modern accounting and management control systems.

With the introduction of modern accounting for all enterprises, this course need to provide only a general introduction to this field for future managers (not training of accountants). With the advent of the wide use of computers, it is important that the future managers, depending on their future specialization, are familiar with the latest management information systems (MIS) decision support systems (DSS), various other systems and office automation.

By introducing students to the modern systems available on the world market, it would help them in the selection, installation and organization of information systems when they join an enterprise after completion of their studies.

4. Investment and Financial Planning and Evaluation.

This is a very important area for any student who, regardless of his original field of studies, plans to be involved in the management of assets and in investment decision.
This course should include methods used to select investment alternatives and the financing of such investment. It should also include the impact of taxation and inflation on the investment decision. This course should allow students to specialize in investment planning, analysis, cash flow, break even point and return on funds invested or planned to be invested.
As there is a shortage of capital for the development of Latvian industry and the economy generally, it would help to utilize the capital available for investment in the most productive areas, ensuring that the borrowed capital establishing of the break even point.

5. Marketing.

It is regarded in the industrialized, developed word that one of the hardest tasks in any management structure is the development of good marketing plans and strategies.
If Latvia is to successful in its drive for exports, it is necessary to have good marketing specialists. This course ought to introduce the students to the basic requirements for developing marketing skills: the importance of knowing the market, market research methods and approaches, knowledge of product(s) to be offered, understanding the idea that the "customer is always right" and the ways of planning and controlling marketing efforts. Depending on the availability of lecturers, an introduction to advertising and promotion methods used in marketing would be a very useful addition.

6. Inventory control and logistics.

This is an area, witch due to philosophical reasons has never been well developed in Latvia during the past fifty years. However, it is very important (although not always appreciated) in the running of a successful enterprise. This specialization should provide effective methods of controlling movement of goods, proper transportation, reduction of loses through wastage, damage, pilferage and spoilage. It should also give the student a good training in inventory management, stock take, reconciliation of theoretical stock against actual, understanding the importance of meeting set delivery goals (the latest 'just in time' method), warehouse design, material flow and general management of the inventories.
It should also cover the full understanding of cost of investment in inventories and the effect of these costs on the successful operation of any enterprise.

7. Practical Office Management and Control.

It is expected that a great deal of business dealings will be with western countries and, for that matter, also with the new independent Eastern European countries. To compete on the world market, training in office management function would be very useful. This short course should cover general business principles, arrangement of simple agreements, ordering and shipping procedures etc. Some additional training in commercial English might also be helpful. The basic topics to be covered are:
a) Office systems, intercompany communications, importance of proper and clear communications.
b) Simple agreements between companies covering the basic supply agreement details and the importance of each - quantity, price, delivery date and any penalty if the delivery date is not meet (e.g. refusal to accept the delivery), agreed quality (usually based on sample supplied beforehand), any guaranties etc.
c) Ordering procedures, shipping instructions, payment terms etc.
d) Terms used in international trade (such ETA, ETD, FOB etc.) and the full meaning of these terms.

Summary

With Latvia entering the market economy and with it the associated competitions on world markets, it is essential that trained people are available to fill the positions, requiring special skills and knowledge.

Suggestions in this short article could form a basis for developing courses and programs to prepare students to fill this gab. My suggestion would be initially to place more emphasis on practical training and for the first couple of years give the research a relatively low priority. Once the basic training has been completed and tried in practice, the emphasis can be changed.

A Blood Component Analyzing Method for Estimating the Correlation Between Some Fat Soluble Vitamins in Persons who Live in Latvia and Could be Subjected to Some Oxidative Stress

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A method has been developped for blood plasma optical properties measurements in order to obtain information about some fat soluble vitamin relations.

632.blood samples were taken during the period of 2 years from 632 persons,most of them were males,between the ages of 30-40, who have taken part in Chernobil disaster liquidation campaign. Most of them were without definite illness syndromes. In heptane extract, vitamin A can be observed as a peak at 327nm. measured from a line connecting absorption minimums (2). Retinol had its absorption maximum at 327 nm and had no absorbency at 448nm. Beta - carotene showed its maximum at 448nm, but still had absorbency at 327nm. The difference between the measured absorbency at 327nm and the calculated one represents various carotenoid degradation products which are characteristic to oxidative stress. It has been observed that illumination causes not only the bleaching of carotenoids in visible range, but also an increase of absorption in near ultraviolet range (1). Pretreatment of heptane extract with $NaBH_4$, was only partially effective in suppressing the autoxidation, didn't suppress the oxidation of alpha tocopherol, and this was used for more precise vitamin E determination. E vitamin quantity was characterised as absorption values at 296nm. The correlations are: :A327 = 0.35.A448 + 0.04, which reveals that elevated carotene levels parallels elevated A vitamin level

$A_{296} = A.0.163.A448$, that shows a positive correlation between alpha tocopherol and carotene levels. A negative correlation exists between hemoglobin concentration in blood plasma(,measured as Sorret values in 0.1cm sample) and carotene level :A.Sorret $_{0.1} = -0.473.A448 + 0.38$, which let's to propose, that elevated erythrocyte osmotic resistance can be connected with higher carotene level. However there is a weak correlation between the latest and bilirubin concentration, $A_{460} = A_{448} + 0.3$.

Fat soluble vitamins must be supplied by the diet, which is subjected to seasonal variations. Some serum measurements reflect seasonal changes, ^296, characterising tocopherol quantity. reaches its maximum at late autumn, the peak value, that is ^296, in November. If peak values characterising tocopherol quantity are related to phone values,where the last represents not only tocopherol,but also various other heptane soluble components,then the relation ^296/296 in late autumn reaches 0.23,but in spring the relation is twice lower.In autumn also vitamin A level is higher ,the corresponding ^327 value is 0.025, which corresponds to 20 mkg/dL and is in the range of normal laboratory values (15-60mkg/dl). The lowest values of vitamin A are in winter, were the values are twice lower and are under the limit. Carotene levels are the highest at the second half of September, where the A448 values reaches 0.25. That corresponds 93mkg/dL.The normal laboratory values -50 - 300 mkg/dL. After this period a very sharp decline is to be observed. In the mid of January the values were twice lower. At the end of February 1993 the A448 values were only 0.08, that is three times lower compared of the September ones,which corresponds to 31 mkg /dL and is below the normal limit. In previous winter the lowest values could be observed at March. At that time they were higher of those observed now in February.In previous year the values were 30% higher as now. Perhaps this could be explained by lower beta carotene intake.

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The effect of superproduction delta-endotoxin of Bacillus thuringiensis var.thuringiensis PB1023.

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ABSTRACT

A novel strain of *var.thuringiensis* group from Latvian collection of *B.thuringiensis*, namely PB1023, was synthesized two different major proteins 130 kDa (P1t) and 65 kDa (P2t), respectively. First of them, 130 kDa large protein can be dissolved at both reducing and non-reducing conditions in alkaline buffer pH 9.5. After incubation alkali-soluble fraction of crystals from PB1023 with larval gut extract processed protein band with MW about 60 kDa was observed. Second major protein of PB1023 crystals, 65 kDa in weight, dissolves not in alkaline buffer. Both purificated fractions of PB1023 crystals, 130 kDa alkali-soluble protein and 65 kDa alkali-insoluble protein were toxic to *P.brassicae* larvae in bioassays experiments, and besides, the insecticidal activity of P1t was higher than the toxicity of P2t.

Electron microscopy of the samples from PB1023 sporulated culture showed spores and typical bipyramidal crystals plus ovaloid inclusions. Alkali-insoluble fraction of PB1023 sporulated culture under the same conditions gave only ovaloid inclusion structures. Therefore, a conclusion was made that P2-like 65 kDa protein forms the ovaloid inclusions and P1-like 130 kDa toxin is the main component of classical bipyramidal crystals.

The media for the PB1023 were optimized. Feed-bath culture showed higher yield of the toxin per lactalbumin and shorter time of the process in respect with bath culture.

PB1023 culture was found sensitive to concentration of carbon source at early phases of sporulation. As a result of the pulse of glucose in early sporulated culture, the time of sporulation increased twice and production of the toxin was 5 time higher in respect with control.

The effect obtained could be used for the regulating of toxin and spores ratio at the production of bacillar insecticides.

INTRODUCTION

A general trend in modern environment protection is an limitation of the usage of xenobiotics (e.g.pesticides), and their substitution by biological preparations in agriculture and forestry.

There are a number of *Bacillus thuringiensis* preparations used as commercial insecticide: Dipel (Abbot Lab., U.S.A.), Biotrol-BTB (Nutrilc Products, U.S.A.), Turicide (Sandoz Ltd., Switzerland), Lepidocide, bitoxibacilline (Russia), gomeline (Belorussia); last year the bacillar insecticide L-Bacillon (Solvo Ltd.) have been produced in Latvia. These preparates are successfully used for the control of the pests from *Lepidoptera* and *Diptera* (butterflies and mosquitos). The main prefer of bacillar insecticides is the lack of toxicity for

mammals and human and significant specific toxicity against target insects (Aronson et al. 1986).

During the sporulation phase the Gram-positive bacterium *Bacillus thuringiensis* produces large crystalline inclusions. These parasporal crystals predominantly consist of about 130 kDa protein molecules called delta-endotoxin, or crystal protoxin. Crystal parasporal proteins are often highly toxic to specific insects but not toxic to mammals and humans.

Upon ingestion by insect larvae the crystals are dissolved and the protoxin activated by midgut proteases, resulting in toxic N-terminal 30-70 kDa fragments (Hofte et al. 1986, Schnepf et al. 1985). The formation of ion channels across the midgut membrane of target insects was postulated to account for the lethal effect (Schnepf & Whiteley 1985).

Crystal protein genes are located on large (> 50 kbp) plasmids or on bacterial chromosome (Kronstadt et al. 1983) and are expressed during the sporulation. Parasporal protein synthesis begins at about stage II or III of sporulation: i.e. completion of the forespore septum and the inclusion reaches maximum size by stage V (Nagamatsu et al. 1984).

Crystal proteins with a specific toxicity to *Lepidoptera* and *Diptera* have been described. *B.thuringiensis var.kurstaki* strain HD1 synthesize two kind of crystal, a bipyramidal which is composed of 130 kDa P1 protein toxic to lepidopteran larvae, and flattened cuboidal crystal toxic both to lepidopteran and dipteran larvae, which formed of 66 kDa P2 protein (Yamamoto and McLaughlin 1981; Iizuka and Yamamoto 1983). Other distinct class of dipteran toxin is found in *B.thuringiensis var.israelensis*. It forms usually two to four inclusions per cell which vary in shape and are active against dipteran but not lepidopteran larvae (Gonzalez et al. 1984). There is a broad spectrum of polypeptides extractable from these inclusions including a major 26 kDa protein (Yamamoto et al. 1983) and 65 kDa protein (Hurley et al. 1985) that appear to be toxins.

Krieg et al. (1983) have described a strain of *B.thuringiensis var tenebrionis* that was reported to be toxic to Colorado potato beetle larvae. Bernhard (1986) reported that a crystal protein of approximately 68 kDa was responsible for the toxicity of *var.tenebrionis*. Herrnstadt et al. (1986) have reported a coleoptyeran-toxic strain of *B.thuringiensis var.san diego* with a toxic protein of 65 kDa. The genes encoding these two coleopteran-toxic proteins have recently been cloned and sequenced. (Herrnstadt et al. 1987; Hofte et al. 1987; Jahn et al. 1987; Sekar et al. 1987). Donovan et al. 1988 have been isolated a strain of *B.thuringiensis* EG2158 synthesized two crystalline proteins, 73-kDa protein what forms rhomboid crystals, and 66-kDa protein composing diamond-shaped crystals. Plasmid transfer and gene cloning experiments demonstrated that the 73-kDa protein was encoded on an 88 MDa plasmid and that the protein was toxic to the larvae of Colorado potato beetle.

Because the toxin synthesis is bounded with sporulation events, large-scale periodic cultivation of the bacteria is used in the production of *B.thuringiensis* insecticides. Media used for the cultivation must be optimized for the e. .h case

separately, because different serotypes of *B.thuringiensis* utilize differently the components of media used. Moreover, a various spectra of auxotrophic factors are necessary for the growth of *B.thuringiensis* and these components of media have a different influence to entomocidial activities (Salama et al., 1983). The media used in *B.thuringiensis* cultivation usually contained maize or yeast extracts as growth factors (Nickelson 1974).

METHODS

Bacteria

Strains of *B.thuringiensis* were grown at 30°C in G-tris medium (Aronson et al. 1971) in 250 ml Erlenmeyer flasks (10-15% vol.) or on solid medium contained 1.5% agar (Difco). After 36 h spores and inclusions were harvested by the centrifugation at 10000g for 10 min. Pellets were washed once with 1 M NaCl, three times with destilled water and were finally suspended in water. The parasporal inclusions were examined by phase-contrasting light microscopy.

Cultivation experiments were carried out in a laboratory scale bioreactor 5 l at 30°C. Regulating of pH (7.0-7.2) was carried out automatically by the addition of 10% NaOH. Aeration intensity was registried as to the partial pressure of oxygen (pO_2). It was maintained at 30 % of saturation.
Respiration intensity of the culture was measured by gas balance method, using the gas-analyzing system PGA-2 designed in Institute of Microbiology, Latvian Academy of Sciences (Baburin,I.A. et al. 1986.).

Preparation of larval midgut extract

Midguts from 10 *Pieris brassicae* 4th instar larvae were excised, homogenised in 1 ml 50 mM CBD (Na_2CO_3-HCl pH 9.5; 10 mM dithiothreitol (DTT)), and the homogenate centrifuged at 10000g for 5 min. The soluble gut extract was filtered through a 0.45 mkm Millipore filter. The filtrate was stored frozen at -20°C.

Preparation of lepidopteran toxins

Crystal delta-endotoxin was solubilized and the insoluble fraction removed by centrifugation at 10000g for 15 min. If needed the soluble component was dialysed against 10 mM Tris-HCl pH 7.0 and diluted to concentration of 1 mg/ml. The insoluble component was washed twice and suspended in 10 mM Tris-HCl pH 7.0. at concentration of 1 mg/ml estimated by the SDS-PAAG electrophoresis. The soluble component (1 mg/ml) was activated by incubation with larval gut extract (4:1, v./v) at 37°C for 15 min without dialysis.

Analytical

The protein content was determined by the method of Lowry et al. with bovine serum albumin as the standard. Molecular size of proteins was routinely checked by sodium dodecyl sulphate polyacrylamide gel electrophoresis.

Bioassays

The bioassays with *P.brassicae* (cabbage white butterfly) were performed early 5th-instar larvae on an artificial diet. Crystals, protoxin and toxin were mixed at 60°C with the liquid diet; the mixture was poured into 50 ml glasses. After solidification of the diet, ten larvae were added to each glass. the effect of the toxin was measured after 4 days of incubation at 28°C. The number of dead larvae were determined. The results of the bioassays were evaluated by probit analysis. The lethal dose needed for 50% (LD50) or 95% (LD95) mortality was determined by linear regression. Each sample was tested at least with three different concentrations.

RESULTS AND DISCUSSION

We found that novel strain of *B.thuringiensis var.thuringiensis* group from Latvian collection of *B.thuringiensis*, namely PB1023, synthesize two different major proteins 130 kDa and 65 kDa, respectively (Fig.1, line 4). First of them, 130 kDa large protein can be dissolved at both reducing and non-reducing conditions in alkaline buffer pH 9.5(line 3,5). After incubation alkali-soluble fraction of crystals (line 10) from PB1023 with larval gut extract (line 7) we observed processed protein band with MW about 60 kDa. Therefore, this protein of *B.thuringiensis* PB1023 (var.thuringiensis) may be analogical to P1 crystal protoxin of *B.tuuringiensis var.kurstaki*.

Fig.1 SDS-PAAG protein analysis of crystals of *B.thuringiensis var.thuringiensis* PB1023 with and without treatment. Line 1 - molecular weight markers, lines 2,4 - crystal proteins, line 3 - alkali-insoluble fraction under non-reducing conditions, line 5 - alkali-insoluble fraction under reducing conditions, line 6 - trypsine preparation (Serva), line 7 - processed alkali-soluble fraction; line 8 - *P.brassicae* midgut extract; line 9 - alkali-soluble fraction treated with trypsine; line 10 - alkali-soluble fraction.

Second major protein of PB1023 crystals, 65 kDa in weight, dissolves not in alkaline buffer. It may be interesting to investigate toxicity of this protei for

the dipteran larvae and compare them by P2 toxin of *B.thuringiensis var.kurstaki.*

Both purificated fractions of PB1023 crystals, 130 kDa alkali-soluble protein and 65 kDa alkali-insoluble protein were toxic to *P.brassicae* larvae in bioassays experiments (Fig.2),and beside, the insecticidal activity of PST was higher than the toxicity of P2t.

Fraction	LD50		LD95	Slope	r
P1t	6.9	11.6	2.16	0.93	
P2t	14.1	164.0	0.46	0.96	

Fig.2. Toxicity of purificated fractions of PB1023 crystals. P1t is a 130 kDa alkali-soluble protein and P2t is an alkali-insoluble fraction of crystals. Slope of the regression line and r, the correlation coefficient of the regression line, are determined for each preparation. Preparations were purificated, bioassay experiments and regression analysis were carried out as described in Methods.

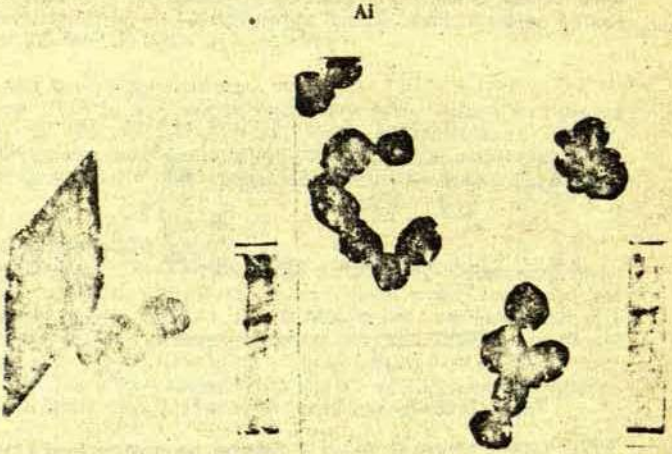


Fig.3. Electron negative-contrasting microscopy (40000 X) and protein patterns of ·per·lated *B.thuringiensis var.thuringiensis* PL1023. Ac - preparation of crystal before treatment with alkaline buffer; Ai - alkali-insoluble fraction

Electron microscopy of the samples from PB1023 sporulated culture showed spores and typical bipyramidal crystals plus ovaloid inclusions (Fig.3). Alkali-insoluble fraction of PB1023 sporulated culture under the same conditions gave only ovaloid inclusion structures. Therefore, we can made a conclusion that P2-like 65 kDa protein forms the ovaloid inclusions and P1-like 130 kDa toxin is the main component of classical bipyramidal crystals.

We isolated plasmid DNA from *B.thuringiensis var.thuringiensis* PB1023. Because there are at least 10 bands of DNA after 0.7 % agarose gel electrophoresis, the localisation of toxin genes may be problematic. We plan to obtain PB1023-derivative strains with reduced plasmids number by the use of mitomycin C treating or incubating master strain at 42°C. We propose that by phase-contrast microscopy and SDS-PAAG protein analysis we could screened both toxinless and plasmidless strains in order to examine the relationship between plasmid and synthesis of toxin.

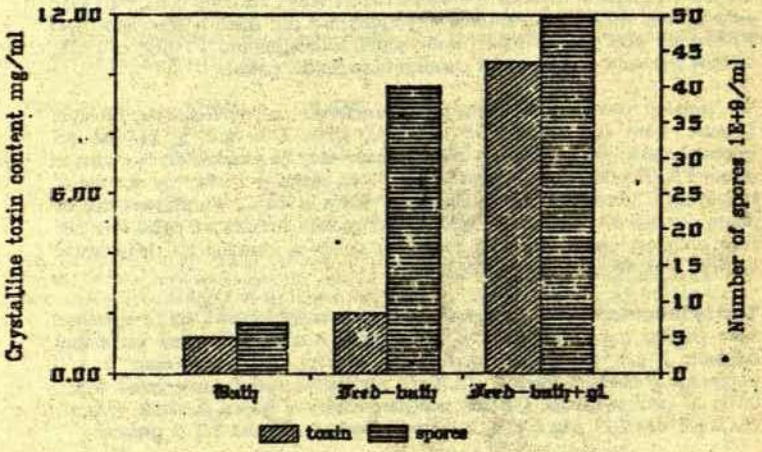
The optimization of the medium for *B.thuringiensis* PB1023 toxins synthesis were carried out in flasks. The LG optimized media contained ammonium sulphate 2 g/l, $MgSO_4.7H_2O$ 0.1 g/l, sodium citrate, dihydrate 1 g/l, phosphate potassium, dibasic 13.7 g/l, phosphate sodium, monobasic 6 g/l, 0.001 M calcium nitrate, 0.1 mM mangane chloride, ferrum sulphate (traces); (final pH was 7.2) plus 0.1 % lactalbumin hydrolysate and 0.2 % glucose.

The growth and sporulation of PB1023 was investigated in the 10 l fermentor bath and feed-bath cultivation. Growth factors and glucose were added during fermentation using gas balance method, adding the substrate at moment when this was a limiting factor of growth (Baburin un citi 1986). Bath culture and feed-bath culture were compaired. The feeding was carried out untill summar lactalbumine amount achieved 10 g/l, as in bath cultivation. Unsurprisingly, feed-bath culture showed higher yield of the toxin per lactalbumin and shorter time of the process in respect with bath culture (Fig.5).

Because B.thuringiensis delta-endotoxin synthesis is bounded with sporulation, detailed investigation of sporulated culture could been interesting. PB1023 culture was found sensitive to concentration of carbon source at early phases of sporulation. Early sporulated culture was estimated morphologically by phase contrast microscopy, then the concentration of glucose was measured (Miller 1959). The sugar was added in media to 1.5%. Because respiration intensity of the culture was not changed at this phase, glucose was not a limiting factor. As a result, the time of sporulation increased twice and production of the toxin was 5 time higher in respect with control.

It could be bounded with effect of glucose inhibition of sporulation of Bacilli. In this statement sporulation genes could be repressed but delta-endotoxin gene(s) could be expressed intensively. On the other hand, it is possible that obtained results could be explained as follows: at early sporulation phase the media contains all components neccessary for the toxin synthesis but there is a lack of some growth factors.

The effect obtained could be used for the regulatind of toxin and spores ratio at the production of bacillar insecticides. Of course, the mechanismes of the effect must be investigated.



Lactalbumin (g/l)	Glucose (g/l)		Number of spores/ml	Crystalline toxin content, mg/ml
1	10	20	$7x10^9$	1.2
2	10	20	$4x10^{10}$	2.0
3	10	20	$5x10^{10}$	10.4

Fig.5. Growth conditions and production of spores and crystalline toxins for *Bacillus thuringiensis var thuringiensis* Pb1023 under periodic fermentation.
1 - Bach culture.
2 - Feed-bath culture.
3 - Feed-bath culture with ipulse of glucose.

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Chemical Characterisation of Sea Buckthorn Berries of Different Forms

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In the past 30 - 40 years extraordinary attention has been paid to sea buckthorn in connection with obtaining of vitamine complex and sea buckthorn oil, a valuable medicinal preparation, from berries. Due to this attention, recently the sea buckthorn is turned from little known plant into a well-known one [1].

Considerable material has been collected about biological peculiarities of the plant, the primary choice of various economically most valuable plant forms has been made, the first sorts suitable for industrial multiplying are acquired.

There are many forms of the sea buckthorn in nature which greatly vary in their biological peculiarities. The quality of the berry harvest of these forms is also very different. Moreover, the observed peculiarities are specific not only for plants of different populations but also for plants within the same population [2,3]

Vast sea buckthorn growths are in the Siberia, in the Caucasus in the Alps and in the sea coast of the Baltic and Northern seas. The largest wild-growing and industrial growths of sea buckthorn are widely distributed in the Siberia along the mountain riversides [1,2].

In the Baltic region the sea buckthorn is cultivated mostly in Lithuania. Here it is applied for soil recultivation of old gravel-pits and dolomite-pits. Next to this investigations are carried out about the role of this plant in medicine [4]. The sea buckthorn growing in Lithuania belongs to the Baltic population and has a large variety of forms.

In spite of its valuability, the sea buckthorn is not cultivated in Latvia on a wider scale. The observations testify that popular Siberian sorts are not suitable for Baltic region because of climatic conditions and susceptibility to deseases. Therefore in the last years in theBaltic states the attention is devoted to Baltic forms. These forms are widely used now

in the Moscow university botanical garden [5] for preparing of new sorts suitable for the European part of Russia and Baltic region.

Particular sorts grown in botanical garden of Moscow university and which are formed on the basis of Siberian and Baltic forms, are tested successfully in Latvia [6,7].

As intensive introduction of sea buckthorn as culture plant in Latvia is only beginning, the aim of our work was to evince the peculiarities and suitability of Lithuanian sea buckthorn forms for cultivation in Latvian conditions. Therefore the task of the given work was to determine some biochemical indices in berries of sea buckthorn plants growing in Lithuania.

Material and methods

Berries for analyses were harvested in September 1991 in the Vilnius District and in september of 1992 in Naujoji Akmene District. The appearance of plants, as well as the shape, colour and size of berries were taken into account by gathering of patterns. The berries were cut together with twigs and were kept in ice chamber till analysis. The berries were characterised by their colour, shape and weight as well as by content of ascorbic acid and sugars, by common acidity and composition of phenolic acids.

Quantitative analysis of Ascorbic acid was carried out by iodometric method [8], soluble sugars by means of picric acid [9] and common acidity by titration with 0,1 N NaOH [10]. Phenolic compounds were extracted with 80 % ethanol and analysed on polyamide thin layer chromatoplates [11]. Quantitative amount of individual compounds was determined by comparing their size on chromatograms and intensity of luminiscence in UV-light as well as by their ultraviolet spectra measured with Specord UV-VIS. For mathematical analysis of data Dospehov's [12] instruction was used.

Results and analysis

It is known that special attention by selection work of sea buckthorn is paid to size of berries, for it determines the productiveness of plants. Literature [3] data shows that for Siberian wild-growing plants the weight of 100 berries is within

limits from 4 to 20 g. Though, there have been discovered separate forms with 100 berry weight to 100 g and more, however, for the majority of sort plants this indice is within limits from 40 to 60 g.

We ascertained that in Lithuania growing plant berry size also may be very different. The data of table 1 shows that these differences may be within limits from 17 to 50 g/100 berries. But the weight of separate berries reached 0,8 g. Consequently, these berries do not differ from many known sort ones.

Table 1. The size and content of ascorbic acid, sugars and organic acids in sea buckthorn berries

Form	Weight of 100 berries (g)	Ascorbic acid (mg %)	Sugars (mg/100g)	Organic acids (%)
		Year 1991		
5L	16,7 ∓ 0,6	85,1 ∓ 0,8	-	2,09 ∓ 0,07
1L	24,0 ∓ 0,2	231,7 ∓ 1,0	-	2,61 ∓ 0,03
LD2	31,2 ∓ 0,5	213,8 ∓ 3,9	-	2,74 ∓ 0,02
LD1	31,9 ∓ 0,3	268,1 ∓ 5,6	-	3,03 ∓ 0,09
12L	34,5 ∓ 0,5	206,1 ∓ 3,9	-	4,19 ∓ 0,04
4L	36,1 ∓ 0,3	200,6 ∓ 3,0	-	3,51 ∓ 0,05
LC1	38,4 ∓ 0,6	100,3 ∓ 1,3	-	2,73 ∓ 0,09
LD3	40,9 ∓ 0,8	165,7 ∓ 5,6	-	2,23 ∓ 0,06
LS1	50,9 ∓ 5,2	110,2 ∓ 1,3	-	2,46 ∓ 0,09
		Year 1992		
U-1	45,2 ∓ 0,2	211,6 ∓ 5,7	10,0 ∓ 0,4	1,16 ∓ 0,05
U-2	44,2 ± 0,4	164,4 ± 2,4	24,1 ± 0,6	1,41 ± 0,02
U-3	37,7 ± 0,4	108,8 ± 1,6	12,7 ± 0,6	1,88 ± 0.00
K-1	44,4 ± 0,5	130,2 ± 0,4	27,9 ± 2,1	1,07 ± 0,02
S-S	34,0 ± 0,5	84,3 ± 1,7	36,2 ± 1,2	0,75 ± 0,02
A	44,2 ± 0,3	52,5 ± 0,2	40,5 ± 1,2	1,43 ± 0,01
SAP	28,5 0,3	186,2 0,3	19,4 0,4	2,17 0,01

The fault of sea buckthorn, grown in Lithuania is their comparatively large thornity. Jeliseyev [3] regards this fault as essential for it diminishes harvesting of berries.

But evidently the chemical composition of berries must be considered as the most important. From this view-point, the content of ascorbic acid is one of the chief indices. In accordance with literature [1] data the content of ascorbic acid may be within limits from some mg% to 1000 and more. Commonly, this

quantity is given within limits from some ten mg% to some hundred mg%.

Our experimental data (table 1) shows that quality of berries from plants grown in Lithuania does not fall behind the Siberian forms or from sorts developed in Botanical Garden of Moscow University. Thus, the content of ascorbic acid for Moscow sorts was from 52,3 to 220 mg% [6], but in our case 52 - 268 mg%.

Two forms (S-S and A) were found at Naujoi Akmene, which were even more rich in sugars than the sorts grown in the Botanical Garden of Moscow University. Unfortunately, the level of ascorbic acid in these both forms was comparatively low. Such a correlation was found also in some other cases, but it can't be regarded as a physiological regularity, so that in the other cases is not valid.

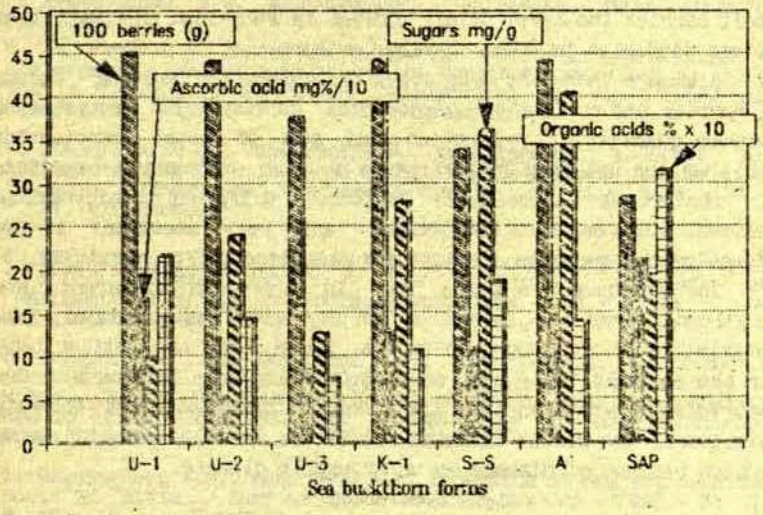

Figure 1. The comparative quantities of berry weight, ascorbic acidand, sugars and organic acids in berries of sea buckthorn growing in Akmene region.

In some cases low level of organic acids in berries correlate with a high level of sugars (see fig. 1) but it could not be noted as regularity.

From figure 1 it can be concluded that there is no definite correlations between the size of berries, and quantitative content of ascorbic acid, organic acids and sugars.

On polyamide thin layer chromatograms more than 25 phenolic compounds were detected which are characteristic for sea buckthorn berries. Most of them were identified as flavonoid compounds. One of them was identified as rutine ($\lambda_{max.}^{96\% EtOH}$ 255, 300 sh. and 362) and second as isoramnetine glucoside ($\lambda_{max.}^{96\% EtOH}$ 257, 267 sh., 300 sh. and 362).

Semiquantitative estimation showed the regularity that in berries with an increased level of ascorbic acid the content of phenolic acids is lower.

We have established (see also table 1) that in especially small berries the level of all indices is very low. Similar data we can find also in works of other authors.

Although there are data about the corelation between colour of berries and content of carotenoids as well as correlation between quantity of β-carotine and sum of total carotenoids [12], we can not find in literature a more exhaustive analysis of reciprocal correlation between different biochemical indices. However, such researches are very necessary if we investigate a question of plant sexualisation and productivity.

The obtained data shows that in Lithuania different sea buckthorn forms are spread which may serve as valuable raw material to be introduced in Latvia. These forms are well suitable for the Paltic climate and do not suffer from diseases. Our observations testimony that the Siberian forms and sorts tolerate very poorly the Baltic climate conditions and sooner or later perish because of disease or unfavourable climate.

Conclusion

It was established that the Baltic population of sea buckthorn has many forms which are widely cultivated in Lithuania. Some of these forms are very suitable for obtaining of good harvest with good biochemical parameters.

Forms with comparatively high level of ascorbic acid and

sugars were found but quantitative correlation between content of different biochemical indices was not found.

Among the investigated forms are such ones which by their biochemical indices do not fall behind the Siberian forms and sorts. These forms may serve as good source for sea buckthorn introduction in Latvia.

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Effect of Storage Conditions on Sea Buckthorn (*Hippophae rhamnoides*) Seed Germination.

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By now it is already known that seed storage conditions and pre-sowing treatment may affect seed germination and plant sexualisation [1, 2]. Unfortunately, such experiments are made mainly with annual plants. Such a problem occurred to us when studying the physiological and biochemical principles of sea buckthorn sexualisation. By seed propagation the most part of the seedlings lose the peculiarities of the mother plant. Therefore it is not profitable to make use of seedlings for direct formation of plantation. Similarly it is not known up to this day how to determine a potential sex of the juvenile seedlings. Previous observations [3. 118 p.] have shown that 50 - 70% of the seedlings develop subsequently as male plants. Thus a practical question arose beside the theoretical one, because the seed propagation remain as a basic means in the selection work of the sea buckthorn.

To follow the fact that the selection of sea buckthorn in Europa is just beginning [3., 74 p.], there is a undoubted future for seed propagation of it. Seed storage conditions and their pre-sowing treatment have an important role in selection work.

There are very few data in the literature up to now about the effect of different conditions on the see buckthorn seed germination. However, it is exceptionally important in case the sea buckthorn must be cultivated in regions, which are not typical for it. The experimental mutagenesis is one of methods how to increase variability of plant properties and also to increase the effectivity of selection work.

Increasing interest has been aroused by application of phhysical or chemical mutagens in combinations with phytohormones. There are observations [4] that seed irradiation with γ-rays increased oil and sugar level in berries. According to G. Privalov's at all [5] data, after seed irradiation with γ-rays and

especially in combination with gibberelline, are obtained plants with increased level of ascorbic acid and oil in berries.

The sea buckthorn seeds preserve their ability to germinate merely 2 - 3 years [6] and there are no data how to prolong this time. There is an opinion that wild plant seeds must be stratified but not the seeds of the culture ones, for they don't have an expressed rest period. However, there is a success in changing the ability of germination by application of different water solutions of 0,02 % succinic acid, 0,05 % boric acid, 0,02 % IAA etc.

The environment temperature also remarkably affect sea buckthorn seed germination [7,8]. Vernik and Zhapakova [8] suggests that the optimum temperature for sea buckthorn germinating is within the limits of 25 - 27°C, however, seeds of some Siberia, Central Asia or Caucasia forms are germinating by strong lower temperature. The dates of collection of berries does not affect the ability of germination. So in laboratory conditions the ability of germinating acnieved in any case (November, December, Yanuary, Pebruary and March) equals 94 - 96 %. Only seeds of berries collected in April showed a lower ability of germinating [8].

Unfortunately, we did not succeed in finding data in literature how different pre-sowing treatment conditions affect further growth and development of seedlings as well as plant sex. To develop this problem the task of given work was to compare germination of seeds obtained from different regions (Siberia, Kaliningrad, Lithuania) as well as to test possibility to use some substances for it modification.

The cultivation of sea buckthorn in Latvia was started not long ago and therefore we lack exhaustive information how to use plants of different populations to form sorts adapted to Latvian conditions.

Material and methods

The berries were collected from plants cultivated in Latvia and Lithuania. Siberia sorts were compared with diferent plant forms of the Baltic population, growing in Latvia and Lithuania.

The seeds of the first group immediately after gathering (end of August) of berries were germinated in laboratory conditions in Petri dishes containing 50 seeds/dish on filter

paper moistened with 14 ml distilled water. Before placing into Petri dishes, the seeds were soaked for 3 hours in a distilled water or in solution, containing 0,05% ethrel or 1% mycrocite (a cytokinine containing substance). The work was made in 4 reiterations. The number of germinating seeds was fixed every day.

Results were evaluated by calculation of the arithmetical mean and the error of arithmetical mean. The results were considered as essential if P = 0,05.

Results and discussion

Results were obtained testifying that storage conditions can considerably change the ability to germinate of sea buckthorn seeds. For example, by storage at room temperature, germination capacity of sorts Chuiskaya and Shcherbinka changed a little in years 1987 and 1988 and was 96 and 93 % in these years accordingly. However, in 1989 germination capacity of seeds of both sorts diminished to 10 %. The germination capacity of seeds decreased exceptionally rapidly when they were stored at reduced temperature (Table 1, Pig. 1).

Table 1. Variability of sea buckthorn germination capacity (in % from common number) depending on storage conditions.

Storage conditions	Date of seed germination		
	18.12.89	26.01.90	23.02.90
ice chambe – 15°C	92,4+-6,3	85,6+-8,2	18,4+-3,9
Room temperature	93,7+-5,1	93,9+-5,6	92,9+-6,7

Pig. 1 shows that storage conditions have influenced both germinating capacity and germinating intensity. The effect of low temperature was much weaker, if seeds were not separated from the pulp of berries. Interesting to note that the best results, however, were obtained if seeds were kept at room temperature.

Sea buckthorn seeds practically do not germinate by low (+5°C and lower) temperature, but germinate very well by heightened (25 - 27°C) temperature. According to Jelisejev [7] even 6 - 7 years old seeds can germinate at such a temperature.

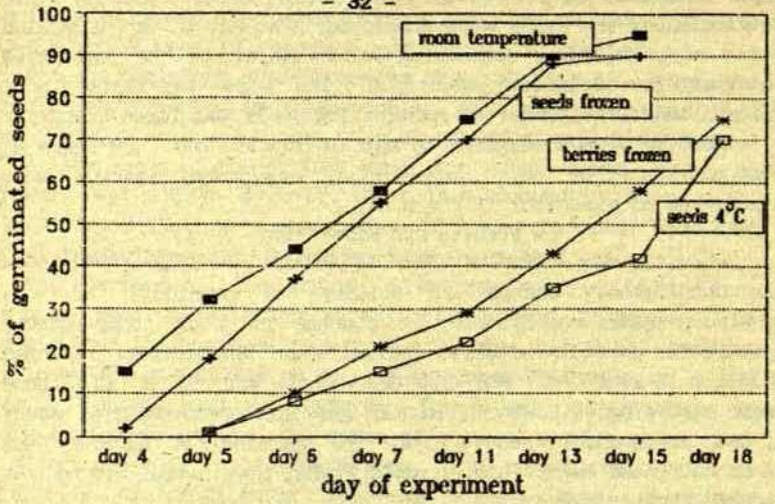

Fig. 1. The intensity of sea buckthorn seed germination depending on storage conditions.

It is believed [10] that such a behavior of seeds is connected with the origin of sea buckthorn from flora of tropical plants. It was stated in experiments with cucumber seeds [1, 2] that periodical alteration of temperature during the storage time, remarkably increased germination capacity, accelerated flowering and stimulated the female sex of plants. It was observed [11] that germination of *Hippophaë rhamnoides* seeds also were stimulated by regular diurnal oscillations of temperature.

Fig. 2 shows that seed germination of different sea buckthorn sorts may be greatly different, however, in all cases we could find the same regularity - the seeds which were kept in changeable temperature showed much more germinating intensity in comparison with seeds which were kept in unchangeable one. For example, in the 9-th day the germinating capacity of seeds of form LC1 was 96 and 35 %, but for form LD5 91 and 45 % correspondingly. On the 13-th day the number of germinated seeds was equal for both variants. That means that the changeable temperature has influenced more the germinating power and less the germinating capacity.

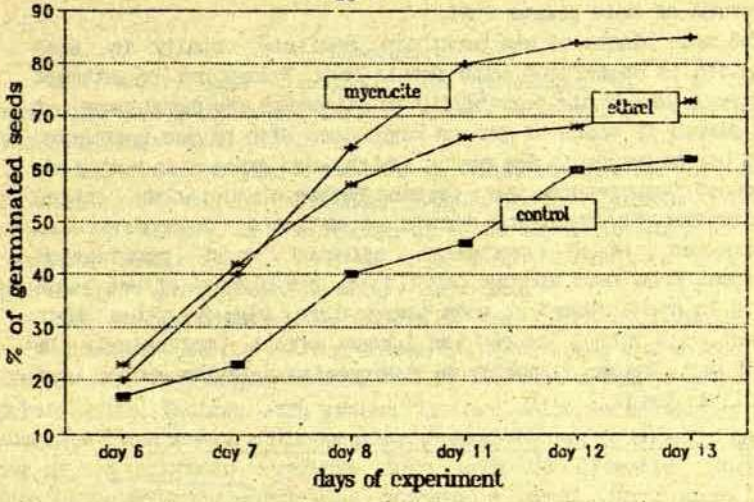

Fig. 2. The influence of variability of temperature during storage on the sea buckthorn seed germination. 1 - seeds kept in changeable temperature (+ 12 and + 22°C); 2 - seeds kept in unchangeable temperature (22 - 24°C). LD1, LD5 and LC1 different forms of plants.

Jeliseyev and Mishulin [15] consider that determinent factor to preserve the viability of sea buckthorn seeds is a water which may not to exceed 6 - 8 %. Thus the seeds with water content 4.1 %, maintained their germination capacity still after 12 years to 78 %, and even after 16 years to 72 %.

There is opinion [12, 13], that seed germination capacity may be modified by means of growth regulators. In accordance with Garanowitsh [14] the seed germination of sea buckthorn was stimulated not only by plant growth regulators - IAA, NAA and gibberellins but also by such simple chemicals as borax, boric acid, benzoic acid, $MnCl_2$, succinic acid etc.

In our previous experiments it was stated, that presowing treatment of cucumber seeds, which were kept at room temperature, by ethrel or mycrocite stimulated female sex. Female sex of hemp was stimulated after seed pre-sowing treatment with solutions of IAA or 6-benzylaminopurine, whereas gibberellin induced

development of male plants [13].

The male plants of sea buckthorn dominate usually by seed propagation in comparison with female ones. Therefore an attempt was made to verify the possibility tc stimulate the development of female plants by means of growth regulators also by sea buckthorn.

In our experiments the seeds, which were exposed in both room and reduced temperature, were treated before sowing with ethrel and mycrocite solutions. For further experiments concentrations, were choosed which maximally affected seed germination independent from seed storage conditions. The biggest effect was observed in seeds stored at room temperature. Fig. 3 shows that treatment with ethrel induced the largest effect, particularly in the 8-th day. The seed soaking in a mycrocite solution gave a less affirmative result.

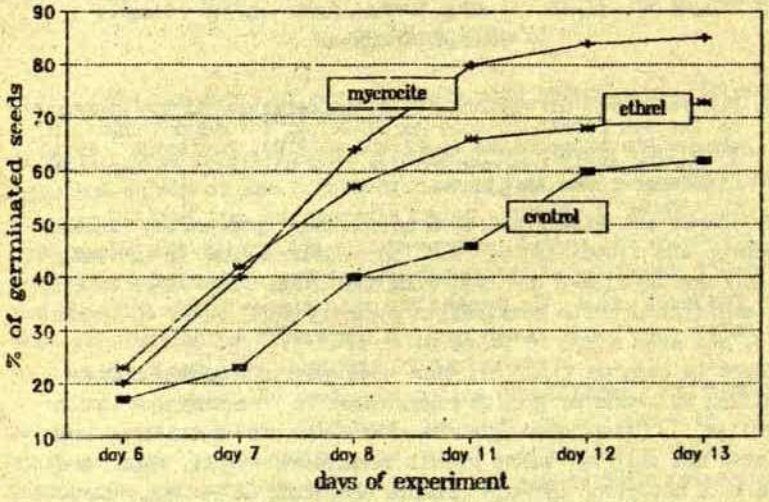

Fig. 3. Effect of mycrocite and ethrel on sea buckthorn seed germination intensity.

The difference between plants of separate variants remained also in further course of plant development. That allows to think that the development of generative buds and sex differentiation also may be influenced. The next experiments will give answer to this question.

Conclusion

The experiments illustrate the dependence of the sea buckthorn seed germination capacity from temperature conditions during storage time.

Results were obtained that the germination capacity of sea buckthorn seeds may be remarkably changed if one regulates seed storage conditions. So the storage of seeds for a two month at a regularly changeable (12 hours at 10-12°C and 12 hours at 20-22°C temperature) germination capacity increased.

The seed germination and growth of seedlings were essentially influenced by seed pre-sowing treatment with ethrel and mycrocite (an cytokinin containing substance). It was stated that growth and physiological indices were similar in them which were observed in adequate experiments with cucumber plants. It can be hoped, that sex of sea buckthorn seedlings also will be diverted toward femaleness. This statement may possess a great importance in selection work of sea buckthorn.

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Testing of Carcinogene Urethane by Conversion of Yeast *Saccharomyces cerevisiae* Gene

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SUMMARY

For environmental pollution screening a specially constructed strain of Saccharomyces cerevisiae with a mutation in a gene that codes for one of the enzymes involved in the synthesis of adenine was used,as a result this strain cannot synthesize adenine,it must be present in the medium for growth to occur. When a conversion caused by environmental pollution factor occurs at the site of this gene mutation,the latter conversion can restore it's adenine synthesizing capabilities and it can be observed by colonies colour change.

INTRODUCTION

The increasing environmental pollution contains a lot of carcinogenes. Most of carcinogenes possess also mutagenic characteristics (approx.90% of carcinogenic substances),and for testing these carcinogenes classical mutagenic identification methods are used. Nevertheless,a part of carcinogenes do not possess mutagenic characteristics or they are expressed weakly. Such carcinogenes can be tested only in experiments with mammals or with the aid of cell cultures from mammals . Experiments of such a kind are very time consuming, therefore they cannot be used for massive screening tests. That is the reason of attractiveness of methods in which nonmutagenous carcinogenes can be tested by the aid of microorganisms. For these experiments yeasts Saccharomyces cerevisiae were used successfully. It was established (1) in experiments with mice cell cultures and yeasts that cancer promoters enhance the mitotic recombination frequency. Intragenic recombination conversion

is the recombination mechanism of genetic material that is important in carcinogenesis and induction of which stimulates the induction of malignant transformation,

Recently with the aid of genetic engineering methods systems were constructed which allow to register the recombination of genes and to apply these events for identification of nonmutagenous carcinogenes. Such system was elaborated with the line of yeasts in which his3 gene changes in a wild type HIS3. Evolution of this system gives the evidence of carcinogenic action of nonmutagenic substances(2).

MATERIALS AND METHODS

In the present investigation the frequency of gene conversion was registered by the aid of S.cerevisiae mutants ade1 and ade2. The auxotroficity of adenine and accumulation of red pigment was the result of mutations in the genes. Estimation of conversion frequency was made by using constructed heteroallelic diploid lines ade1 aned ade2. Such heteroallelic diploids ade1 and ade2 store the red pigment and are not able to grow in nutrient media without adenine. Convertants that emerge as a result of the unreciprocal recombination of genes, are the wild ones, they don't store the red pigment and are capable of growing on media without adenine.

Media for convertant accounting are prepared with limited quantity of adenine. Plating is made with such cell density that the quantity of colonies on Petri-dishes should not exceed 60-90. Heteroallelic adenine deficient colonies are growing so long till the adenine of media is escaped. After adenine is lacking, red cells persist to divide and thus limit the size of the red colonies. On their surface white colour colonies start to grow, because convertants are not sensitive to adenine deficiency.

The effect of different chemicals (in that count also urethane) was carried out by adding these chemicals to nutritient media,or by treating the cells before plating.

RESULTS AND DISCUSSION

Conversion was induced by urethane (ethylcarbamide) $NH_2COCH_2CH_3$. In experiments ad2 gene heterophylic hybrids were used.They were constructed from genetic lines created in St Petersburg. Urethane was added to cultivation ,media at concentration 10mkg/ml,20mkg/ml ,30mkg/ml, etc.
Results were layed out on Fig.1. and Fig.2..
According to published information(Skives,1991) urethane acts as tumour induces and cocarcerogen,and also as radiation induced cancer promoter.It is proposed that urethane requires prior metabolism to go get mutanogenic and carcinogenic.The enzymes responsible for matabolic activation of procarcinogens are principally species of cytochrome P-450.These enzymes are metabolizing urethane to vinylcarbamide,from which reactive epoxides are created.

Urethane > vinylcarbamid > epoxyethylcarbamide

All 4 hybrids reveals an enhancing convertant creation rate if the urethane concentration was increased. The most appropriate for urethane was the hybrid H3,the results of which were further demonstrated.For hybrid H9 without added Urethane the average conversion frequency was 9.2.10*exp -8,but at urethane concentration 3o mkg/ml the conversion frequency was 7.8*10exp-7. Also for hybrid H16 the conversion frequency without urethane was 10exp-6 and at urethane concentration 30mkg/ml it enhanced to 1.2*10exp-7.
It should be mentioned that the induced conversion frequency of respiring mutants without and with added urethane was higher than in stem lines. For example, for hybrid H9 the conversion frequency was 1.2-2.6 times higher than for a hybrid with unchanged phenotype.

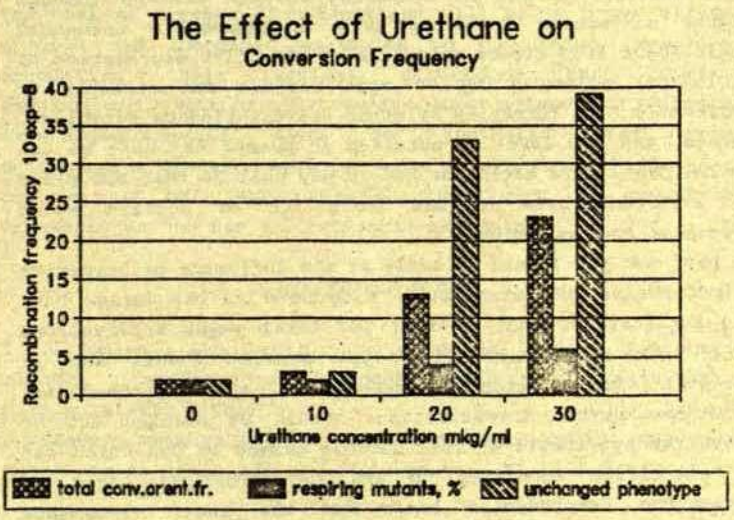
It is believed that urethane does not possess distinct mutagenic capacity,although it was capable to induce mutagenesis ,especially in respiring mutants.

Fig.1.
The effect of urethane, mkg/ml, on conversion frequency of *Saccharomyces cerevisiae* hybrids

			Recombination frequency *10^{-8}			
Nr.	hybrd.	conctr urhtan mg/ml	convertants t. creation frequency	unchanged phenctype	respiring mutants	Respiring mutants %$
1	H16	0	14	---	---	---
1		20	6	---	---	---
4		0	0.2	---	---	---
6		0	3.1	---	---	---
6		20	8.8	---	---	---
9		0	1.9	1.9	0	0
9		10	2.6	2.5	5.3	3.2
9		20	4.9	3,5	7.8	32.6
9		30	6.6	5.4	8.7	39.2
10		0	3.4	1.7	11.5	30
10		10	4.3	1.8	13.5	55.5
10		20	6.9	4.2	24.3	63.3
10		30	10.0	7.6	28.1	76.5
11		0	0.6	0.6	0	0
11		10	1.4	1.4	0	0
11		20	3.8	3.8	0	0
2	H9	0	11.0	---	---	---
2		20	14.1	---	---	---
12		0	9.2	9.2	0	0
12		10	13.2	12.6	14.9	2.0
12		20	32.9	29.3	40.0	4.7
12		30	78.0	42.6	112.0	5.4
5	H10	0	2.7	---	---	---
5		20	14.7	---	---	---
8		0	12.9	6.5	16.6	1.9
8		10	34.7	32.9	46.6	10.4
8		20	54,5	41.3	107.0	5.7
3	H14	0	14.0	---	---	---
3		20	9.0	---	---	---
7		0	7.4	7.4	0	0
7		10	12.9	9.0	38.5	5.8

As the cells on which urethane acted were diploids, then one can speculate, that these mutants were cytoplasmatic ones. Acccrding to some carcinogenic theories,it is the changes in mitochondria which are essential in carcerogenic transformation. It should be mentioned also that yeast respiring mutants are also used as a model for investigating cancer cells. For different hybrids the respiring deficient mutant creation frequancy at the same urethane concentrations was different.

Fig.2.



The highest creation frequency has been observed for hybrid H16-at least 76 % of all colonies were respiring deficient mutants,for hybrid H10-10.4 %,for hybrid H14- 7.4 % and hybrid H9- 5.4 %.

Applying urethane to Ad2 gene conversion accounting system the corresponding results were :urethane 10mkg/ml,20mkg/mland 30mkg/ml creates the enhancement of gene conversion proportional to urethane concentration. In accordance with the knowledge,that urethan acquires carcenogenic character as the result of metabolic activation,the results,of urethane capability inducing respiring deficient mutants ,and enhancing the conversion frequency ,lets to suggest,that in yeasts th urethane metabolic activation is due to cytochrome P450.

The current investigation reveals that yeasts can be used for identification carcinogenes whose character is only revealed after activation.Yeast cells possess a further advantage in that not only toxic and mutagenic effects can occur ,but effects on cell growth ,morphology and differentiation are

also readily visibl. In respiring mutants uretane induces higher conversion frequency than in phenotypic unchanged cells.Apart from direct oxidative or reductive degradation of biological molecules by O2-,this radical can directly or indirectly (via formation of other radicals)induce mutations. Indeed, the low level of mutation in yeasts may well be due to the protective action of SOD.It may well be that SOD plays an important role in the inhibition of certain aging processes and mutations.

We have not yet looked directly at the influence of oxygen on mitochondrial DNA damage.Could mitochondrial DNA damage have any implications what so ever for total yeast survival?One must look at mitochondrial type genetic events and at survival in mutants defective in mitochondrial functions.Further investigations should be carried out to prove the hypothesis of free radical damage to DNA which can be classified according to the two principal sites of attack,the heterocyclic bases and the sugar -phosphate backbone .Base damage by altering the nucleotide sequance of the DNA template disrupts the many processes operating under genetic control.Disruption of phosphodiester bond caused by damage to the DNA -backbone produces single and double strand breaks,releases intact or altered bases or nucleoside fragments and concomitant loss of integrity of the DNA structure and conformation. The nucleic acid bases are considerably more radiosensitive than the sugar -phosphate backbone,reflecting their higher reactivity towards primary species attack. It is known that cytochrom P-450 is generated by cultivation at low O2 pressures.Enhancing O2 pressure will lead to lowering of P450 content and that reveals complications of confirming this hypothesis.

It will be the task of future investigations in yeast system which is after all a eukaryotic one being exploited in the presence of appropriate biochemical activation of promutagenes to further this comparison of the repair of chemical adducts to DNA with the repair of radical injury,using the oxygen effect as a probe.

The task of further investigations will be to prove various organic and inorganic molecules on conversion frequency, accounting their various structures that confers carcinogenity.Most of them require prior metabolism to become carcinogenic.Recent work has indicated that yeast mytochondria contains such proteins as cytochrome P-450 which can be used for enhancing the sensitivity of conversion frequency method for environmental screening.

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Correlation of Some Physico-Mechanical Factors of Leathers with Ultrasound Propagating Speed.

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INTRODUCTION

Ultrasound - it is resilient oscillacions and waves whose frequency exceeds 1.5.104 - 2.104 Hz or 15 - 20 kHz. Propagation of the ultrasound obeys basic laws which are general for acoustic waves of any frequency range. A characteristic peculiarity of the present level of the ultrasound's physics and technics is the vast multiplicity of its application encompassing the frequency range from the audible sound up to the ultimately attainable high frequencies and the capacity region from fractions of a miliwatt up to tens of kilowatts [1].

Ultrasonic methods are used in investigation of properties and structure of substances, in evincing of the processes occurring in them on the macro- and microlevel, in determination of resilient and resistant characteristics [2]. The ultrasonic method is a non-destructive control method and takes up a leading role among a number of novel methods of nondestructive control. The peculiarities of the ultrasonic method and its wide application in non-destructive control of non-metallic materials are connected with its following characteristics:

- method is made use of in tests of manufactures with various form and thickness;
- method is used in study of manufactures and materials accessible only from one side;
- using this method enables to obtain information on the material's quality both in the process of its operation and in the process of its manufacturing;
- method is harmless, it does not require biological

protection and measurements by this method demand little time.

METHODS

Samples selection.

Samples were selected according to demands of USSR State standard 938 0-75 "Leather. Acceptance rules. Methods of sample selection" in the Laboratory of the joint-stock company "Cosmoss" [3].

Samples preparation.

Samples were prepared for physico-mechanical tests according to demands of USSR State standard 938.12-70 "Leather. Method of samples preparation for physico-chemical tests" [4].

Samples investigation.

Stretching test

Examination was performed in accordance with demands of USSR State standard 938.11-69 "Leather.Method of testing as to expanding" [5].

Ultrasound test

The principle of quality measuring by the ultrasonic method of the leather samples under test is based on the fact that the piezoirradiator sends impulses of ultrasonic waves with the corresponding frequency into the investigation object. Ultrasonic oscillations pass through the leather samples and, influencing the transformer-receiver, are transformed into electrical oscillations which upon passage through the amplifying juncture are communicated to the indicator of electronic beams [2].

As the rate of the ultrasound impulses and the resilient properties of the material are interconnected, then the rate of longitudinal waves as well as of transverse and

superficial waves can be used in non-destructive control of natural leathers.

An indispensable condition of ultrasonic measurements is to create an acoustic contact between the ultrasound transformer and the manufactured item to be controlled. As an ultrasound transformer the ultrasonic appliance UK-10P was used with point contacts (Fig. 1), but for the control, natural leather of ox-skin and pig-skin.

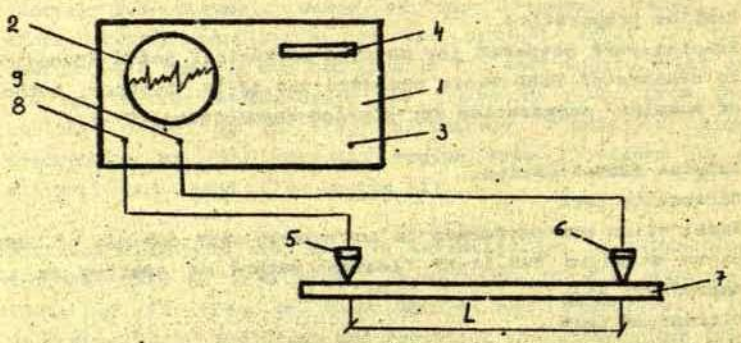

Fig.1. Block-scheme of the ultrasonic appliance.
1 - ultrasonic appliance,
2 - electronic ray indicator,
3 - regulator of the signal increase,
4 - electronic counter of time,
5 - piezotransformer-receiver,
6 - piezotransformer-irradiator,
7 - sample under study,
8 - entrance of the generator,
9 - exit of the generator.

RESULTS

In practice most often the rate of impulses of the longitudinal waves is used, because they are easiest to measure.

The rate of propagation of ultrasonic oscillations in leather is determined by the formula

$$C = \frac{L}{T - \Delta T} \cdot 103, \qquad /1/$$

where C - rate of the ultrasound propagation in the material, m/sec;

L - distance between the piezotransformers, mm;

T - time of propagation of the ultrasonic wave, μsec;

ΔT - delay of the time of propagation of the ultrasound, sec.

The delay of the time of ultrasound propagation is determined by the method of longitudinal profiling.

Delay of time for pigskin ΔT - 97 μsec (Fig. 2a),

for oxskin ΔT - 249 μsec (Fig. 2b).

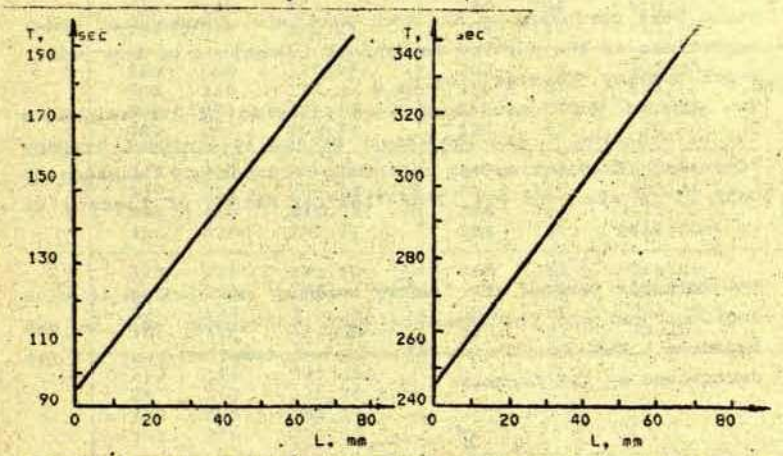

Fig.2 Diagram of the correlation dependence of the time of ultrasonic waves propagation in dependence on the measurement basis: a - in pigskin, b - in oxskin.

Leather is an anisotropic material, therefore during the tests longitudinal and transverse directions of scission of the samples were taken into account.

The coefficient of anisotropy is determined as a ratio between the passage rate of ultrasound in the longitudinal direction and the passage rate of ultrasound in the transverse direction:

$$K = \frac{\uparrow c}{\rightarrow c} \quad /2/,$$

where $\uparrow c$ - passage rate of ultrasound in the sample with longitudinal direction, m/sec;

$\rightarrow c$ - passage rate of ultrasound in the sample with transverse direction, m/sec.

Calculations of the anisotropy coefficient are shown in Table 1. After ultrasound was passed through the leather samples, they were subjected to physico-mechanical tests to determine the tear resistance.

Tests were performed on the tear machine - dynamometer under conditions of the physico-mechanical laboratory of the joint-stock company "Cosmoss" [6].

The samples were used which were selected by lab-asistants for examination in the Laboratory of the joint-stock company "Cosmoss". Examination was performed accordingly demands of USSR State standard 938.11-69 "Leather.Method of testing as to expanding".

The workable part of the leather samples was divided in five sections and the thickness of each odd-number section was measured. The resistance of samples towards tear is determined by the formula:

$$\sigma = \frac{P}{S} \quad /3/,$$

where P - load on the sample in the moment of tear, H;

S - area of the transverse section in the moment of tear, mm^2.

Table 1. Results of experimental measurements of samples of pigskin and oxskin by ultrasonic method.

Sample number	Pigskin T, μsec	T-ΔT, μsec	C, m/sec	K	Oxskin T, μsec	T-ΔT, μsec	C, m/sec	K
1	278	181	353,59		364	115	556,52	
	253	156	410,26	0,94	359	110	581,82	1,12
	259	162	395,06		375	126	507,94	
	250	153	418,30		375	126	507,94	
2	293	196	326,53		361	112	571,43	
	253	156	410,26	0,73	364	115	556,52	1,00
	222	125	512,00		361	112	571,43	
	224	127	503,94		364	115	556,52	
3	251	154	415,38		365	116	551,72	
	242	145	441,38	0,99	365	113	566,37	1,07
	245	148	432,43		368	119	537,82	
	245	148	432,43		374	125	512,00	
4	264	167	383,23		333	84	761,90	
	264	167	383,23	1,52	331	82	780,49	4,27
	344	247	259,11		305	156	410,26	
	359	262	244,27		301	152	421,05	
5	605	508	125,98		343	94	680,85	
	632	535	119,63	0,82	356	107	598,13	3,84
	568	471	135,88		326	77	169,76	
	489	392	163,27		335	86	165,80	
6	325	228	280,70		347	98	653,06	
	339	242	264,46	1,27	376	127	503,94	1,28
	383	286	223,78		396	147	435,37	
	411	314	203,82		386	137	467,15	
7	323	226	283,19		363	114	561,40	
	332	235	272,34	0,66	373	124	516,13	3,25
	244	147	535,37		340	91	703,29	
	255	158	405,06		341	82	780,49	
8	242	145	441,38					
	241	146	138,36	1,00				
	323	226	283,19					
	313	216	296,30					
9	243	146	438,37					
	233	136	470,59	1,19				
	285	188	340,43					
	248	151	423,84					

Table 2. Results of experimental measurements of samples of the leather carried out by physico-mechanical method and by ultrasonic method

Smp. Nb.	Pig-skin P, N	S, mm²	σ, N/mm²	E, mm	σ*, N/mm²	Ox-skin P, N	S, mm²	σ, N/mm²	E, mm	σ*, N/mm²
1	27,0	1,4	1,92	12	1,73	45,6	1,5	3,04	18	2,39
	30,2	1,4	2,16	14	1,73	36,3	1,4	2,59	16	2,48
	32,8	1,4	2,34	13	2,24	27,8	1,3	2,14	22	2,12
	28,2	1,4	2,73	12	2,31	29,6	1,4	2,11	10	2,12
2	25,8	1,4	1,84	22	1,72	18,3	1,2	1,52	18	2,44
	26,6	1,3	2,05	23	1,73	16,8	1,2	1,40	20	2,39
	45,0	1,4	3,21	11	2,54	26,2	1,2	2,18	12	2,21
	49,6	1,4	3,54	11	2,52	30,2	1,2	2,52	11	2,19
3	25,4	1,4	1,81	13	1,73	34,4	1,3	2,65	16	2,37
	25,4	1,4	1,81	12	1,73	33,2	1,3	2,55	17	2,43
	33,2	1,4	2,37	11	2,34	28,8	1,4	2,06	20	2,16
	33,4	1,4	2,39	10	2,34	30,3	1,3	2,33	18	2,13
4	19,7	1,4	1,41	11	1,73	45,0	1,4	3,21	10	3,15
	24,5	1,0	2,45	8	1,73	43,6	1,4	3,11	9	3,22
	23,0	1,0	2,30	9	2,16	24,0	1,4	1,71	23	1,90
	24,8	1,0	2,48	9	1,87	22,8	1,4	1,63	22	1,99
5	20,0	1,2	1,67	16	1,72	40,0	1,3	3,08	10	2,85
	21,0	1,2	1,75	16	1,72	41,8	1,3	3,22	11	3,54
	27,0	1,3	2,08	11	1,60	22,7	1,4	1,62	26	1,65
	26,0	1,3	2,00	11	1,67	24,0	1,4	1,71	27	1,64
6	15,2	1,4	1,09	12	1,72	33,8	1,5	2,25	10	2,75
	17,0	1,0	1,70	8	1,73	25,4	1,4	1,81	12	2.20
	16,4	1,0	1,64	13	1,81	24,0	1,5	1,60	17	2,01
	16,8	1,0	1,68	13	1,76	25,4	1,5	1,59	17	2,06
7	18,0	1,0	1,80	6	1,72	34,7	1,4	2,48	12	2,41
	18,6	1,0	1,86	7	1,73	35,2	1,4	2,51	12	2,24
	24,2	1,1	2,20	9	2,34	26,7	1,6	1,67	24	1,39
	19,8	1,1	1,80	10	2,27	25,0	1,5	1,67	24	1,50
8	17,0	1,1	1,55	11	1,73					
	21,2	1,2	1,77	11	1,72					
	15,6	1,2	1,30	15	1,96					
	17,6	1,2	1,47	16	2,00					
9	14,7	1,0	1,47	10	1,73					
	13,2	1,4	0,94	11	1,73					
	17,6	1,1	1,60	11	2,11					
	19,4	1,0	1,94	9	2,31					

σ* - hypothetical

The data of the passage rate of ultrasound and tear resistance of leathers are given in Tables 2 and in Figures 4, 5, 6, 7.

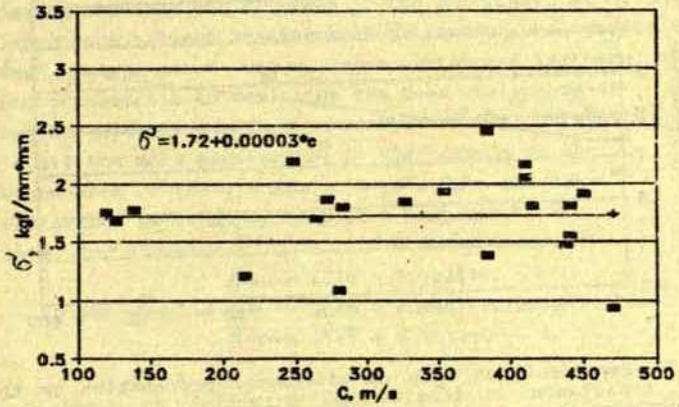

Fig.3. Dependence on rate of ultrasound propagation on the tear resistance in longitudinal direction of cutting of pig-skin samples.

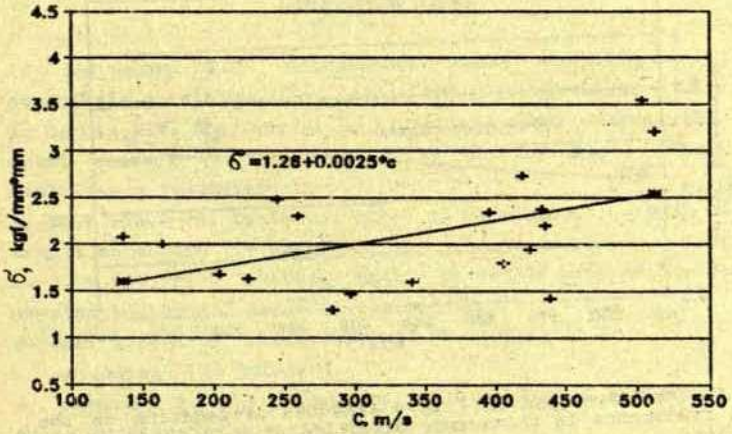

Fig.4. Dependence on rate of ultrasound propagation on the tear resistance in transverse direction of cutting of pig-skin samples.

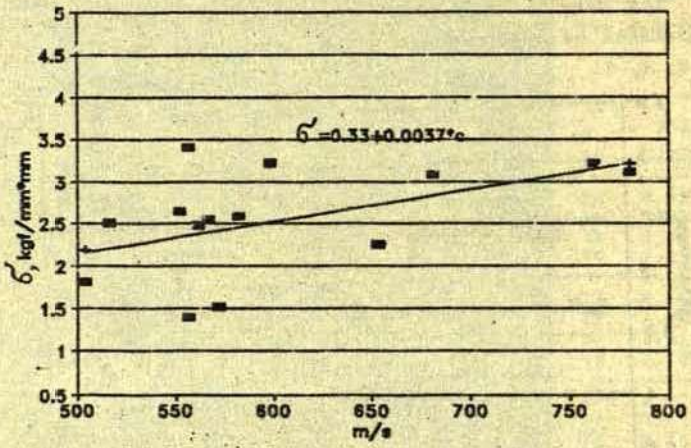

Fig.5. Dependence on rate of ultrasound propagation on the tear resistance in longitudinal direction of cutting of ox-skin samples.

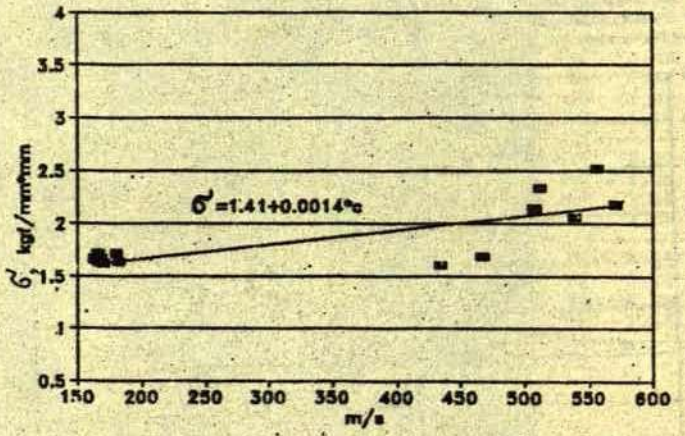

Fig.6. Dependence on rate of ultrasound propagation on the tear resistance in transverse direction of cutting of ox-skin samples.

CONCLUSIONS

1. Coefficient of anisotropy of the dependence equals for pig-skins - 0.01, for ox-skins - 2.26.
2. Having obtained the diagrams of the correlational dependence between the rates of ultrasound propagation and tear resistance of various types of natural leathers, it became possible to determine the tear resistance of leather without destruction of the samples. Determination is all the more accelerated if the formula of the dependence of the ultrasound's rate upon the tear resistance is found by help of a computer.

Thus, for pig-skins $\sigma_{\uparrow} = 1.72 + 0.00003 * C$ (N/mm^2),

$\sigma_{\rightarrow} = 1.26 + 0.0025 * C$,

for ox-skins $\sigma_{\uparrow} = 0.33 + 0.0037 * C$ (N/mm^2),

$\sigma_{\rightarrow} = 1.41 + 0.0014 * C$

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Dehydrated Bakers' Yeasts Possess a Cytochrom b in Low Reduction Rate, as Estimated by Reflectance Spectroscopy

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SUMMARY

Reflective and transmission spectroscopy revealed that in desiccated yeasts the cytochrome b is in a partially oxidized state. Low cytochrome b reduction rate in desiccated yeasts may lessen superoxide radical formation possibilities.

INTRODUCTION

The structural organization of the respiratory chain has been subject of considerable study because that gives information about yeasts functional state ,what is important for regulating cultivation conditions to prepare a product that can be used for producing dehydrated yeasts.

Bulk of our knowledge about yeasts has been acquired from studies in aqueous solutions. The cytochrome reduction rate depends upon the function of the respiratory chain. Electrons flow from substrate through a series of cytochromes to molecular oxygen.It represents a dynamic equilibrium. Our previous observations revealed that high quality rehydrated yeasts possess in a dynamic equilibrium, where oxygen does not limit the flow ,partially reduced cytochromes ,where the highest reduction level corresponds to cytochrome c ,(50-70% reduced), and the lowest to cytochrome b (30-50% reduced). Cytochrome a at the first moment after rehydration is in a more reduced state, but after some minutes it enters a more oxidized state with a reduction quotient of 30-50%. Cytochrome b gets more oxidized when endogenous substrate is exhausted(1). NADH addition to rehydration media enhances

cytochrome b reduction. In such a way cyt b reduction rate is substrate -dependant. Obviously NADH can enter the outer cell membrane and mitochondrial membrane. Bubbling the rehydrated yeast suspension with 100% oxygen increases NADH oxidation rate and approx mately 4 times enhances oxygen consumption. Presumably cytochrome b reduction state in rehydrated yeasts depends upon NADH availability (2,3). Measurements of cytochrome b reduction degree were carried out in a rehydrated state, at which the enzyme molecules are located in a media with a little water content (6-12%) and that does not suffice to determine the optical properties of cytochromes, because the disturbing scattering of light is not low. The aim of these observations was to get more information about cytochrome b reduction state in dehydrated yeasts where the light breaking properties of cell constituents are very significant and that results in increased light scattering ,as a result background absorption increases with decreasing wavelength.The result is transparency decrease. One must take into account distortion possibilities by estimating cytochrome absorption values (4). To avoid disturbancies connected with low transparency these investigations were carried out by using reflected spectra which, by applying Kirchof's sphere, do not possess this disadvantage, because all light is collected (4). These investigations are interesting from the view-point that a method of analysing reflected light to estimate food products quality is getting popular at this time,because such analysis does not require sample preparation which is time-consuming and therefore does not suit for mass analysis. The second reason for these investigations is that experiments carried out with a good optical instrument give also information about other cell ingredients,which in reduced state can serve as a donor of electrons to cytochrome b, whose reduction state was interesting to observe in various commercial yeasts. The theoretical basis for such investigations is the literature material, that enzymes can function as catalysts in dehydrated yeasts containing little water (5). If yeasts

are dehydrated, the enzymes are suspended, but not dissolved (as in water). Such heterogeneous dispersions may be plagued by the hindered diffusion of substrates to the enzyme particles. It is yet not clear how much water do enzymes need. Investigators (5) have published data that enzymes act when there is not a true layer of water, but rather just a few clusters (presumably around charged groups of the protein). Alcohol dehydrogenase ,tyrosinase and alcohol oxidase are catalytically active in organic solvents when several hundred water molecules are bound per enzyme molecule. These levels approach a monolayer coverage and are comparable to the water contents required for the onset of catalytic activity of dry enzymes without any solvent. A little water is used as bound water that enhances the enzymes conformational flexibility which in turn should promote catalysis. Hydration accelerates enzymatic reactions. Enzyme resistance to undesirable factors depends only on its bound water, and not on the free water in the solvent, as long as the essential bound water is present. Heat-induced unfolding of enzymes requires ample confirmational mobility. All reactions causing irreversible thermoinactivation of enzymes involves free water. Thus both processes should be drastically hindered by dehydration. In hydrated state enzyme is inactivated at a lower temperature than in the dehydrated one,but some are not. Some enzymes exhibit marked storage stability in dehydrated state.It is not known why some enzymes are more sensitive to dehydration than others and why some enzymes for their functioning require more water than others'.These questions cannot be solved by investigation the dynamic equilibrium state of cytochromes,and don't enable to determine the result of enzyme function at different velocities. The aim of this work was only to find information about cytochrom reduction state in dehydrated yeasts, which can be used for further investigations to explain the low reduction rate of cytochrome b as a tactic manoeuvre by the cell to preserve themselves from free radical damage.

Water can also have an effect on free radical quenching efficiency by various scavengers . Plato C.Lee and others(6) have shown the life time of singlet oxygen and its quenching by azide ions and tryptophan in normal micells ard reverse micells. At w > 30 the water pool size increases up to 50.10-10m in diameter ,the quenching behaviour of azide ion is practically the same as that in bulk water. As w decreases below 30,the quenching efficiency by azide is diminished. The above results clearly indicate gradual changes of water structure and mobility from fluid-like at w >30 to ice-like at w< 30.

Free radicals can damage yeast cells (6,7) .It is not ruled out that superoxide radical can be scavenged by cytochrome c,by reducing it and that reveals the necessity of cytochrome investigations in dehydrated state,what was possible by reflectance spectroscopy.
Results were also achieved by transparent light measurements. Although expressions have been developed for the interpretation of spectrophotometric measurements of granulated materials (4), little attention has been paid to the effects of scattering of the incident relation. Measurements may be made via varying amounts of the scattered radiation received (our unpublished observations with UV VIS,Carl Zeiss Jena).
Results of various authors(4) have shown that the peak of an absorption band will be depressed when measured in a suspension.

MATERIALS AND METHODS

Commercial bakers yeasts were analysed; Oetker - Which were considered as yeasts with high cell viability rate ,and molasse yeasts, a product of Druva with a low cell viability rate, ethanol yeasts 05,06 ,cultivated on ethanol ,and 16.06 and 17.06 molasses yeasts in Institute of Microbiology, Latvian Academy of Sciences. In most cases the cells were dried by dry air, temperature 37°C, and the process was

finished in 30 to 40 minutes, these samples were marked with "r"(rapid), in several cases the samples were dried in a moisture air, and the duration was prolonged to 24 hours - "sl" (slow).

The water content was 8 - 12 % .

In one case the cytochrome spectra of yeasts were compared with that of rats heart. Isolation of mitochrondria implies chopping of heart muscle with scissors,washing with sucrose to free it of blood as much as possible,homogenization of tissue in order to disrupt the cells and release of the subcellular components and removing the nuclei,whole cells,cell debris and red blood cells. Nevertheless the mitochondrial fraction so obtained contained some hemoglobin.

The reflected light was analysed by the aid of Perkin's Elmer lambda scan 9,by using a sphere for diffuse reflected light measurements.

The setcings for cytochrome determination in alpha region are :

01 ord/absc mode	d1/WL
03 scan speed	120 nm/min
04 response	0,5 s
05 lamp/det	VIS /860,8 nm
06 cycles/time	1 / 0,05 min
07 delta wavelength	1 nm
08 peak threshold	0,2 D1
09 recorder	off
10 ord min/max	- 500,0/5,000
11 abscissa min/max	500,0/650.0
12 abscissa format	20 nm/cm
13 printer	/ scale /

Other investigation were carried out in a 0,1 cm cuvette, using transparent light measurements by Beckman spectrophotometer.

The main setting for scanning mode and abscissa/ordinate data are given in graphs .

Cell clusters were grinded that enabled a better filling of cuvettes, the rate of which was 0,7g/1cm3.

Scanning Results are plotted as relations between cytochromes. The results are plotted on graphs, representing phenomene in such a mode which enables to summarise the cytochrome's b/c relations in dehydrated and rehydrated

yeasts. The relations are not formed as molar one's,but as absorption values - heights,measured from the base to top (distance from the foot to peak)between the basis line, connecting the lowest absorption values and the maximal values.

Some estimations of cytochrome b reduction state in dehydrated yeasts were carried out under mineral oil immersion that lowers the dissipation rate of light and permits to carry out measurements by light path in cuvett of 4 mm. In this case absorption determinations were made by a Carl Zeiss spectrophotometer UV VIS,which possesses a cuvette position near the photomultiplier,which increases the light reception angle and permits to analyze more thick samples.Some samples were allowed to desiccate in room temperature with high moisture content,in order to prolong the dehydration stage till 24 hours and might have an effect on cytochrome reduction state.

RESULTS

1.The measurements of absolute reflectance are adequate to characterize cell constituents,whose light absorption are slowly changing with wavelength,e.g., riboflavine derivates, but are less sensitive for observation of tiny absorbance changes such as cytochromes.

Such conclusions can be made by analysing reflected light from two commercial dehydrated baker's yeasts : "O" ,which represents "Oetker's" product, and "631",which is a product of" Druva"(name of enterprise in Riga).

From Fig.1 it is evident that "O" reflects light much better than "631",and that enables to determine the absorbing peaks.One can see that "O" possesses a broad absorption band between 230 -and 300 nm. This band lies in a range where aminoacids absorb. Some peaks superimposed on a wide peak can be attributed to cyclic aminoacids,such as phenilalanine, tirosine,and tryptophan,but it is not ruled out that in this region can also absorb eventually added antioxidant. A very tiny and flat absorption band can be seen at 343 nm.That

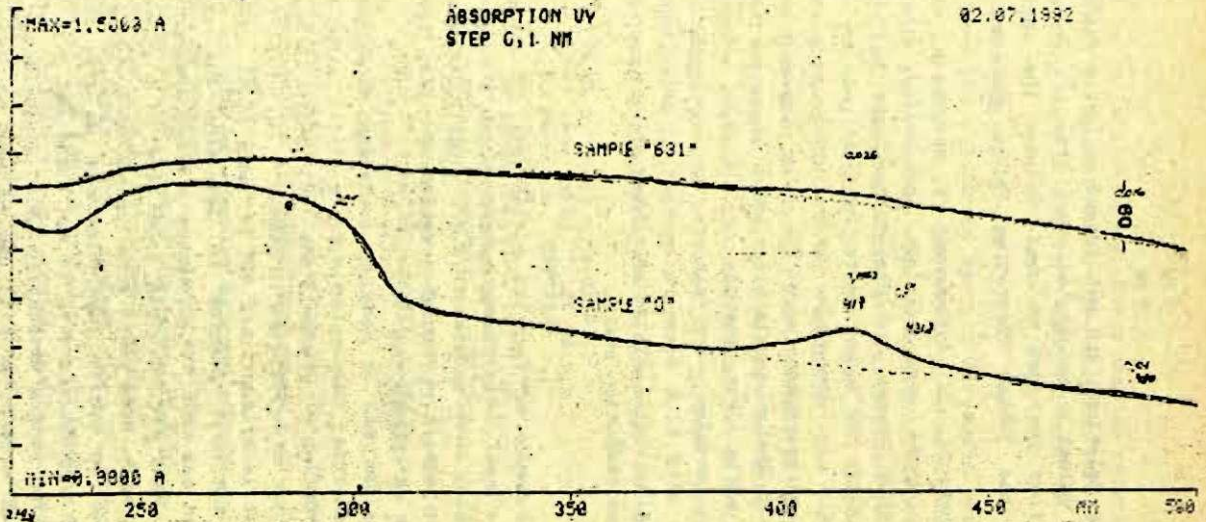

Fig.1. -log(reflected light) from two rehydrated yeast samples in relation to wavelenght in UV

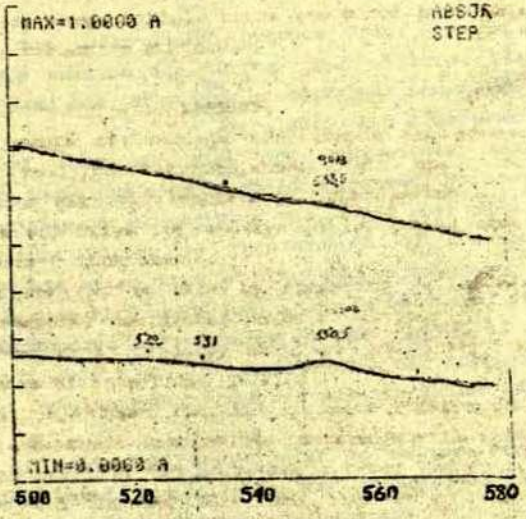

Fig.2. -log(reflected light) from two rehydrated yeast samples in relation to wavelenght in VIS

could be the reduced NADH .It is not easy to distinguish between reduced NADH absorption and oxidized riboflavin absorption .That such possibility exists, reveals the absorption band at 480 nm .Riboflavine absorption bands are more evident in sample "631".This is even more to feasible considering the higher background of "631",which in accordance with Munk's equation lowers the superimposed peaks values.It may be that the higher background of "631"is the reason why cytochromes in this product are less evident. However,estimating the cytochroms at various wavelength it is evident that cytochrome b in dehydrated yeasts is on a low reduction rate.Otherwise the peak in Soret at 430 nm, where cytochrome b absorbs, should be the predominant maximum, but not the 417 one,which is near to the absorption band of reduced cytochrome c.

In experiments with reduced cytochromes (not shown)the A431/A417 value is 1.1, but in the current experiment in dehydrated yeast the value is only 0.54 ,and that points to a low cytochrome b reduction state.
The same conclusions can also be made by observing corresponding absorption bands in beta and alpha ranges,which can be seen from Fig.2. For example, the corresponding values to cytocrom b and at wavelength 563 and 551 nm are O,004 and O,031, consequently the ratio of both peaks are O,004/O,031= O,13,but in a reduced state (not shown) it should be O,7.For "631"it is not so easy to estimate the cytochrome reduction state because the peak values are very low, therefore the next step was to analyse the derivated spectra.

2.The performances of using first derivative is the great amplification,because its baseline is less changing with wavelength than the absolute characteristics,therefore great amplification can be applied without descaling ,and that gives the possibility to distinguish between cytochroms.
The results of Perkin-Elmer lambda 9 scan are rapped as first derivative (Fig.3and4).That gives the opportunity to

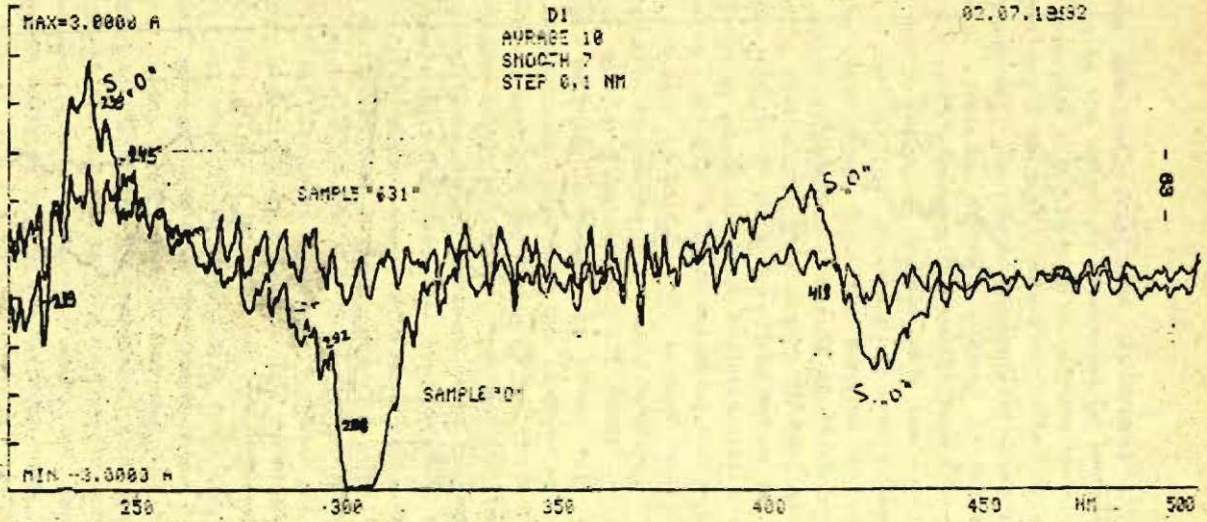

Fig.3.The 1st derivat. of -log(reflected light) from two rehydrated yeast samples in relation to wavelenght in UV

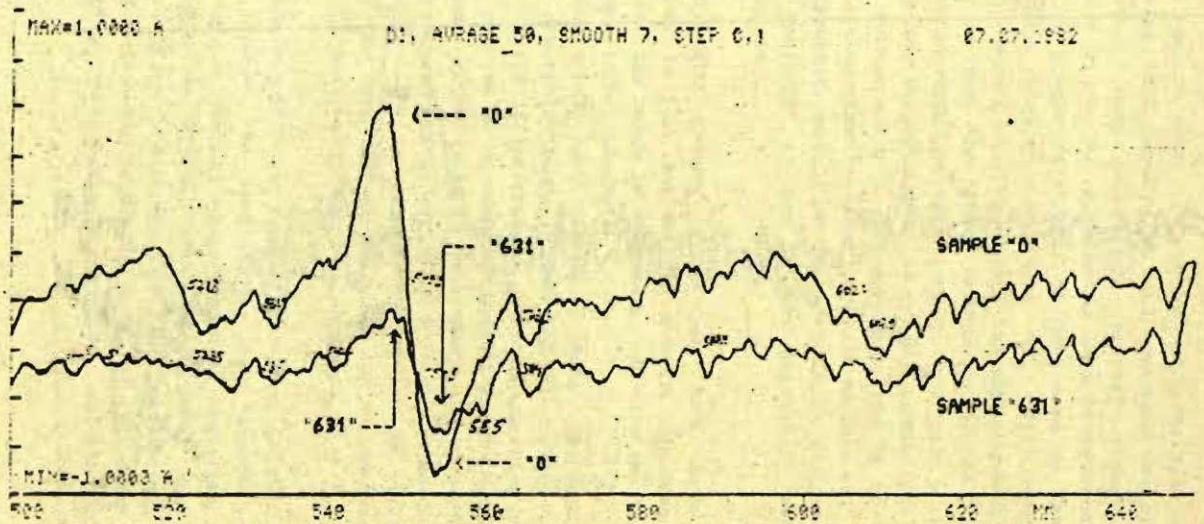

Fig.4.The 1st derivat of -log(reflected light) from two rehydrated yeast samples in relation to wavelenght in VIS

eliminate absolute reflectance differences ,the cause of which can be the different smoothness of surfaces. Oetker's yeasts possessed a little more reflectance than Druva's yeast ,and that could be due more tiny cells or scrambles of cells (detreet) in latter.Although reflectance values are very high (visually Oetker's product is coloured like a paper, but Druva's product is somewhat more gray-yellow.) The yellow tuning slowly changes with the wavelength.It is evident from the first derived,where both samples lie under "0" line ,that means ,that the absolute reflectance has a slope ,which reflect the lowering of reflected light with decreasing wavelength. This effect is more pronounced for "631".For sample "0" ,therefore this change is less felt as disturbance by using the first derivative.Nevertheless, if a peak is on a material with an absorbing background, the peak is not so easy to distinguish, because reflected light is attenuated in accordance with Munk's function. That partially explains the observation that all peaks corresponding to "631"are decreased,although it does not explain the difference between peaks which are in close neighbourhood. At longer wavelength (in the region of red light) the bases of both graphs nearly coincide. That enables to distinguish between the peaks, which represent rapid reflectance changes with wavelength. The fact that the baseline of the graph practically does not changes with wavelength, enables to use high amplification. Without such as amplificacion the cytochrome peaks are too tiny to be distinguished. This great amplification has some technical problems in registration process, because noise level is comparable with the information signal. To overcome this feature, repeated scanning was used. It is evident from these graphs,that alpha and beta absorption band of cytochroms b and c can be seen. In beta region tiny peaks at 509 and 512 nm are to be seen.The derivated for transmission light scans reveals maximums at 510 and 515 nm (Fig.5). Transmission light scans in reduced by dithionite rat heart mitochondria reveals absorption maximums at 508 and 514,5 nm where the height of

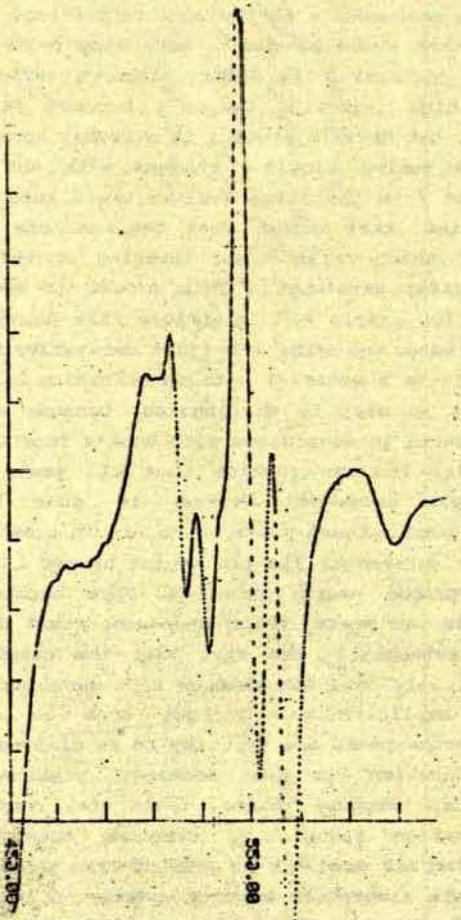

Fig.5.The 1st derivat. of wet yeast sample 05.08r in reduced state. light path 0.1cm

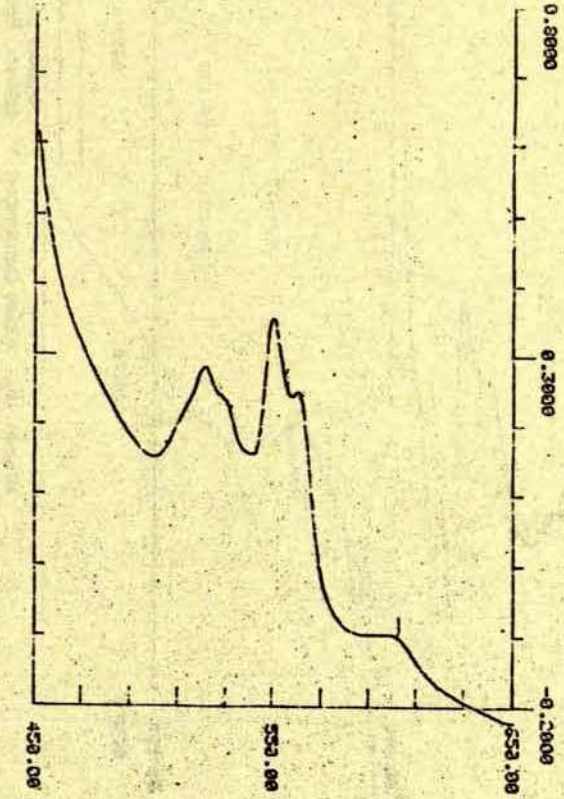

Fig.6.The absolute absorbance of wet yeast sample 05.06r in reduced state,light path 0.1cm

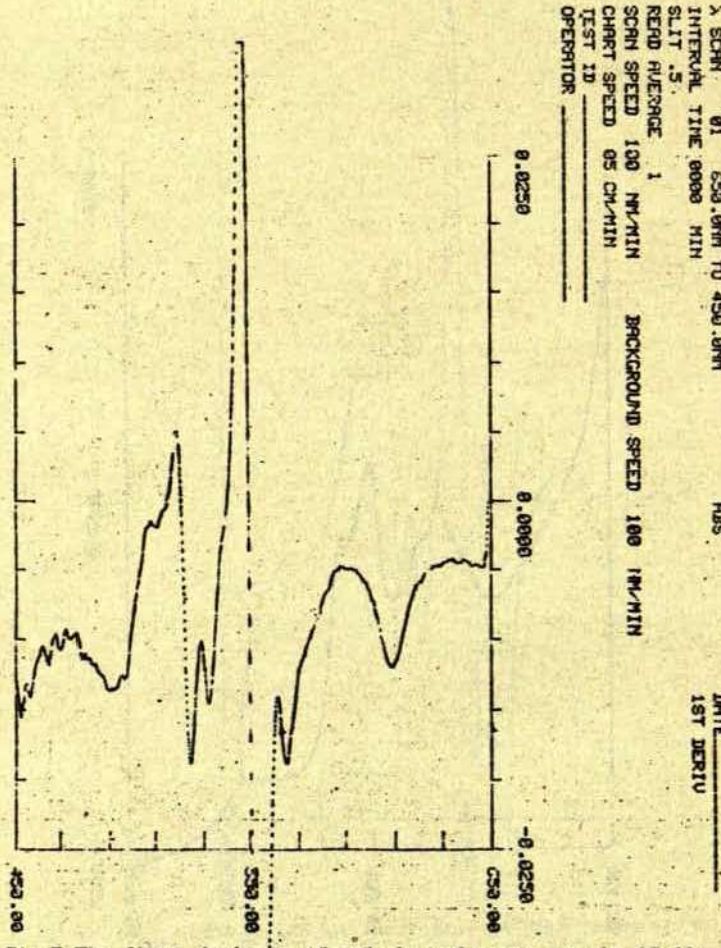

Fig.7.The first derivat of rehydrated yeast sample 05.06r in native state (the cytochromebis partially oxidized),light path 0.1cm,cuvett filled with ground yeasts

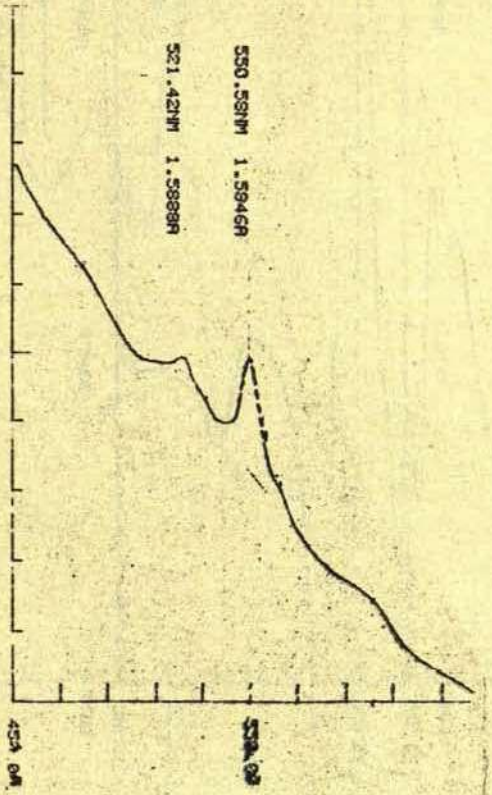

Fig.8.The first absolute absorbance of rehydrated yeast sample 05.06r in native state (the cytochromehis partially oxidized),light path 0.1cm.cuvett filled

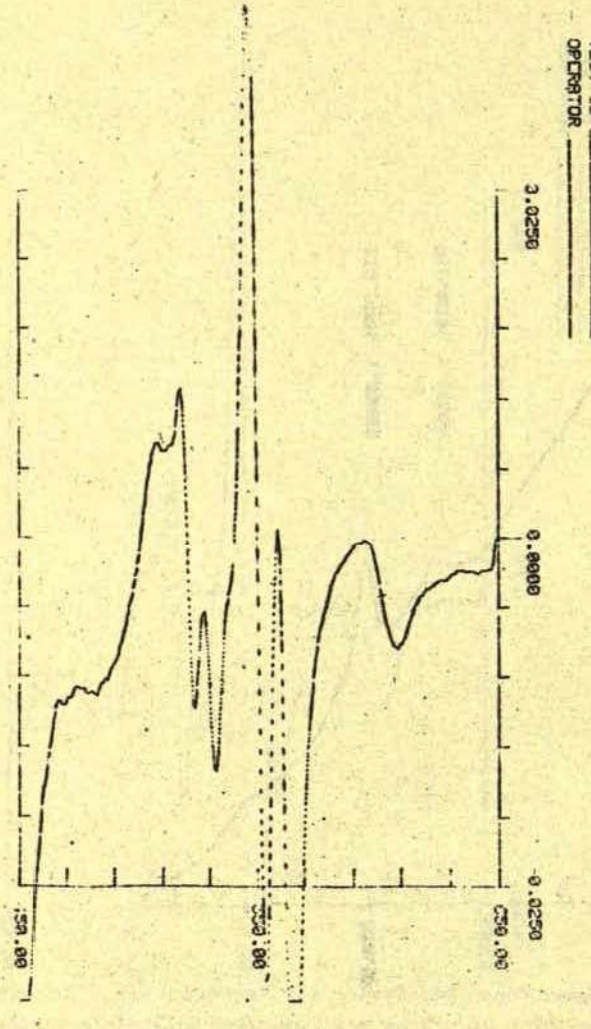

Fig.9.The 1st derivat ,wet yeast sample 05.06sl in reduced state,light path 0.1cm

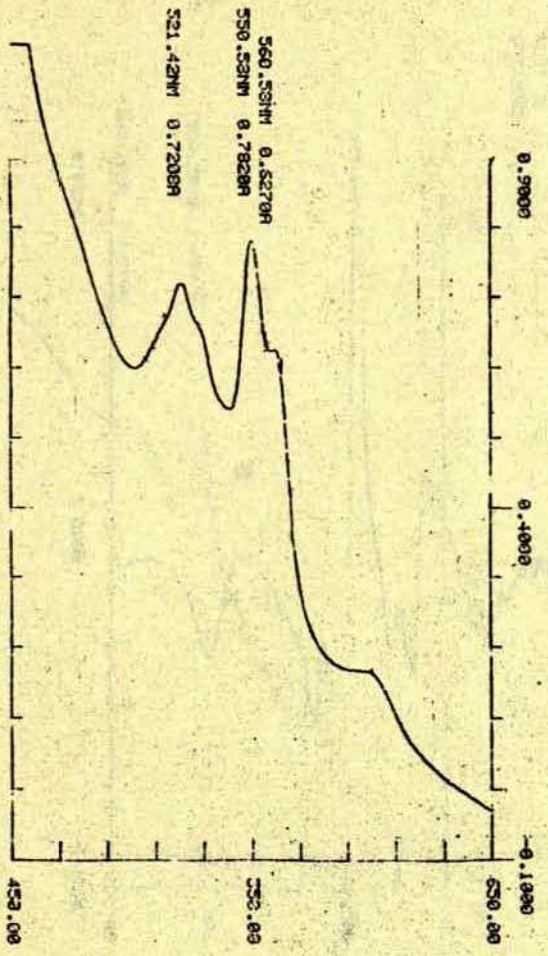

Fig10.The absolute absorbance of wet yeast sample 05.06s1 in reduced state,light path 0.1cm

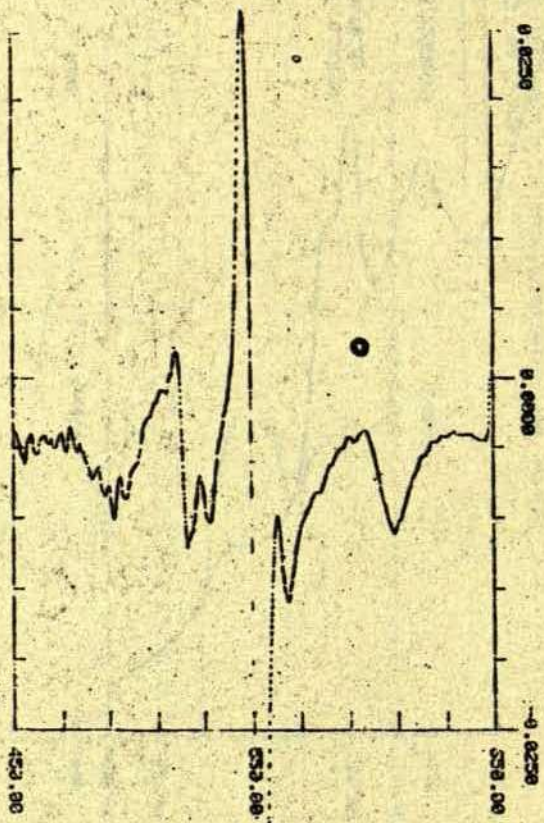

Fig.4. The first derivat
of rehydrated yeast sample
05.06e1 in native state(the cytochrome_b_
is partially oxidized),light path
0.1cm,cuvett filled with ground yeasts

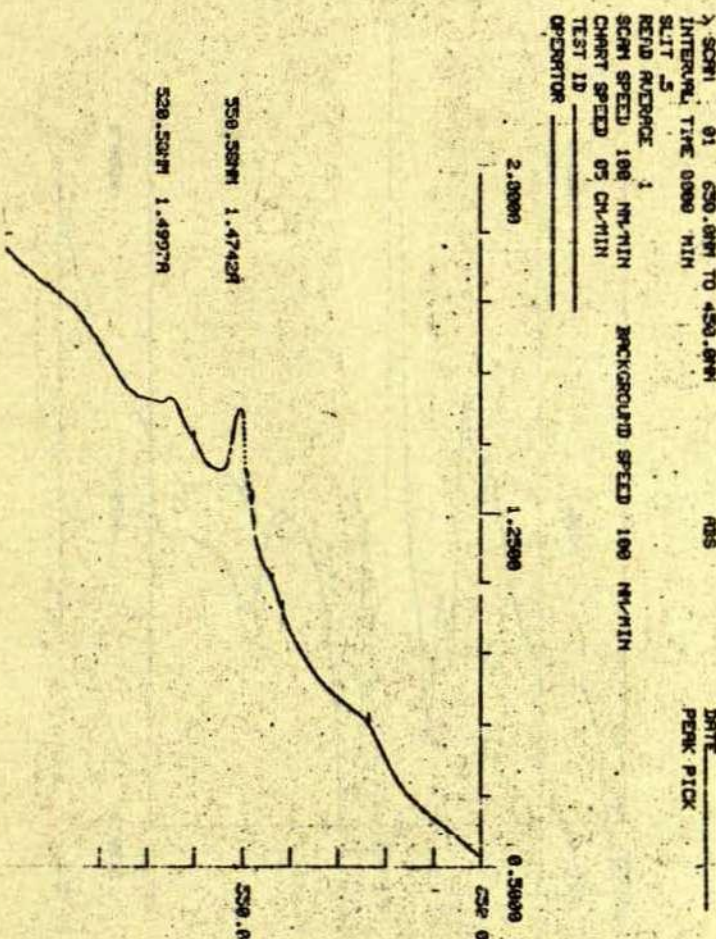

Fig.12. The absolute absorbance of rehydrated yeast sample 05.06e1 in native state (the cytochrome b is partially oxidized), light path 0.1cm, cuvette filled with ground yeasts

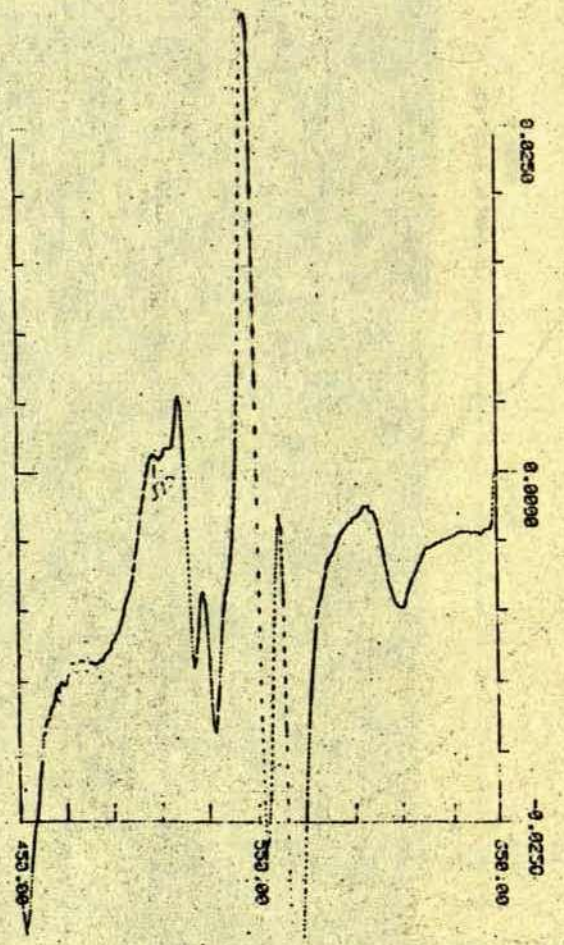

Fig.13.The first derivat
of rehydrated yeast sample
06.06r in nati'e state(the cytochrome b
is partially oxidized).light path 0.1cm

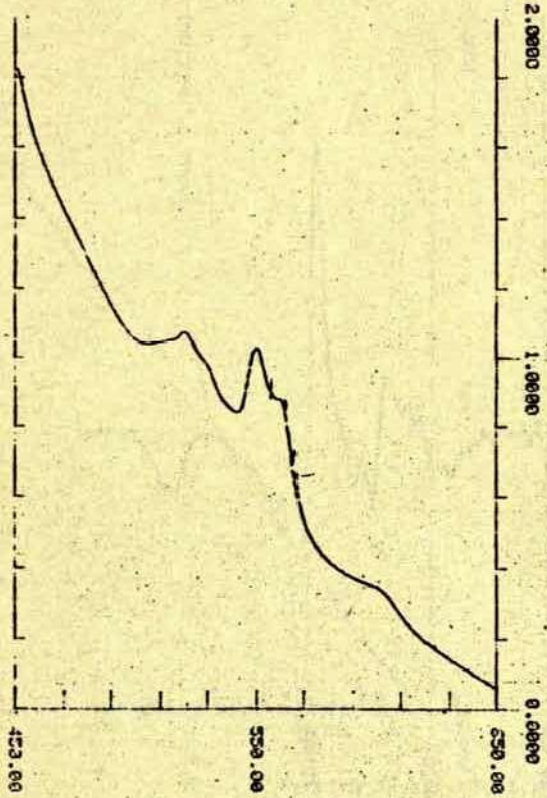

Fig.14.The absolute absorbance of rehydrated yeast sample 05.06r in native state(the cytochrome b is partially oxidized), light path 0.1cm,cuvette filled with ground yeast

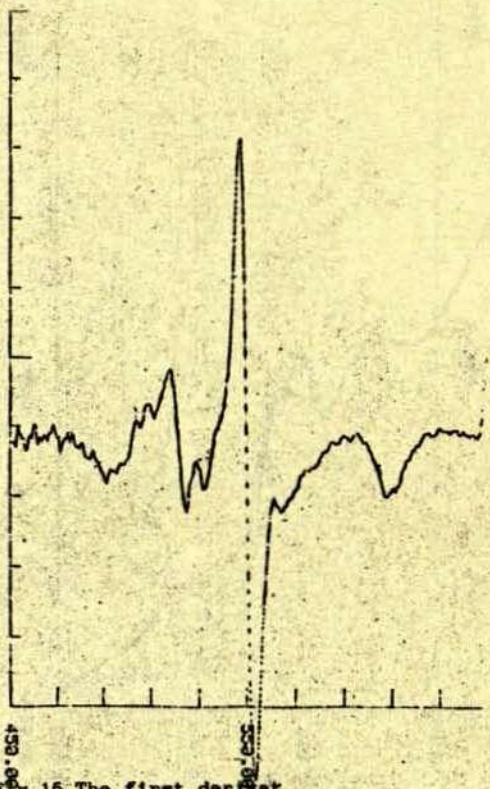

Fig.15.The first derivat of dehydrated yeast sample 16.06r in native state(the cytochrome b is partially oxidized),light path 0.1cm,cuvett filled with ground yeasts .

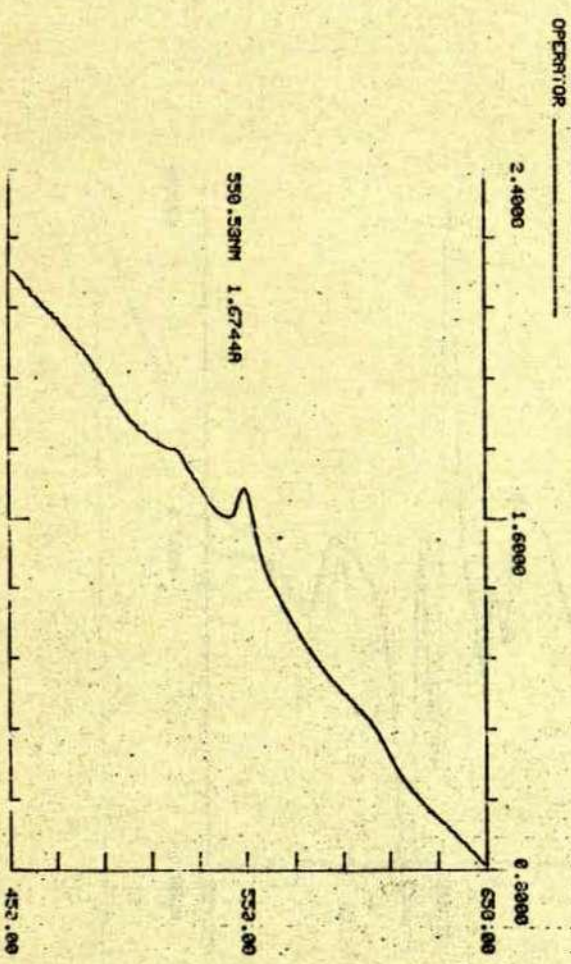

Fig.16.The absolute absorbance of dehydrated yeast sample 16.06r in native state (the cytochrome b is partially oxidized), light path 0.1cm,cuvette filled with ground yeasts

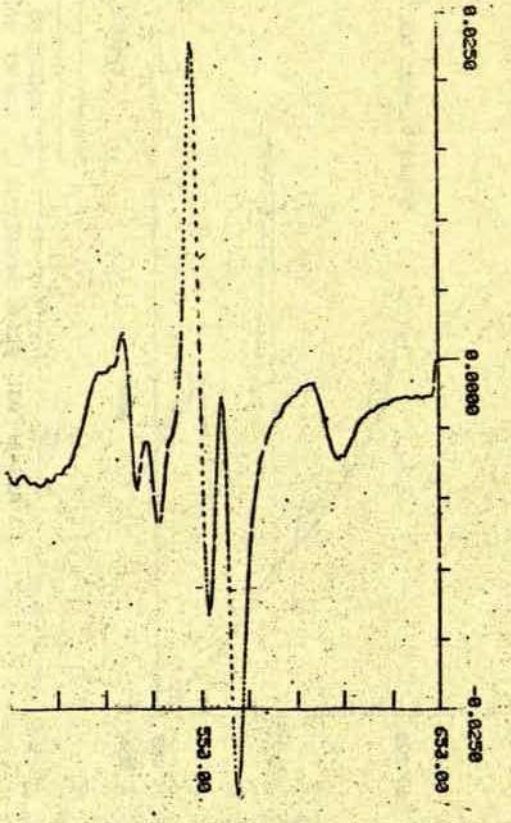

Fig.17.The first derivat of rehydrati yeast sample 05,06s1 in reduced state, light path 0.1cm,

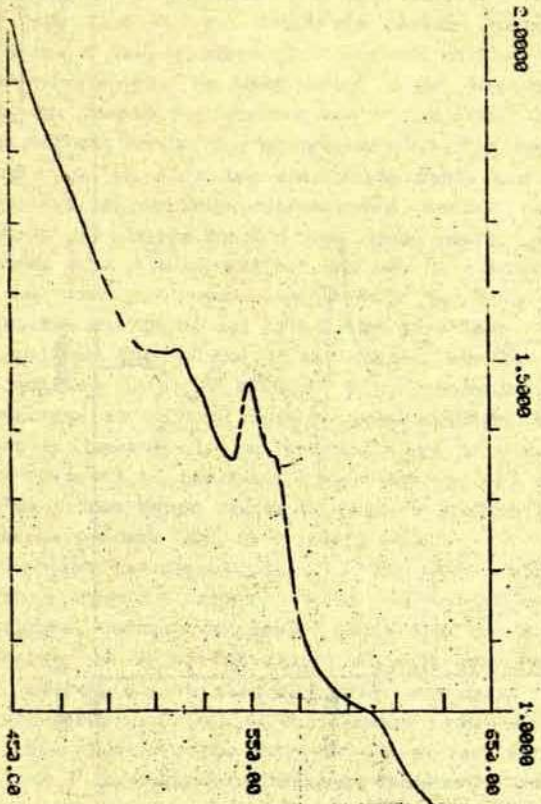

Fig.18. The absolute absorption of rehydrated yeast sample 16.06s1 in reduced state, light path 0.1cm

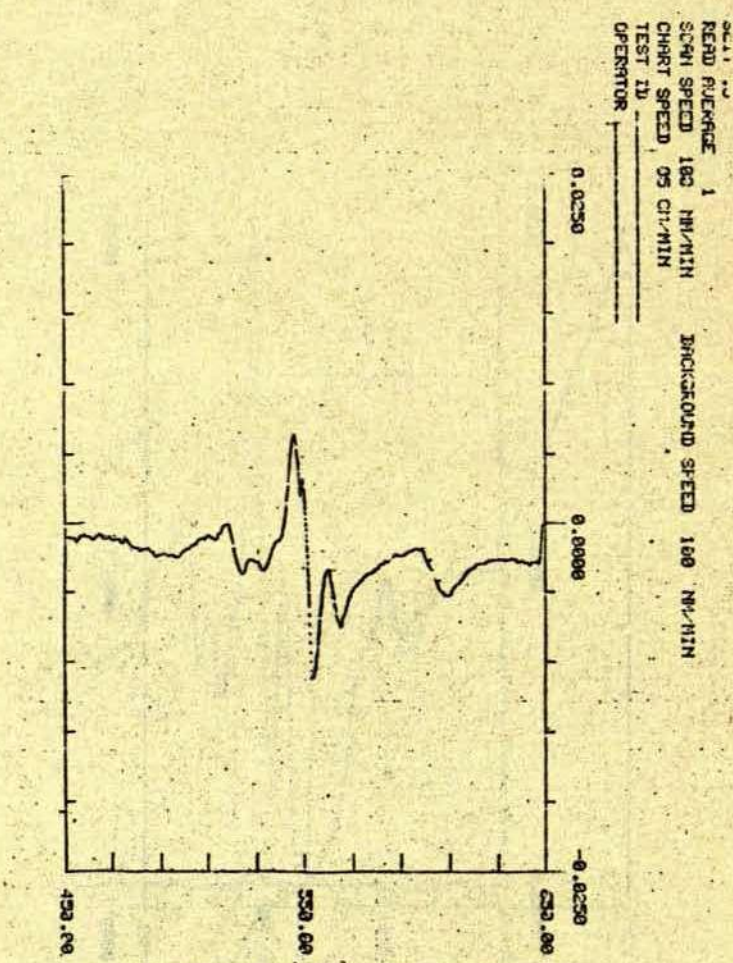

Fig.19.The first derivat. of rehydrated yeast sample 16.06s1 in native state(the cytochrome_b is partially oxidized), light path 0.1cm,cuvette filled with ground yeasts

the first maximum is twice less than the corresponding value of the second(not shown). In yeasts both values have the same dimensions .The main beta region bands reveal two maximums - at 522 nm and 530 nm which corresponds to cytochrom c and cytochrom b complexes.The first has a higher value than the second one There is a slight difference between corresponding wavelength for both samples.For sample"O" the bands corresponding to cytochrome a and cytochrom b complexes are 521,8 and 531,5 nm .For "631" the maximum corresponding to cytochrome c has an absorption maximum shifted to a little longer wavelength. In transparent light the corresponding values for sample "0" are 522 and 532 nm. The corresponding values for rat heart mitochondria are 521,5 nm and 527,5 nm
In alpha region also two absorption bands are to be seen ,characterizing at 551nm cytochrome c complex, and at 564 nm cytochrom b complex.Besides the main peaks, a more blue cytochrom b at 555 nm and 542,6nm can be distinguished.This peak is more pronounced in Druva's product. In Druva's product the values of all cytochromes are less .In alpha and beta regions the b and c cytochroms can be practically distinguished only in reduced form, therefore from these observations it is not easy to judge whether the cytochrom content is lowered or the cytochroms are in a more oxidized state. This can be estimated from gamma region. From graph 2 is evident that gamma region of Druva's product reveals less cytochrome content than in Oetker's yeast.
That permits to suggest that on the outer side of yeast granules Druva's product could be more subjected to proteolysis, because rehydrated cells does not reveal such a difference, or it is the result of more gray background of "631". On both graphs also cytochrom a complex can be seen. The ratio of cytochrom a to cytochrom c is higher in Oetker's yeast, what confirms the observations on rehydrated yeasts (not shown).If analysing the exact wavelength one must bear in mind that if the peak is on the slope,then tiny shift of the maximum can be occurred.

The effect of noise is specially evident when scanning is undertaken at shorter wavelength,because reflected light decreases with decreasing wavelength and the noise is considerably high compared with the signal (Fig.4.).Although the results of repeated scanning reveals that the peak in Soret range is at 419 nm ,and only a very tiny, if at all, peak is at 429 nm and that confirms that the reduction state of cytochrome b in rehydrated state is indeed very low. When trying to approximate the derivatived an absorption band at 399 nm is to be seen. Such a band is evident also on graph 1. where absolute recordance is plotted. It is likely that the haem with such a short absorption band is a haem which can not be reduced and an elevated relative content of this haem is not a sign of yeast quality.
Fig.4 reveals also a maximum of absorption band at 298 nm ,245 and 239,8 nm which are much higher in "0" than in "631"

3.The next graphs represents the results in yeast cytochrome analysis in transmission mode. In transmission mode the dissipation of light is appreciable ,therefore basis line in absolute measurements declines with decreasing wavelength.That results a slope which incline is the result of light scattering which increases with decreasing wavelength.It can be estimated that this increase is higher in Oetker's yeasts but lower in yeasts cultivated on ethanol .That permits to suggest that Oetker's product at dessication (dehydration) process forms structures that can be measured with light wavelength,which results in enhanced light scattering.It is not ruled out that these structures represent sugar or lipid granules,because these materials possess considerable light refractance.The light scattering has also a consequence that the absorption peaks in gamma or beta region are corresponding lower than the peaks in alpha regions where light scattering is less pronounced. As a consequence we can propose a new method for estimating dried yeast quality .

All measurements show that cytochrome b in all samples is only partially reduced. Such results can be obtained by comparing cytochrome b to cytochrome c peak ratios in dehydrated yeasts and in these samples after rehydration and complete oxygen consumption. The difference can be seen in a" more red" cytochrome b which in reduced state is responsible for oxygen radical formation (not shown). Presumably,there exists some mechanism which cuts off the dehydrogenesis feeding the cytochrome b .Such proposal can be made by observation that flavine compounds in all dry samples are in an oxidized state ascumed from the existence of the peak at 480-485 nm. Yeasts in a dehydrated state possessing cytochrome a/c peak ratio 0,4 revelled a peak 480/peak 550 ratio = 0.11, but yeasts with an average a/c ratio 0,2, the corresponding peak 430 to peak 550 ratio was 0,18. It is likely to propose that cytochrome b oxidation in dehydrated yeasts is an adaption reaction which can protect from active radicals formed when "more red" cytochrom b is in a reduced state. In Oetker's yeasts this adaption reaction seems to be more expressed than in ethanols yeasts . The absolute value of flavines representing peak at 480-485 nm reverse correlates with cytochrom a content.

Flavines could always be observed in dehydrated yeasts under mineral oil immersion.

Table 1. Absorption values of dehydrated yeasts by the light path length 4mm., Values are expressed as peak heights measured from the nearest minimums

	480nm	521nm	532nm	551nm	563nm	603nm
Oetker	0.0125	0.022	0.007	0.1457	0.035	0.0493
"16.06r",dehydrated in 40min	0.037	0.027	0.01	0.048	0.005	0.01
"16.06s1,dehydrated in 24h	0.029	0.048	0.02	0.106	0.04	0.03
"05.06r",dehydrated in 40 min	0.023	0.046	0.012	0.14	0.025	0.019
"05.06s1,dehydrated in 24h	0.02	0.07	0.035	0.173	0.04	0.036

It can be calculated that the peak 563(corresponds to cytochrome b absorption) ratio to peak 552(corresponds to cytochrome c absorption)does not exceed 0.4 and that is a much lower relation than when the cytochrome b is reduced. These observations reveal that at a prolonged dehydration the cytochrom b reduction rate is increased. It can be suggested that low cytochrome b reduction rate in dehydrated yeasts corresponds to higher viability and high cytochrome b reduction rate in dehydrated yeasts is a sign that they are not good for storing.

The results of Fig.5 and Fig 6 show that the 1st derivative and absolute absorbance of wet yeast sample 05.06r in reduced state,light path 0.1cm reveal corresponding values:

peak 604/551 ratio, absolute =0.139, derived 0.094
564/551 " " =0.85 " 0.67
532/564 " =0.322 " 0.264

The results of Fig.7.and Fig.8.gives the
first derivative and absolute absorbance of rehydrated yeast sample 05.06r in native state (the cytochrome b is partially oxidized),light path 0.1cm, cuvete filled
with ground yeasts

peak 604/551 ratio, absolute =0.13, derived 0.1
564/551 " , " =0.21 " 0.062
532/564 " =0.6 " 0.93

The graphs of Fig.9.and Fig 10 permits to calculate the first derivate and absolute absorbance of rehydrated yeast sample 05.06sl in reduced state,light path 0.1cm,

peak 604/551 ratio,absolute =0.138, derived 0.11
564/551 " , " =0.764, " 0.57
532/564 " =0.254, " 0.283

The data of Fig 11 and Fig.12 gives the values of the first derivative and absolute absorbance of rehydrated yeast sample 05.06sl in native state (the cytochrome b is partially oxidized), light path 0.1cm, cuvete filled with ground yeasts

peak 604/551 ratio,absolute =0.16, derived 0.114
564/551 ratio, =0.28, 0.15
532/564 " =0.22, 0.54

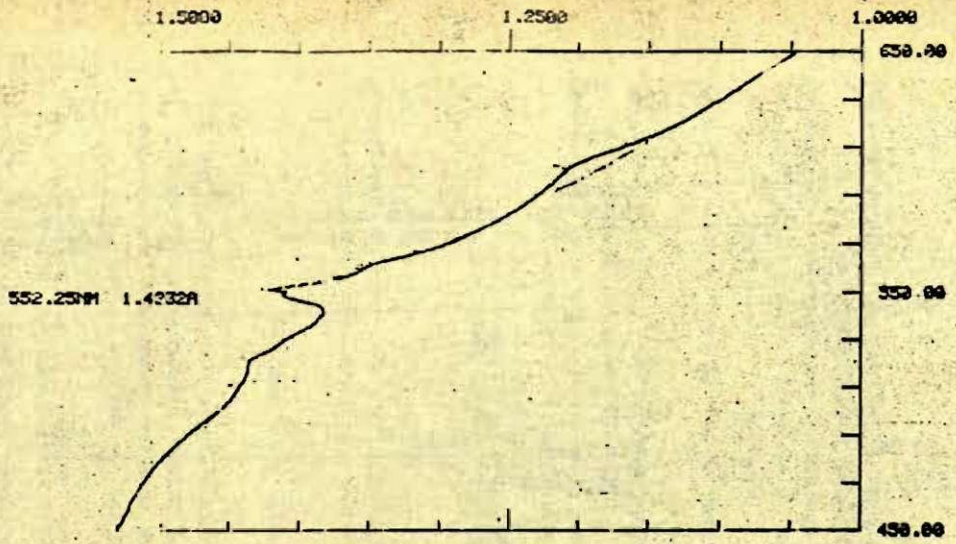

Fig.20.The absolute absorbance of rehydrated yeast sample 16.06a1 in native state (the cytochrome_b is partially oxidized), light path 0.1cm,cuvette filled with ground yeasts

λ SCAN 01 650.0NM TO 450.0NM RDS DATE
INTERVAL TIME 0000 MIN 1ST DERIV
SLIT .5
READ AVERAGE 1
SCAN SPEED 100 NM/MIN BACKGROUND SPEED 100 NM/MIN
CHART SPEED 05 CM/MIN
TEST ID _____
OPERATOR _____

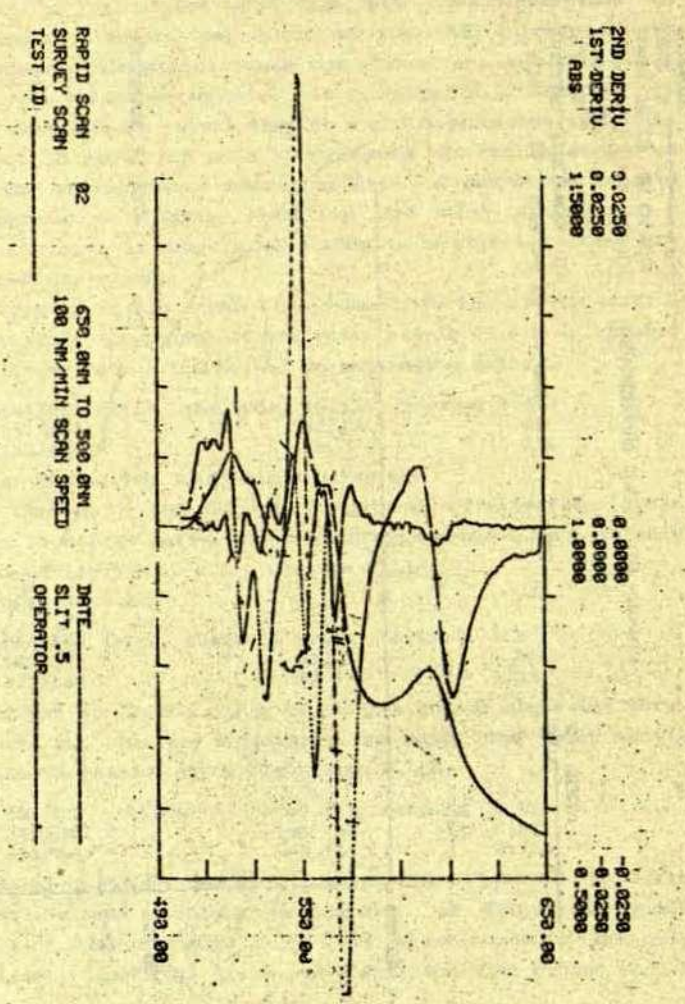

Fig.21.The first and the second derivat , and the absorbance of rehydrated yeast sample Oetker in red. state, light path -0.1 cm

The graphs of Fig.13.and Fig 14.permit to calculate the first derivative and the absolute absorbance - of rehydrated yeast sample 16.06r in reduced state, light path -0.1 cm :

peak 604/551 ratio,absolute =0.17 , derived 0.123
564/551 " =0.833 " 0.69
532/564 " =0.33 0.24

The data from Fig.15 and Fig 16. permits to get the first derivative and absolute absorbance of rehydrated yeast sample 16.06r in native state(the cytochrome b is partially oxidized),light path 0.1cm, cuvete filled with ground yeasts

peak 603/551 ratio,absolute =0.22, derived 0.1
peak 564/551 ratio, =0.09, 0.02
532/564 " =1 2

The lay-out of Fig.17.and 18.gives the opportunity to calculate the first derivative and the absolute absorbance of rehydrated yeast sample 16.06sl in reduced state,light path -0.1cm:

peak 604/551 ratio,absolute =0.15, derived 0.13
563/551 " =0.885 0.69
532/563 " =0.26 0.21

Material on Fig.19 and Fig.20. permits to calculate the first derivative and absolute absorbance of rehydrated yeast sample 16.06sl in native state(the cytochrome b is partially oxidized),light path 0.1cm, cuvete filled with ground yeasts

peak 604/551 ratio,absolute =0.296, derived 0.22
563/551 ratio, =0.49, 0.23
532/563 " =0.238 0.21

Data gathered on Fig.21 gives values of the first and the second derivative, and the absolute absorbance of rehydrated yeast sample Oetker in reduced state,light path 0.1 cm

peak 603/551 ratio, absolute =0.498,derived 0.406sec.derived 0.11

563/551 " =0.91 0.885 0.64
532/563 " =0.36 0.24 0.3

Data of Fig.22 shows that the corresponding values of rat heart

mytochondria cytochromes have a lower cytochrom b content than yeast mytochondria,but the relations of peaks determined in alpha and beta regions are proportional to each other.

The data presented on graphs gives the opportunity to summarize the cytochrome's b/c relations in dehydrated and rehydrated yeasts. The relations are not formed as molar ones, but as absorption values - heights, measured from the base basis line,connecting the lowest absorption values and the maximal values . The corresponding values in beta region change between 0.3 and 0.7, with an average ratio for dried yeasts 0.39, for dehydrated ones - 0.65. In alpha region the corresponding area of observations carries 0.15 - 0.9, the average ration for dried -0.33, for rehydrated 0.84. The numeral proportions prevailing between two sets corresponding to beta band varies 1,7 times, but for alpha 2,5 times.
One must bear in mind that the corresponding values are formed by connecting the common baseline for both cytochromes b and c,what means that they can interfere one with other.The difference between the wavelength for cytochrome b and c in beta region is less than in alpha region ,therefore the interference of both cytochromes in beta region is more distinctly marked.When estimating (comparing) similarities or dissimilarities of slowly dehydrated and yeasts dehydrated at a normal speed,then the former had a higher cytochrome b reduction rate.

The y' ratio for cytochromes b to c in beta region varied in a broader diapason than the absolute values .The range for y' was 0,2-0,7 for dehydrated the average value was 0.27, for rehydrated ones -0.67 the ratio is 2,48.In alpha region the whole range extends from 0,06 to 0,9,the mean values for dehydrated 0,13,and rehydrated 0,73,though the corresponding ratio is 5,6 .Consequently,the first derivative secures in alpha band 5,6/2,5 =2,24 times better resolution than the absolute values .Correspondingly,in beta region the resolution increment is 2,48/1,7=1,46 x .In such a way alpha

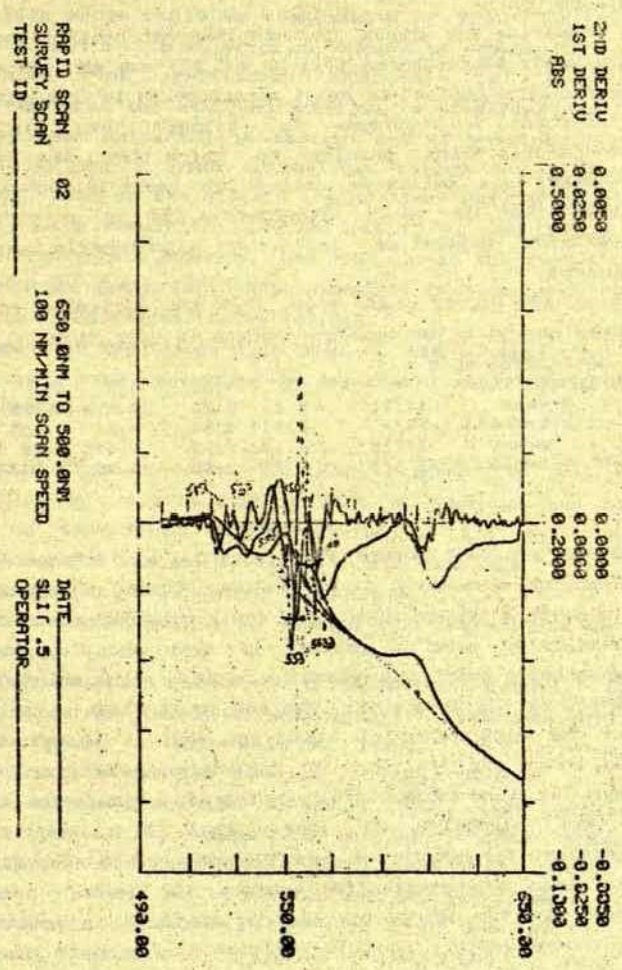

Fig.22.The first and the second derivat , and the absolute absorbance of rat heart mytochondria in reduced state

region gives better resolution for reduced cytochrome b than beta region.

By comparing the graphs obtained from rat heart mitochondria with yeast mitochondria (Fig.21 and 22) one can see that the cytochrome a content in heart mitochondria in comparison with cytochrome b content is higher than in yeast mitochondria,which permits to think,that the electron transport via cytocrome oxidase in heart mitochondria is higher than in yeast mitochondria,and as a consequence superoxide generation in yeast mitochondria could be enhanced.

Fig.21 and Fig.22 shows also, that the derivated values in comparison with the absolute values in both type of samples do not differ much :

wavelength band		521.5	532	551	553	604
Oetker	Y1/Y	0.147	0.12	0.213	0.168	0.05
Heart mitochondria	Y1/Y	0.133	0.09	0.24	0.152	0.08
Oetker	Y''/Y'	0.192	0.29	0.275	0.24	0.256
Heart mitochondria	Y''/Y'	0.214	0.06	0.24	0.256	0.1

DISCUSSION

One can see that Perkin Elmer "lam'da scan 9" operating in reflection scan mode gives the opportunity to resolve the wavelength more precisely than spectrophotometers running in transmission mode.The reason is that when operating in transmission mode ,the operation is on a slope,which can give a shift of the wavelength. The effect of slope makes evident that the middlepoint of the first derived of cytochrome b lies under the "0-line". By reflection mode scanning that cannot be observed.The disadvantage of transmission scanning is the discaling of reading,when high amplification ,necessary for cytochrome detection,is used.In some elder two beam spectrophotometers(for example ,UV VIS,Carl Zeiss,Jena)there are two sample places ,one of which is located near the photoreceptor. In this position the bulk of dissipated light is gathered and the effect of false absorption increasing (due to scattering) is lowered.The disadvantage of this system is sensitivity to luminescence ,because no monochromator is included between sample and light

detector,and the luminescent (emission) light can decrease the real absorption values.In two beam spectrophotometers the dissipation slope could be compensated by inserting in the reference beam a slip of paper,or benthonite suspension . The disadvantage of reflection scanning is the low sensitivity,when the analysed material is gray.Yeast samples are gray when measuring is carried out at wavelength were amino acids absorb (near ultraviolet region and a shorter wave one). Even in the visible region lower quality products are gray as a result of some oxidation at dehydration of some carbohydrate moieties ,and that lowers the sensitivity of cytochrome detection .This undesired phenomenon is very marked in gamma region (Soret region)

Independent of the observation mode, it can be seen that the cytochrome b type cytochrome in desiccated material is in a low reduction state.

To explain these results, one must respect the published information by various authors that cytochromes can be a source of superoxide radical production and in cells adaptation reactions can be elaborated by two ways :

1) the enzymatic activity can be changed,
2) the quenching efficiency by various cell ingredients can be changed.

The chief question, however, is about mitochondrial H_2O_2 production as the result of mitochondrial reduction status. H_2O_2 is formed in mitochondrias largely from superoxide radicals ,which can be removed by the presence or the specific enzyme superoxide dismutase (SOD), that generates H_2O_2 by dismutating superoxide radical(O_2^-).The question arises from where cytochromes O_2^- is generated. Except of cytochrome oxidase, the cytochroms are usually classified as dehydrogeneasis. In respiratory chain ,they are involved as carriers of electrons from flavoproteins on the one hand, to cytochrome oxidase on the other hand. There can be distinguished seven electron transport components: two b- type cytochromes, two flavoproteins,two iron sulphur

proteins and ubiqinone. Of these components, however, cytochrom b566 has also a direct reaction with oxygen, if the cytocrome is in a reduced state. This observation conflicts with the dogma that cytochrome oxidase is the only respiratory chain component which can bind and reduce oxygen. Since cytochrome b566 is a monovalent redox catalyst, the primary product or this oxygen reduction should be O_2^-. The test conditions required a reduced state of this bv type cytochrome. Reduced state could be achieved in different ways. R. Murray concludes that the rate of respiration of mitochondria can be controlled by the concentration of ADP. This is in the case when oxidation and phosphorylation are tight coupled. Chance and Williams have defined 5 conditions that can control the rate of respiration in mitochondria. Generally, most cells in the resting state are in state 4, and respiration is controlled by the availability of ADP. In this state cytochrome b566 gets reduced. Parallel to the degree of cytochrome b566 reduction a H_2O_2 formation has been observed. In coupled mitochondria the redox state of cytochrome b566 changes with phosphate potential. When work is performed, the phosphate potential is lowered, because ATP is converted into ADP, allowing more respiration to occur, which in turn replenishes the store of ATP. As respiration increases, the cell approaches state 3, where cytochrome b566 is fully oxidized. Correspondingly, the H_2O_2 production is very low and also a concomitant H_2O_2 production is essentially zero. The cytochrome b566 reduction state could be also enhanced by feeding mitochondrial respiration chain with succinate, but lowered by feeding with malonate, because malonate is a competitive inhibitor of succinate dehydrogenase. Much information of the respiratory chain has been obtained by use of inhibitors. Dimecaprol and antimycin inhibit respiratory chain between cytochrome b and cytochrome c. All respiratory inhibitors blocking electron flow on the oxygen site of the b-cytochromes, reduces this cytochrome and enhances peroxide production. Other inhibitors prevent the oxidation of substrates blocking electron flow on

the substrate site of the b type cytochromes ,e.g., rotenone, dibromothymoquinone,reduces b type cytochromes and reduces their reaction with molecular oxygen. Whenever cytochrome b566 is in a reduced state H_2O_2 production is low .The well known cytochrom aa3 inhibitor - cyanide,binds not only to cytochrome aa3 ,but also to cytochrome b566.Also carbon monoxide can bind to cytochrome b566 when it is in a reduced state. Thus cytochrome b 566 in a reduced state can bind CN-,CO and this inhibits the reaction of this cytochrome with O_2, and ,since this cytochrome is a monovalent redox catalyst , also O_2^- production ceases.

There are also some observations that yeasts contain two cytochromes b, one of which has a longer wavelength as the other h (8).

Baker's yeast mitochondrial cytochrome b-564 is characterized by exhibiting both a labile pH-dependent high potential form (Elb,pH=7 = +190 mV)and a stable pH dependent (pKa = 6,8 low potential form (Eb,pH++7OmV The different behaviour of these two forms of cytrochrome b-564 versus pH seem to be a decisive factor for transformation of redox energy into acid-base energy in oxidative phosphorylation site 2.

In order to identify the cytochrome b which is affected by ATP, the absorbance changes in the spectrum region between 558 and 566 nm were determined every two manometer in baker's yeast between mitochondria subjected to two critical redox potentials under two extreme phosphate potentials. When the redox potential in the sample cuvette was adjusted to -200 mV -enough by itself to reduce both forms of cytochroms bT as well as cytochrome bK -two peaks were always observed at 560 and 564 nm either in the absence or in the presence of ATP.On the contrary,when the redox potential was adjusted to +1oo mV - a potential value at which exclusively the high potential form of cytochrome bt an absorption maximum appeared at 564 nm only if the sample had been previously incubated with 10 mM ATP,no peak being observed if the sample had been incubated without ATP. In accordance with these results it

seems that the cytochrome b changes its redox potential depending on phosphate potential and that which is probably implicated in the transduction of redox energy in oxidative phosphorilation is the one exhibiting a maximum absorbance at 564nm.

There are some treatments that convert the high potential form of cytochrome b-564 into its low potential one .Thus heating or sonication of baker's yeast mitochondria - treatments which disrupt membrane structure -produces the same effect as addition of either PCCP (with or without ATP) or ADP plus Pi.These deenergizing treatments convert all the mitochondrial cytochrome b-454 -which initially is one third in the high potential form - into the low potential form .In contrast ,ATP increases very significantly the proportion of the high potential form which after the addition of the nucleotide amounts to about 90 per cent. The addition of the ATPase inhibitor oligomycin neutralizes the ATP effect.

The high potential form of mitochondrial cytochrome b-564 is pH-dependent and in acid media it is not easy to reduce. Our observations show that in yeast culture cytochrome b is not always fully reduced even in anaerobic conditions .It gets fully reduced only by elevating pH of the buffer solution.

The question to be answered is whether low cytocrome b reduction rate in desiccated yeasts is the result of intracellular acidification or the result of lowered ATP level, or the result of lowered electron transport to the cytochrome. Whatsoever it should be it is likely to suggest that more oxidized cytochrome b tends to lessen superoxideradical formation and that gives a chance for maintaining viability of desiccated yeasts.Such presumption is in accordance with observations that yeasts with higher viability after dehydration show a lower cytohrom b reduction rate.

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Determination of dried baker's yeast quality by using a fluorescent method in reflectance mode

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SUMMARY

It has been estimated that dried commercial yeast quality can be analysed by using the fluorescent method, the optimal wavelength ranges for illumination and fluorescence were determined.

INTRODUCTION

Methods of quality assay in the branch of food chemistry has become a classical tool in food chemistry. There is an increased interest of securing baker's yeast viability after prolonged storage, which is demanded from consumers in nutrition and food processing. Recently, more interest has been generated regarding optical methods in dried yeast quality criteria. Fluorescent methods offers the opportunity for simultaneous determination of several compounds, which can be easily estimated. The most common investigated fluorescent compounds are aminoacids. One aminoacid, which fluorescence intensity is more marked is tryptophan. Krasnikov (1) summarizes its luminescence characters.

Native tryptophan can be excited at two wavelength:	218nm ,280m,
the emission maximum	353nm
Dehydrated tryptophan can be excited at three wavelength	280,295,305nm
the emission maximums	333,460.
Triptophan oxidized,. excitation maximum	320nm
the emission maximum	430nm
Triptophan dehydrated oxidized, excitation maximum 360nm	
the emission maximums	470nm

These data show that if the analysed material are dehydrated yeasts, then the emission wavelength is shifted to a shorter wavelength (from 353 -to 333nm).

The second conclusion is, that the propriety tryptophan luminescence observation wavelength shoud be 330nm, but not 460nm, where the luminescence is low.

The third conclusion is, that when tryptophan is oxidized, then the total luminescence is weakened.

This summary reveals, that rehydrated material has two luminescence peculiarities :

1)it reveals a finer absorption and emission spectral structure than dissolved in water material. That enables to carry out analysis at room temperature, instead of liquid nitrogen temperatures,

2)by analysing dehydrated yeast product ,one should mind, that even in dehydrated state,yeasts possess some water content, which, in order to sustain cell viability, should not be lowered under 8%. The exact moisture content can vary under air conditions, and that can change the exact excitation and luminescence wavelength. Therefore the aim of these investigations is to carry out excitation and emission observations in a wider diapasone, and less attention should be admitted to covere the luminescent ingredients, as to luminescence pattern differences in better and lower quality product.

Yeast cells possess also membrane structures which includes fatty acids. In accordance with data presented by Krasnikov(1) linoleic and linolenic acids can be evaluated by using two wavelength excitation method:
linoleic acid gives maximal luminescence, when excited at 325nm,

but linolenic, when excited at 355nm. It is known, that good rehydrated yeasts have a lower unsaturated acid content, therefore it should be interesting to observe the fatty acid luminescence values observed at emission wavelength 400nm. If linolenic acid is oxidized to peroxide state, then the luminescence wavelength is shifted from 400nm to 405-420nm. Indeed, the emission intensity, observed at 250nm, relation by excitation at 418nm and 400nm for "0" =0,54, but for "631 = 0,74.

If the linolenic acid is oxidized to aldehide state, then excitation maximum is shifted to 435nm, and emission maximum to 435nm.

If linolenol acid is oxidized,then luminescence maximum is shifted to 480 and 490 nm.

These dates from literature show, that as a result of oxidation the deep excitation peaks in the diapason 320 - 360 are lost, luminescence appears only when excitation is accomplished with longer wavelength light and that will be used for interpretation our results.

MATERIALS AND METHODS

A novel analytical system has been applied by using a sensitive appliance, which permits to make the illumination by such a low energy light source that illumination does not harms the analysed product sample and the results are reliable.

The fundamental requirement for a fluorimeter calls for two optical systems, one for excitation, the other for the fluorescence radiation.

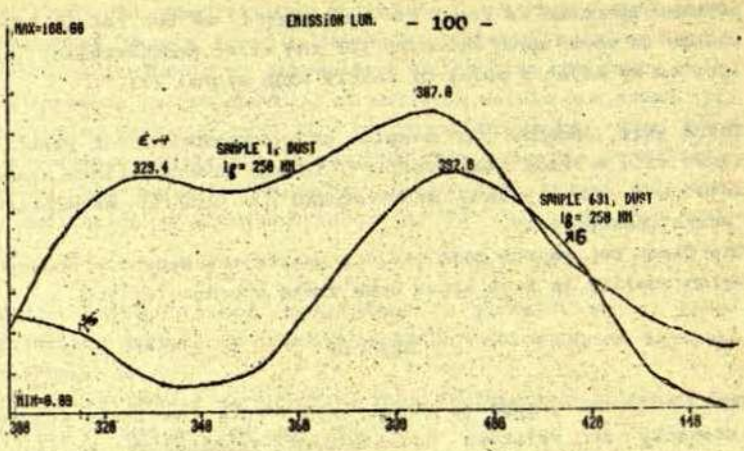
The operation of a spectrofluorimeter usually follows some procedure as this- By rough preliminary observations, a suitable wavelength in the emission spectrum is chosen, and the second monochromator set at this point. An excitation spectrum is then plotted by scanning the second monochromator with the first set at a suitable value. With most substances

neither spectrum is found to vary greatly as the result of a change in wavelength selected for the other monochromator ,as long as it is at a point of fairly high signal (3).

There were analysed two samples of commercial dried yeasts: Yeast with a trade name "Oetker", in our investigations named as"O",and dried yeasts manufactured in Riga by enterprise "Druva",named "631".
The first represents good quality yeasts,the second - product which quality is lost after some weeks storage (4)

RESULTS

The first two graphs of Fig.1. represents the luminescence intensity in relation to incident (excitation) light wavelength. In the first graph the luminescence intensity was determined by a detector with a maximum sensitivity at 346 nm. The light emission from the sample "631" was low in comparison with light emission from sample "O", therefore higher amplification was used - the amplification was increased by a factor 4,5. The excitation luminescence wavelength pattern can be interpreted with corresponding absorption and emission bands. If the emission band is registered by a photoreceptor with maximal sensitivity at 340 nm, then "631"shows a distinct excitation maximum at 253nm. This is a maximum, if compared with the minimum at 260nm. At this minimum however linolenic acid should reveal appreciable excitation sensitivity. In "631"the minimum is only 1/2 of the corresponding to 253 value, in sample "O"the value is of the same order of the maximum at 253nm. The excitation diapason 280-305nm corresponds to a low luminescence when light detector with maximal sensitivity at 390nm is used, because the wavelength of tryptophan luminescence - 333 nm is far from the detector sensitivity maximum. If a detector of maximal sensitivity at 340 nm is used, then triptophan luminescence is better evident. However tryptophan luminescence in "631"is 9 times less compared with triptophan



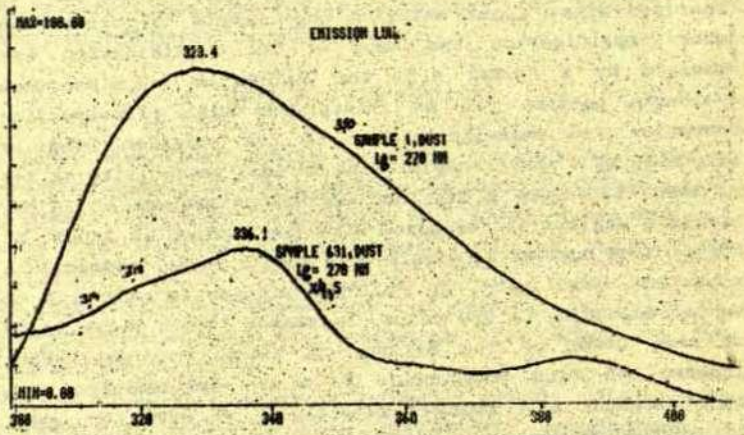


Fig1.Emmission intensity(in relative units) by varying excitation wavelength,nm. The sensitivity is enhanced 6 times by scanning sample 631 (the upper graph).in the lower graph- 4.5 times . Luminescence was observed at 396nm-upper graph,and 346nm -the lower graph.

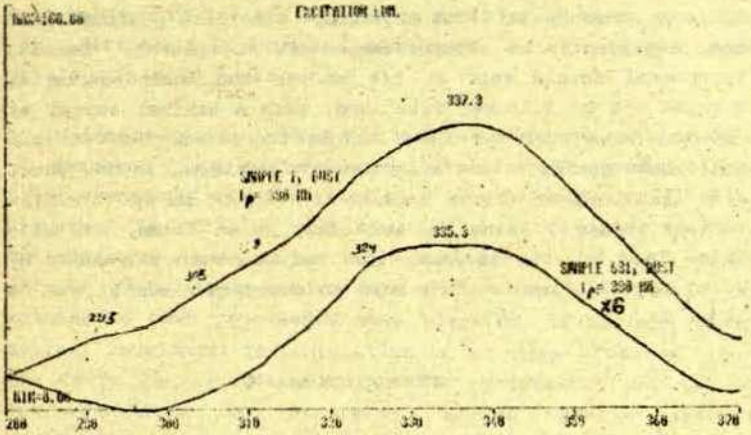
luminescence in "0". If tryptophan is oxidized, the excitation bands can be observed at a longer wavelength 320 and 360 nm. In this case also luminescence is shifted to a longer wavelength, therefore the detector of 390 nm sensitivity maximum gives higher luminescence values. In another report (3) there were estimated also yeast absorption values in region where cyclic aminoacids absorb. In "0" the corresponding absorption values where more marked as in '631". That harmony with the luminescence measurement results. Although some of the excitation bands which are related to absorption bands, are shifted to somehow more red wavelength :for ex., the absorption band at 245 nm, but the maximal luminescence occures, when excitation was carried out at 253nm.,the absorption bands in 280-300nm could not be separated. According to literature (1,2) absorption wavelength of tryptophan solution in neutral water has the chief maximum at 279,5nm, a shoulder at 271nm and a sharp peak at 288nm. Our absorption wavelength lies under 300nm. However, it has been reported that all three maximums of dehydrated tryptophan were shifted to longer wavelength by 0.5-7nm and the excitation spectra shod reveal three maximums at 280,295 and 305nm. The current observations permits to suppose, that there could be in this region three excitation maximums (the graphs for sample "0"), but mini ums are washed out,supposing that there could take part also other luminescating substances. In Fig.1.,upper graph are represented excitation wavelength if luminescence was observed with a detector with the maximal s ısitivity at 380nm. In this case there was practical no luminescence of sample "631", if the excitation was carried out in diapason 290-300nm. That means, that aromatic aminoacids have luminescenc with a shorter wavelength and therefore cannot be detected. That means that there is a lack of other substances that can be excited in this diapason. To such substances could be related ,f.ex.,tocopherols - one of the most effective membrane stabilisator. However tocopherols emit at 440 nm,but that should be registered by the first

detector. However any excitation band at 305nm, which is characteristic to alpha tocopherol, nor corresponding emission band, has been observed. The diapason in which one sample is unsensitive to excitation, but the other is sensitive, can be illustrated by the following observation:

if the excitation is carried out at 291,3nm , then the luminescence ratio of "0" and "631"is 62x,
by excitation wavelength 303nm , the corresponding luminescence ratio is 61, at 309nm the corresponding ratio excitation wavelength is 24; at 324nm excitation wavelength, the ratio is 10,at 338 nm the ratio is 10,5. In this point one must mind,that 291nm is the excitation wavelength of linolenic acid.Linolenic acid has an excitation minimum at 330nm,but linoleic acid absorbs at this wavelength.

Perhaps this wavelength is good for practical use, because the luminescence intensity gets maximal values. It is likely to believe, that at this wavelength NADH absorbs. The observations from literature reveals that NADH coupled to enzyme in reduced state has not an absorption maximum at 340 nm, but is shifted to a shorter wavelength, even till 320nm and the luminescence maximum could be somewhere in the diapason between 430 and 460 nm.

It is known,that NADH absorbs also at shorter wavelength. The exact absorbency maximum depends upon the state of NADH in the cell. If NADH is bound to enzyme, then its maximal absorption is shifted from 260nm to 280nm and these wavelengths can also be used for excitation purposes. On lower graphs of Fig.1 one can see ,that when excitation wavelength is 250nm, then emission band at 440nm can be seen:in "0" it is 38x more powerful as in "631". By using excitation at 270 nm, no luminescence can be observed in "631"when the wavelength is longer than 410nm.



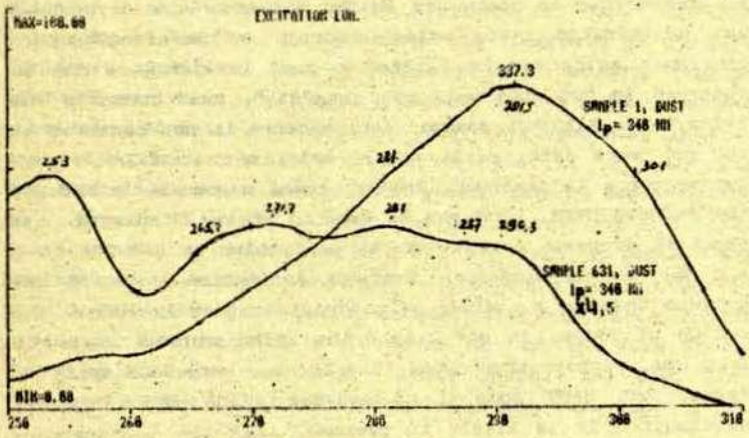


Fig.2.Emmission intensity (in relative units)by varying the wavelength of analyzed light.Excitation was carried out at 250nm -upper graph, and 270nm - lower graph

If illumination is carried out by a lamp with a maximum at 250 nm., then two emission bands can be observed -one at 329,4 nm, another at 387,6 nm(Fig.2). The first maximum could not represent the luminescence of tocopherol, because tocopherol should emit at 340 nm. If the luminescence is carried out by a longer wave lamp, with a maximal energy at 280 nm,the maximum at 329,4 nm is increased. The maximum ratio between the Oetker's and Druva's products, if the short wave illumination lamp is used is at 330 -335 nm is 52 x, the minimum ratio by using the same lamp is at 414nm, and it's value is 6,2x. The maximum ratio between both products, by using the illumination lamp with an energy maximum at 270 nm is 11 x.

DISCUSSION

A trend exists in modern analytical laboratories to employ automation whenever it can be economically justified (4). Our results show, that dried baker's yeasts can be analysed by direct luminescence methods. If excitation is changed, it can be recommended to change it in the diapason were tryptophan and unsaturated fatty acids absorbs. At wavelength,where linolenic acids can be excited a good luminescence can be observed in "0", but very poor in "631", that concerns the 291nm band. Although maximal luminescence is not observed at 400 nm, were fatty acids should emit, but at 333nm, where luminescence is observed. Another trial was made to observe the reduced NADH. NADH can be excited at two diapasons. The shortest diapason corresponds to excitation at 260-270 nm , and the second diapasone includes excitation at 340 nm. Between them is a minimum of absorption, that includes the reqion of 297nm. In our graphs the 297nm minimum is not a deep one, perhaps tryptophan interferes. Pure NADH emits at 460nm, but NADH coupled to enzymes emits at a shorter wavelength. It is likely to propose, that the luminescence can be observed at 440 nm. A distinct thiamine absorption band at 345 nm and emission at 460 nm has not been observed,

therefore it cannot be a disturbance for NADH determination. Presumably NADH in both samples in an oxidized state, because nor distinct excitation wavelength, nor luminescence wavelength could be found.

If luminescence is observed in a shorter wavelength diapason - detector sensitivity maximum at 346nm, then the fluorescence of cyclic aminoacids could be observed. This diapason is however less suitable for yeast quality determination, because the luminescence ratio is less, as in reduced NADH determination assay. The same is related to wavelength that represents some vitamins. Udenfriend gives optimal wavelength for excitation of B6 group vitamins. They are 340nm for pyridoxine, 335nm for pyridoxamin and 330 nm for pyridoxal. By analysing "631"we observe maximum excitation at 335,3nm, by analysing "0"the wavelength was 337,3nm. Udenfriend (1) gives optimal wavelength for emission: for pyridoxin - 400 nm, the same for pyridoxamin, and 385nm for piridoxal. Our observations reveal maximum emission at 392nm for "631", 387,6nm for "0", and the intensity of latter is 7,5 times the intensity of former. Pyridoxin, pyridoxal, and pyridoxamin are 3 closely related pyridine derivates. Pyridoxal phosphate is the major coenzyme expressing B6 activity. In yeasts they are especially used in the metabolism of ethanol to acetaldehyde. It is not ruled out that fluorescent analysis of yeasts can be employed not only for dried yeast quality determination, but also for yeast metabolism investigation. But for such purposes the excitation wavelength and the corresponding luminescence should be investigated in a broader diapason where longer wavelengt' luminescence is accounted. The current work was to investigate some wavelength which could be used for commercial yeast analysis. These wavelengths were found .

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A Shift of Dried Baker's Yeasts Water Absorption Peak in Near Infrared Reflectance Spectra

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SUMMARY

Near-Infrared reflectance spectra was used to analyze dehydrated commercial baker's yeast. A wavelength shift of absorption peaks has been observed between baker's yeast of various quality.

INTRODUCTION

Near-Infrared reflectance spectra (NIR) is often used for determine food quality (1). NIR is commonly used in measuring the nutritional composition of foods containing carbohydrates such as starch and cellulose. Spectra are usually gathered at room temperature and due to the relative intensity of the spectra to minor deviations in temperature, quantitative relationships between absorption and moisture content are developed. Under functional quality questions of baker's yeast it is viability after desiccating and rehydration. There is an interest of solving the mechanism which concerns how yeast cells can tolerate desiccating. Large progress was made when it was observed that cells of *Saccharomyces cerevisiae* are able to accumulate glycogen and trehalose when placed under special environmental conditions(2,5). These compounds were synthesized in tolerant organisms during the drying process. The hypothesis was raised that polyhydroxy compounds might protect membrane during desiccating by substituting hydrogen bounded water as the water is removed (1). The theory supported the finding that trehalose did indeed protect membranes during dehydration. Evidence regarding the mechanisms of such protection emerged when physicochemical techniques were used. It was proposed that

water in disperse systems has properties which derived from the usually observed "bulk" properties. Indeed, there are several states of water in contact with a surface, also often a crude distinction into "free" and bound water has been used (2). Conveniently NMR measurements are used to characterize the mobility of different species of water molecules: those in direct contact with the carbohydrate (i.E., the monolayer), those in the multilayer, and molecules of bulk water. However, NMR measurements are difficult, slow and expensive to perform. NIR spectra on the other hand, are relatively fast and simple to collect and are especially sensitive to the water absorptional bands (3). Delewiche has shown, that NIR methods are much faster and simpler than NMR ones, much faster and simpler methods are the optical ones(3). In NIR techniques both transmission and reflectance methods are used. One of a means, which can be used for estimating the water interaction with hydroxycarbons is the Near -Infrared Scattering transmittance spectroscopy (3). Diffuse reflectance NIR is elaborated method for estimating the nutritional composition of foods containing carbohydrates such as starch and cellulose and water. One of a tool, which can be used for estimating the water interaction with hydroxycarbons is the Near -Infrared Scattering transmittance spectroscopy (3). Spectra are usually obtained at room temperature, because variations of temperature can change the water content, but for specialised purposes also hermatic cameras have been designed. Delewiche has suggested that NIR spectra reveals much information when hydrogen bonding is present. NIR can be used to quantitace the temperature dependancy of non-hydrogen -bonded OH groups, and has gathered information on temperature induced changes to the NIR spectra of carbohydrates in the solid state .Authors (3) revealed that water absorption spectra measured in the range 1400 -1500 nm, and 1900 - 2000 nm are changed when temperature is varied. From -80 to 60°C, the 1900 -2000 nm band shifted approximately 10 nm and 15 nm towards shorter wavelength in starch and microcrystalline cellulose,

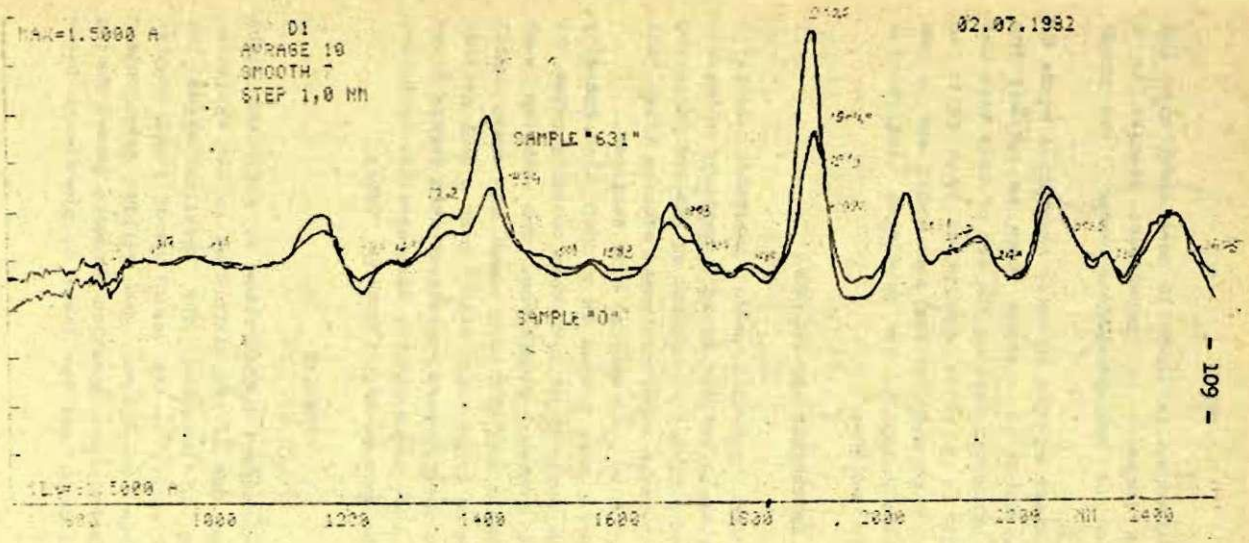

Fig.2. The first derivative of diffuse reflectances (R) transformed to apparent absorbances (log 1/R) from two rehydrated yeast samples in relation to wavelength in NIR IR range

repectively. The direction in shift is consistent with the hypothesis that an increase in temperature results in a decreased proportion of hydrogen-bonded water - to water molecules.

Information on trehalose changes in water absorption bands of baker's yeasts dehydrated to a state which is optimal for product storing is currently lacking.The aim of this work was to show that there is a water absorption band shift in yeasts, compared to that reported for pure water and it can be estimated relatively rapidly for providing information about baker's yeasts quality.

MATERIALS AND METHODS

Two commercial yeast products were analysed: Oetker's product, which was named "O",and Druva's product, which was named "631". "O" had a trehalose content of 18%,and preserved a very good viability even after prolonged storage time, "631 " of - 12%, the viability with storage time declined.

Sample of each product were placed in a contr lled humidity media - 40% relative humidity at 24°C This humidity range was common to natural storage conditions. The samples were allowed to equilibrate at 25°C for about ten weeks until weight changes were no larger than $2*10^{-3}$ over a week period. Diffuse reflectance spectra were registered with Perkin Elmer Lambda-scan-9. The absorbance spectra were obtained by taking the logarithm of the inverse of reflectance value.

RESULTS

Graph.1. shows that direct registration of reflected light reveals distortions. One of the distortion is the variation in the absorbance of baseline. The baseline shows two effects. The major effect is the variation of light scatter due to the different smoothness, and yellow- gray colour characteristic to amino-sugar reaction products formed during the dessication process, and the level of absorbance base-

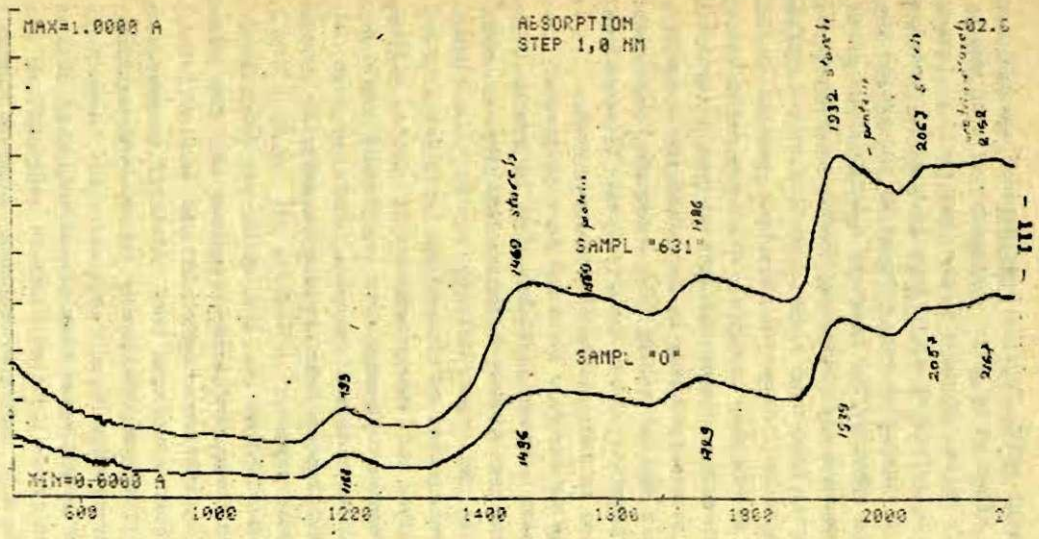

Fig.1.Diffuse reflectances (R) transformed to apparent absorbances (log 1/R) from two rehydrated yeast samples in relation to wavelength in NIR IR range

line due to unwanted noise. Diffuse reflectance spectra are normally strongly affected by light scattering effects due to different particle size. Obviously the "631"has a lesser particle size and a surface of a distinct lowered reflectance capability. These disturbances were removed by converting the spectra to derivate spectra. The derivate spectra shows noise characteristics, because analytical information contained in the NIR spectra comes from only a small portion of the total spectral signal obtained. Therefore spectral processing is usually necessary in NIR data analysis, which are designed to remove the non-relevant variance and extract the analytical information. This is made possible by repeating the scanning. This was done till the obtaining the necessary precision level to get information about water - cell interactions which induce changes in NIR spectra of adsorbed water .

The NIR -detectable water could be found in both samples .In sample "631"the watar content is higher,than in sample "O". That could reflect the different water activity in both products. Presumably the water activity in "631" is higher than in "O". Without further investigations it is not easy to make any speculations of the character of water affine components. Still one can suppose, that glycerole could be generated, in the technological process where product is pressed before cutting in small peaces in order to start rehydration, and lacks oxygen.

The main observation however is the shift of water band absorption wavelength compared to that reported (3) for pure water adsorbed on polystirene beads. It is evident that "O"absorbs at a higher wavelength than "631". If we compare these observations with that reported by (3), then absorption maximum wavelength increment,is adequate for lowering temperature. It can be suggested that the direction of shift is consistent with the hypothesis that sample"O"has a increased proportion of hydrogen bounded water-to water molecules, as compared with sample "631". Another feature is to decide . which wavelength range is more suitable for moisture determination in yeasts. Dried baker's yeast

possesses great amounts of polycyclic hydroxy compounds, therefore attention should be paid to the fact if there is interference between these components and water absorption bands. Data reported by Delewiche (3) shows that if water is adsorbed on polystirene beads, the highest absorption values are to be measured at 1935 nm,although there is also an absorption band in the shorter wavelength region. If water is absorbed on a starch, then the shortest wavelength band maximum corresponds to 1461,5nm, if the water content is 24,5%, then against a background A=0,32 the absorbance peak is A= 0,076, other measurements revealed that 15,3% water content corresponded to a peak value A=0,06. The longer wavelength absorption band at 1923 nm at a moisture content 24,5% reveals the absorbance peak of A= 0,63 on a background A= 0,2, but 0,21 at a moisture content 15,3% on a background 0,6. This shows that if water is adsorbed on a starch then the sensitivity of method depends upon the background, and the wavelength. At wavelength 1923 nm the method is much more sensitive as at the wavelength 1346,5. At the latter the method for starch measurement is inadequate, because starch absorbs in this region that gives high background values and lowers the sensitivity of method. Our measurements show that the increment of absorption slope permits to distinguish between water absorbtion and the absorption related to other cell compounds, the maximum of the first derivate is located considerably higher upon the "0"line,than the minimum, which means that the ascending part of the peak is steeper as the descending one and this part can be used for moisture measurements. However the 1910-1940 nm diapason is more sensitive for water measuring purposes,than the shorter wavelength diapason. Of course, the wavelength diapason depends also upon the scanning method and the detector, which can be employed. Si-detectors can be applied in the region from 800 to 1200 nm,they have a very high signal - to -noise ration (4). Our observations show that at 840 nm absorbance values of both samples differ. A problem in this region, however, is that spectra are even less selective than they

are in the higher wavelength region and the visual information in the spectra is almost useless(6).

CONCLUSIONS

NIR reflectance spectroscopy can be used for dried baker's yeast analysis. Equilibrated to room conditions, samples of lower quality reveals higher moisture content and a shift of wavelength. Observations are consistent with hypothesis of Delewiche, and others (3,7) that water can be associated with the carbohydrate matrix and an increase or decrease in hydrogen-bonded water molecules results in a decrease or increase in the vibrational frequencies of the water OH bonds, and that can be used for yeast quality determination.

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Porosity Studies of the Gypsum Forms Produced by Lease Enterprise "Rigas Porcelans"

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INTRODUCTION

Latvia is famous for the traditions of porcelain manufacturing. At present, too, the lease enterprise "Rigas Porcelans" is producing a wide assortment of porcelain manufactures and is exporting it to several foreign countries, mainly to Lebanon, Mongolia, Kuwait, Saudi Arabia etc..

In order to obtain high-quality porcelain with the casting method, qualitative gypsum forms are needed. On order from the lease enterprise "Rigas Porcelans" the factors were determined which influence the gypsum forms' porosity. The management of the enterprise wanted to know he reasons of the diversity of the forms' porosity which are produced by different masters and the possibility of grouping or sorting of the gypsum forms' with equal or very close porosity.

METHODS

In order to obtain high-quality manufactures, great significance pertains to properly manufactured and applied gypsum forms. The gypsum forms were cast in the lease enterprise "Rigas Porcelans" by three different masters. From each master two probes of the form manufacturing were taken, then these gypsum samples were brought to the plant's laboratory where the ultrasound apparatus UK-10 was set up and measurements were performed(Fig.1.).

In the case of the penetrating of gypsum forms' surface the surface waves rate C_R was determinated according to the formula:

$$C_R = \frac{1}{\tau - \Delta\tau} \quad \text{m/sec} \quad /1/,$$

where 1 - the measurement basis, mm;

τ - total time necessary for the waves to propagate from the irradiator through material to receiver, sec;

$\Delta\tau$ - time delay in the concentrators, sec.

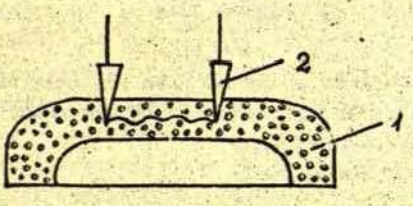

Fig.1.Gypsum form and piezotransformers.
1 - solidifying gypsum; 2 - piezotransformers.

Such action allowed in an uninterrupted manner to follow the process of gypsum solidification which on the average lasted 10 -14 min, in dependence on the ratio the gypsum/water (g/w). Measurements were realised after each ten minutes. The gypsum porosity was determined, measuring the maximum water amount that imbibed in samples by the formula:

$$W = \frac{m_w - m_o}{m_o} * 100\ \% \quad /2/$$

where m_w - freshly formed mass of gypsum;

m_o - absolute mass of a dry sample, g.

For evaluation of acoustic factors and porosity parameters the methods of data mathematical processing were made use of.

The mean arithmetical values were calculated:

$$\bar{x} = \frac{\sum x_1 \dots x_n}{n} \qquad /3/,$$

where $\bar{x}$ - the mean value of the factor to be measured;

x_1 , x_n - separate measurements (samples);

n - number of measurements or samples.

The mean quadratic dev'ation was calculated following:

$$\hat{S} = \sqrt{\frac{\sum (x - x_i)^2}{n - 1}} \qquad /4/,$$

where $\hat{S}$ - mean quadratic deviation from the mean value;

$\bar{x}$ - mean value of the factor to be measured;

x_i - actual measurements;

n - number of measurements.

DISCUSSION

Permanent control of solidification kinetics of the gypsum composition g/w = 1,2 was performed by use of the method of ultrasound surface waves.

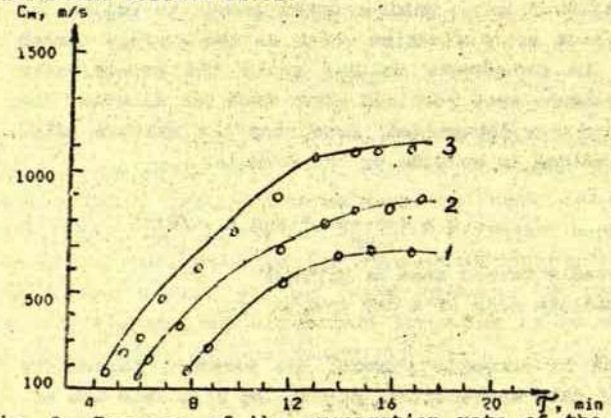

Fig. 2. Increase of the propagation rate of the ultrasound surface wave in gypsum during solidification time. 1, 2, 3 - gypsum composition made by different masters.

From Fig. 2. it is to be seen that solidification of gypsum lasts on the average 14 minutes, which is indicated by the circumstance that the time of ultrasound propagation after 14 minutes long solidification of gypsum virtually ceases to decrease. In Fig. 2. the change of the propagation rate of the ultrasound surface wave is shown in gypsum with ratio g/w = 1,2 in dependence on the solidification time, using the previously given formula for calculation of the ultrasound rate /1/. As it is evident from Fig. 2,
three manufactured gypsum compositions solidify in very different ways. First of all, the induction period in solidification process is different: for the samples made by the first master it equals 4 min, of the second - 6 minutes, of the third - 8 minutes. This induction period shows the time of solidification which is necesary for formation of the minimum strength in the gypsum manufactured item. When this minimum strength was created, on the oscilloscope of the ultrasound apparatus UK-10 an ultrasound wave a small amplitude appeared. In the case of solidification the rate of ultrasound propagation rapidly increased from 100 m/sec to 700 m/sec, 800 m/sec and 1150 m/sec respectively in the manufactures made by the first, second and third master. In the final stage of gypsum solidification, i.e., after 14 minutes, the ultrasound rate changed insignificantly, therefore the conclusion of solidification process is characterized by the parameter dc/dt = 0. The very diverse ultrasound propagation rates at the end of gypsum solidification point to the very different porosity which the various manufactures possess.

The different results obtained in experimental study of the samples made by different masters suggested to us that we should perform additional experiments in the plants laboratory with the same gypsum taken by the plants masters. We decided to establish the correlation in a wide range of the ratios g/w in dependence on the manufactured item's

porosity. By changing the ratio g/w it is possible to purposefully change the manufactures porosity. In the plant laboratory the following compositions of g/w ratios were prepared:

g/w = 0.9; 1.0; 1.1; 1.2; 1.3; 1.4.

Knowing the sample s porosity and the ratio g/w in these samples, the relation between those two values was taken and depicted graphically in Fig. 3.

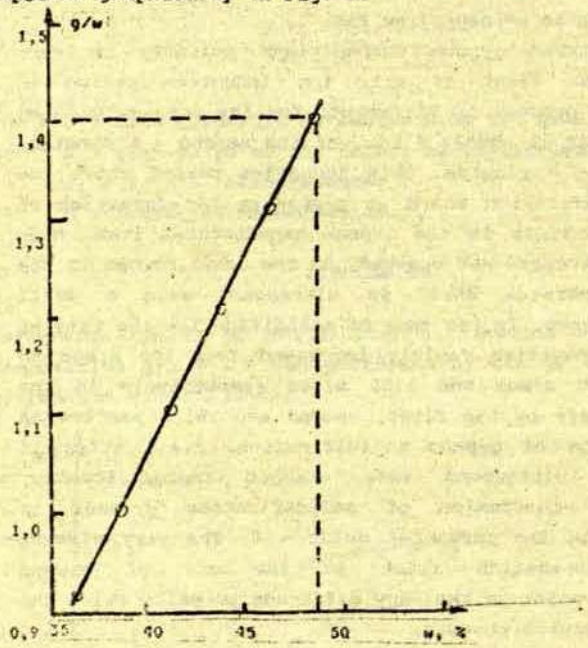

Fig.3. The dependence of gypsum manufactures porosity of determinated experimentally on the ratio g/w in lease enterprise "Rigas Porcelans".

As it is evident from Fig.3., changing the ratio g/w from 0.9 to 1.4 enables to monitor the manufactures porosity from 36.7% to 49%. According to demands set for the porosity of

gypsum forms it must be within limits of 43% to 47%, thus in a rather wide range.

As it is obvious, the lesser porosity can be ensured with the ratio g/w equal to 1.16, but the larger one - with 1.31. Laboratory studies showed that an accurate keeping to the g/w ratio ensures a precise porosity to the gypsum forms. Further on we clarified gypsum forms of that porosity are produced by the three masters at the lease enterprise "Rigas Porcelans".

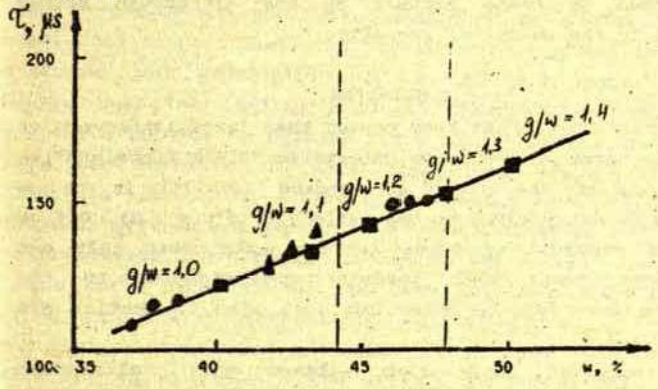

Fig.4. Dependence of the propagation time of the ultrasound surface wave upon the manufactures porosity in gypsum forms:

- o - forms of the first master;
- ▲ - forms of the second master;
- ● - forms of the third master;
- ■ - the additional experiment carried out at the plant's laboratory with different g/w ratios.

On the diagram the ultrasound propagation time in solidified gypsum manufactures is indicated in dependence on the manufactures porosity. On the diagram the porosity of all samples obtained by the three masters well as some results which were attained in the enterprise s laboratory for the sake of comparison. As it is to be seen from Fig.4., only one

of the masters working at the plant manufactures forms according to the demands of the standard. It is evident from Fig.4, that there is a linear relation between the ultrasound propagation time and forms porosity.

For the enterprise to get out of the unfavourable situation, it is necessary to ensure that the masters work in an honest manner, or there is a second solution: to sort out the forms manufactured by these masters by the ultrasound method according to the extent of porosity.

CONCLUSION

1. Experimentally it has been proved that large inadequacy of the gypsum forms at the lease enterprise "Rigas Porcelans" to the demands of the plant' s standard (43-47%) is to be explained by not keeping to the ratio g/w (g/w = 1.2). Out of the forms produced by three masters under test only one manufactures forms with porosity corresponding to the standard's demands, the other two work with the ratios g/w 1.0 and 1.1.
2. The acquired correlation between the ultrasound propagation time and form s porosity can be practically used in the plant in sorting out of the forms in dependence on the porosity.
3. It has been found experimentally that the gypsum forms' porosity can be monitored purposefully by slightly changing the ratio of g/w composition. There is a linear relation between these values.

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BUSINESS of SATELLITE RECEPTION SET-UPS in LATVIA

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INTODUCTION

Although appearance of satellites can be dated back to several decades and the direct reception satellites to one decade, still the industry of satellite set-ups all over the world has been developing only in the last years. It is assumed that the leading place in receiving of satellite broadcasts belongs to Germany where in 50% of the new flats there is installation with connecting clips for the collective reception system. In Germany the collective reception systems are popular therefore because in many cases the architects don't permit to put up the satellite mirrors at the house facades, but the set-ups placed on the roof rather often are subjected to the wind's voltage.

According to the rules prevalent in Germany, referring to "Kathrein 90 Kataloque", the aerials for household use if they are put up lower than the 7th floor, have to endure 800 N/m which corresponds to the wind speed 35,8 m/s, i. e., 129 km/h or wind strength 12 balls.

In 1990 in Germany the satellite industry production amounted in value to 1,7 million DM, colour TV sets- 6,4 million DM. The average price of a TV set is 1200 DM, but of the satellite set-up - 600 DM, i. e., the price of one TV set equals the price of two satellites.[1;3]

In a year 900 000 disks are sold. In 1992 more than a million will be sold. It is assumed that nowadays there are 2 million functioning disks. Most of all the manufactures of Kathrein, Amstrad, Technisat are sold: Kathrein 31,7%, Technisat 12,9%, Hirschman 9,7%, Fuba 8,4%, Amstrad 10%, Nokia 8%, Wisi 7,8%, the others- 7,2%. The installed production: Kathrein 29%, Technisat 18%, Amstrad 10%, Hirchman 8%, Wisi 8%, Nokia 4%, Fuba 7%, the others 16%. [1;4]

The studies in Taiwan are developing most rapidly of all at the Computer and Communication Research Laboratory (CCRL), at firms Proton Electronic Industrial Corp., Shampoo Technology Corp., Dating Corp.. In the research institutions 16 millions dollars are invested, during next 5 jeers the investment will amount to 200 million dollars.[1;5]
At the Berlin International Communication Means Exhibitions were demonstrated the set-ups which receive 28 programs Pall, 8 - D2 Mac, 3-HD Mac. D2 Mac has two formats 16/9 and 3/4. All the programs come from RTL Plus, Sat 1, 3 Sat, 3 Plus. D2 Mac comes from TDF-1-2, Canal Jot, La Sept, Euro Music, Cinema TV, Canal Plus.[1]

Television technique is passing from Pall over to D2 Mac, then to HD Mac, afterwards to HD TV.[1]
Post ministers of France and Japan have decided to work out a converter which would operate according to the standards both of HD Mac and of the Japanese High Vision. It is supposed that this converters will be ready in 1997, but it is possible that at the Olympic Games in Barselona in 1992 something of the kind had been tried.[1;6]

RESULTS AND DISSCUSION

There is a complete lack of information on the satellite set-ups produced in Latvia, their types, prices and technical parameters which constitute the basis for the present report. Information was gathered during visiting to the users of set-ups, collecting from them particulars on the adjustment time, picture quality, sound quality, the most viewed programs as well as critical remarks on the set-up's operation.
Having examined the construction peculiarities of the aerials manufactured in Riga, it was found that as the aerial's material in 80% of cases aluminium is used, in 15% - epoxide resin framework with an agglutinated aluminium foil, in 5% - polystyrene framework. Comparing these data with the aerials produced in Germany, it turns out that there polystyrene with

a special coating is used as the main material which prevents corrosion preserving, high reflection capabilities.

Determining these size of aerials put up in Riga , it has been established that in 15% of cases they exceed 2m, in 75% the size is from 1,2-2m, in 10% aerials with the size from 0,9-1,2m.

Having investigated the fastening peculiarities of the aerials installed in Riga, it was found that in 85% of cases a three-point system with a stationary azimuth is applied, in 10% two screws are used for putting up which are hand-operated and those position stability is ensured either by a slit in the screw or by an elastic undultorsocket made of spring bronze. In 4% the direct-current motors are used with in connection element bridge transmission, but in the remaining cases the step motors are used.

Evaluating the exploitation properties of aerials according to details supplied by the users it has been found that in 80% of cases complaints refer to the instability of the large (more than 1,5m) aerials' position caused by wind deformation, because turning by 1 ' which corresponds to turning only by 15mm causes for an aerial with 1,8m diameter the amplificivation coefficient equals to that of an aerial with diameter of only 1,2m and the quality of the 1,8m diameter diminishes by 3 dB which signifies decrease of signal two times. In 20% of cases the complaints refer to the deterioration of the aerials' properties during the operation process if they are made of epoxide resin, which could be explained by a heterogeneous mixing of epoxide resin with the solidifying agent or by a heterogeneous irradiation from sunlight. Having studied the production process of aerials, it has been established that in 80% of cases the aluminium aerials are formed with a little roll by inclusion of terminal processes.

Having investigated the fastening system of the converter it has been determined that in all cases it is fastened in the aerial's focus and in 50% of cases its proper fastening is

judged from the signal fading upon change of the aerial's orientation towards satellite.

In none of cases examined by me, there was installing of several convertors in the aerial's mirror.

Evaluating the polarization set-ups, it has been found that the examined users apply the ferrite set-ups (Faraday polarizator).

Further data were acquired, referring to the producers of the set-ups. It has been found that in 90% of cases the receivers are manufactured on the spot in Riga by 9 firms which produce 85% of receivers "with manual tuning", in 10% of cases such receivers are produced where the channels are chosen by remote monitoring board, in 5% such receivers are produced where the program is selected by press-buttons. All the native companies are selling the foreign produce, too. Most of all the manufactures of Echostar, Arcon and Kathrein are merchandized.

Tuning of the foreign receivers to the necessary program in all cases is performed by preliminary programmed press-buttons which are located on the remote monitoring board that functions in the IR ray regime and has a 100% stereo performance.

It is not always that companies supply exhaustive information on the receivers' sensitivity, homogeneity of sensitivity in the frequency range and automatic monitoring of amplification. In all the cases there are Low Noise Converter (LNC) feeding clips which allow to use a mechanical polariser and the Faraday one.

Having studied the documentation of convertors, it has been found that there exists data on the noise number, but there is a lack of data on amplification and its homogeneity as well as on the heterodynes stability and on the distance of mirror frequencies. In 90% of cases the convertors made abroad are used.

Information was obtained from the manufacturers concerning the price of set-ups which is given in 6 tables.

It is evident from Table 1 that the majority of aerials are made of aluminium. The aerials' price doesn't increase in proportion to the aerial's aery, what suggests that the metal isn't the limiting factor for the price. It is of interest to point out that optimum under our circumstances are the aerials with diameter 1,8m and aerials with larger diameter than 1,8m are not more expensive.

From Table 2 it is to be seen that the polarisers' prices are rather similar, what can be explained by the fact that in the CIS quite good ferrites are available which can compete with the foreign analogues.

It follows from Table 3 that convertors usually are within the region of 10-12 GHz, and their prices, too, are very much the same. Relatively higher are prices for the convertors with several ranges.

It can be assumed from Table 4 that the receivers as to their price are rather different, which can be explained by the automatization degree.

In Table 5 the distributors' prices are presented. It needs to be mentioned that the active distributor stimulate the amplification of the signal.

From Table 6 it is evident that the cheapest optimum satellite set-up costs 22 512 roubles.

Having analyzed the price fluctuations for satellite set-ups, we can see that the prices of set-ups' haven't increased in proportion to other household electronic goods, which corresponds to the trend in the world for the satellite set-ups to become simpler.

Table 1 Survey of parabolic aerials and their prices

Aerial's diameter[m]	0,9	1,0	1,2	1,4	1,5	1,6	1,8	2,0	2,1
Companies:									
Stella roubles					*3500		*3500		
by transfer					*6000		*6000		
Walket roubles				4000-6000					
							100		
Baltsat $			45			50			
Roboteks roubles			^5000	5000	5000				
Furnieris $	^50	50		50	^50				
Unisat $			*20	*25			*50		*50
Latsat $	^40		55	70	^70			#90	
Motor Museum DM	100	100	100	100	100	100	100	100	

*- without mechanism
^- glass plastic
#- carbon plastic
Aerials' mechanisms
1500-2700 roubles
15-25 $

Table 2 Survey of the polarisers' prices

Company	Polariser	$	DM	Roubles
Latsat	mechanical	15		
	impulse	20		
	ferromagnetic	18		
Baltsat	ferromagnetic			1600
Furnieris				1500
Walket	mechanical with impulse control	20		
Stella	ferromagnetic			1500
Unisat	electromagnetic	15		

Table 3 Prices of different converters

Company	Parameters	$	DM	Roubles
Latsat				
Kathrein GFR				
UAS-211	0,98-1,1 dB	130		
UAS-171 with 10,95...11,7 GHz 12,5...12,75 GHz	1,4 dB	220		
UAS-52 Ofset		230		
Gardiner USA	0,8-0,9 dB	140		
Dxontene Jap.	0,8-0,9 dB	140		
Feinwerk	4 GHz,30k	350		
Roboteks				
Echostar	0,8 dB	130		
Gardiner USA	0,7 dB	136		
	0,8 dB	126		
	0,9 dB	116		
	1,0 dB	110		
Baltsat	0,9 dB	125		
	1,0 dB	120		
IGE				
Yagi Jap.	1,0 dB	120		
Walket	1,1 dB	130		
	1,4 dB	95		
Stella		90-120		
Radij Moscow				8000
Unisat		90-130		
Elar Latvia				8800
Radij Moscow		60		

Table 4 Survey of satellite receivers and their prices

Company	Receiver	Manufacturing Country	$	DM	Roubles
Latsat	SR 100	Latvia	70		
	SR 200	Latvia	70		
	Wisi OR 31	GFR	250		
	Kathrein GR 8.	GFR	320		
	Echostar SR 1500	Netherlands	300		
	Sankyong	South Korea	280		
Roboteks	SR 1010	Latvia			5500
Walket	Arcon P 600	USA	250		
	Echostar SR 1500	Netherlands	300		
Baltsat	Baltsat	Latvia			6500
Furnieris		Latvia			9000
Stella	Stella	Latvia			5500
Unisat	SR Q SV	Latvia	40-60		
			80-100		
Latrek	Latrek	Latvia			10 000
Mottor	ArconP 600	USA		390	
Museum	ArconP 900	USA		480	
	Prosat			530	

Remark: in some prices the turnover tax is not includ 1.

Table 5 Prises of distributors

Company	Distributor	$	DM	Roubles
Baltsat	passive			1000
	active			1500
Latsat	passive	12		
	active	15		
Stella	passive			1200
	active			4800
MottorSAM	1418		93	
MuseumSAM	1418T		125	

Table 6 Prices of satellite set-ups in the shop "Solan".

Name	Price (roubles)
Set: Receiver "Stella"	5 912
Converter SMW LNB 1112 PRO, 1,2 dB (Sweden)	9 884
Parabola aerial	5 104
Feromagnetic polariser	612
in total	22 512
Decoders	4 448
Stereoprocessors	4 448
Spliter	1 612
Receivers: Origo	36 040
Orion	32 248
Unisat SR Q SV	6 304
Stella	7 560
Mercury Star Sat	10 340
Baltsat	7 088
Furnieris	7 560
Converters: Gardiner, 0,9 dB (USA)	16 008
Echostar, 1,1 dB Dual Model BKJ 1352	22 120
LNB 1210 Ku Band	8 896
HHO Saturns, 1,8 dB	5 332
M 55 121-2	6 024
Vorter HDB-600	10 631
Polarisers: Walket	556
Baltsat	1 788
Diameter of parabola aerial 1,6 m	5 448
1,8 m	6 336
Amplifier 5 dB Prosat frequency 1,3-1,7	1 112
Translation TV cables 25 m	250

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Determination of Some Basic Physico-mechanical Parameters of Leatherettes by the Aid of Nondestructive. Testing Problems with Ultrasound of Such Composite Materials as Leatherette

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In the present time the leatherette and film materials are economically significant materials for Latvia. They have found wide application in light industry and are accepted as materials with special wide use for export goods production. They consist of fibre and non-fibre layers, including composite materials, such as fibre reinforced resins, and textile fibres. Both fibres and plastic filling materials can be produced in Latvia, because we have fundamental raw material base. Their fundamental mechanical properties depends upon the used materials and the bond between them. A nonexistent or faulty glued bond in the leatherettes is easily revealed by its lower transmittance. In the USA industries the wheel probes are used for the continuous testing of plywood by the transmission method /1/. Quality tests of leatherettes have not so far found practical application, because the attenuation is quite high, compared with metals.

The most actual problems to be solved are the homogeneity of the product.

According to manufacture's method soft leatherettes and synthetic leathers point are produced with deformation methods from the polymer liquids, solutions and dispersions, by pressing them into a fibre carcass, this procedure should be controlled by examining the product.

According to polymer shape the leatherettes and synthetic leathers may be with resin, polyamide, nitrocellulose, polyvinylchloride, polyurethane and with union coverlet. The mechanical properties complex of the leatherette with polyvinylchloride coatings are near to the leather.

The polyurethanes industries make able to obtain soft leatherettes with high deformation-resistant properties, with

good cold stability with small upper density and satisfactory hygienic characteristics.

Depending on the used materials and technology the leatherettes may be monolithic, porous-monolithic, porous. In these experiments it was tried to get an express method for testing monolithic materials.

MATERIALS AND METHODS

As frequency generator a standard IK-10P defectoscope was used, receipted signal amplifier and travelling time detector was equipped with special made transducer equipped with exponential pole shoes, whose contact area was about 1 square mm /2-19/.

In all cases material is a heterophase material, which greatly increases the attenuation, only frequencies around 0.01-0.1 Mc can be considered. Beside of bonding of plastics to carcass another problem is the elastic characteristics of polymer material. For measuring them perhaps higher frequencies should be applied, what was not made in these experiments.

To receive a high attenuated signal a good couplant is needed. Couplants containing oil or water for leatherettes are usually undesirable. In our case a good acoustic contact has been assured by using very smooth shoes. The coupling pressure is so high, that no coupling liquid is required. In any ultrasonic test system the shape and roughness of the test surface is of decisive importance. In the case of direct contact where the probe is pressed into a specimen covered with a thin film of coupling liquid, foreign particles or layers are very disturbing. It is therefore necessary to remove any dirt of the surface. Generator frequency was 60 kHz. Pulse time was 1 ms. In most cases the carcass was very near to the analysed surface and in some cases it was even visually evident, that enables to interpret the results as carcass depending.

RESULTS

The method provides information obtaining about material quality in its manufacture process and also in exploitation; it may be used for the diversified configuration and thickness elaborations testing; it is economical in the time and expenses; it doesn't demand biological protection.

The ultrasonic method gives exactitude and quick information about the investigated materials physico-mechanical properties. The obtained results about ultrasonic waves propagation through the leatherettes samples may be used to figure out elasticity module and shear module, what are the basic characteristics of the leatherettes elastic and resistant properties. On the three leatherettes samples are shown, how to determine basic resistant characteristics - the elasticity module E and shear module G. The ultrasound propagation time difference in the all samples of one shape doesn't allow to overfulfill +5%. Measuring basis for the all samples should be identical and was equal 5 cm.

Young module E was determined using Rayleigh wave velocity c_R:

$$E = \rho c_R^2 \frac{2(1+\mu)3/2}{(0.87+1.12\mu)^2} \quad /1/ \ /19, \text{ p. } 29/,$$

where ρ - material density, kg/m;
c_R - Rayleigh wave velocity, m/s;
μ - Poissen coefficient.

The shea module G is determined also using Rayleigh wave velocity c_R:

$$G = \rho c_R^2 \left(\frac{(1+\mu)}{(0.87+1.12\mu)} \right)^2 \quad /2/ \ /19, \text{ p. } 30/,$$

where ρ - material density, kg/m;
c_R - Rayleigh wave velocity, m/s;
μ - Poissen coefficient.

The longitudinal wave's rate c_l in the environment with density ρ is determined by the elastic module E and by Poissen coefficient μ:

$$c_l = \sqrt{\frac{E}{\rho} \frac{1-\mu}{(1+\mu)(1-2\mu)}} \quad /3/ \ /14, \text{ p. } 14/.$$

The transverse wave's velocity C_t in the environment with density ρ is determined by the shear module G or by the module E:

$$C_t = \sqrt{\frac{G}{\rho}} = \sqrt{\frac{E}{2\rho(1+\mu)}} \qquad /4/.$$

According to the equations /3 and 4/ we have a ratio:

$$\frac{C_t}{C_l} = \sqrt{\frac{1-2\mu}{2(1-\mu)}} \qquad /5/.$$

If the longitudinal and the transverse wave's velocities are known, from the formula /5/ Poissen coefficient is calculated:

$$\mu = \frac{C_l^2 - 2C_t^2}{2(C_l^2 - C_t^2)} \qquad /6/.$$

For the determinate elasticity and shear module it is necessary to know the density of investigation samples. From /16, p. 24-26/ is known the leatherettes surface density. Their thickness is known from /10, p. 2-31/, /8, p.2-31/. The density was found dividing surface density on thickness (Table 1).

Table 1. Some of Physico-mechanical Characteristics of the Some Leatherettes

Name	Vinyl-T	Vinylurethane-T	Vinyl-T poriferous
Colour	yellow	light blue	light yellow
Group	9	14	19
Thickness, mm	0 5	0.9	1.4
Surface density, g/m²	650	470	950
Density, kg/m3	1300	522,2	678,6
Poissen coefficient	0.43	0.22	0.37

In all cases was used the leatherettes for haberdashery. Their articles were: for the first sample - 1R7-68 and 1R4-68, for the second sample - 1R7 and 1R31, and for the third sample - 1R3, 1R31 and 1R1.

After determine time of appearance of the straight longitudinal wave front on the appliance screen and after measure of the transmission mode basis between transducers,

we may to figure out the longitudinal wave propagation velocity by the formula /7/:

$$C_l = \frac{l}{\tau_l - \Delta\tau_l} \qquad /7/\ /19,\ p.34/,$$

where $\Delta\tau_l$ - delay of the impulse in the transformers and in the passable layers.

The delay $\Delta\tau_l$ was determined by the longitudinal profiling experimental method, when transducer with exponencial wave guids is putting up immobile, but the basis l of the transducer-receiver is changing. From the results of measurements on the fixed basis we have experimental straight line (Fig.1). For the yellow sample $\Delta\tau_l$ = 145.5 μs, for the light blue sample $\Delta\tau_l$ = 185 μs, for the light yellow sample $\Delta\tau_l$ = 165 μs.

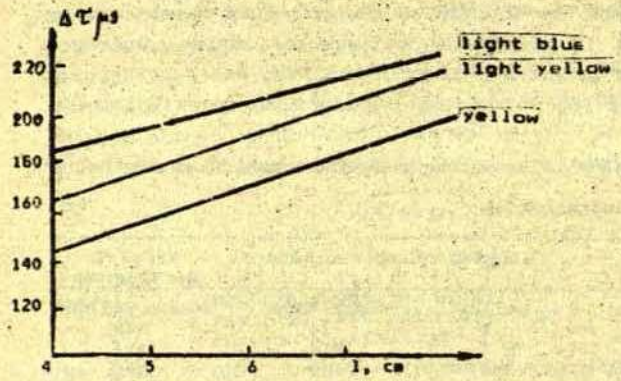

Fig.1. Dependence on time of ultrasound wave's propagation through the leatherette samples in the longitudinal profiling on the distance between ultrasound irradiator and receiver.

In the thinwall elaborations may be various types of waves. In the simplest case surface waves passage into symmetric and unsymmetric Lemba waves. Between the bend C_U and surface C_R waves velocity and the elaboration thickness there exists the definite dependence. In the thinwall elaborations the

ultrasound velocity depends upon the elaboration thickness: if thickness is smaller, ultrasound velocity also is smaller. So for the non-destructive control of the thinwall elaborations the bend wave may be used only in the case, when elaborations thickness is changeable. According to the data of the velocity C_U and C_R and the testing leatherettes samples thickness h data should be determined by the formula /8/ coefficient K for the surface waves velocity for the each sample and then carry out the control.

$$K = \frac{C_U}{C_R} \qquad /8/\ /19,\ p.35/,$$

where C_U - ultrasound bend wave velocity, that is transmission through investigated thin sample, m/s;
C_R - ultrasound surface wave propagation velocity, that is transmission through the thick samples, m/s;

Coefficients K values for the investigated samples were: for the yellow sample - 0.35, for the light blue sample - 0.60, for the light yellow sample - 0.4.

After experimental determination of the ultrasound velocity in the 15 samples of the leatherettes the third type of each and using another calculated data we can exemplify the corresponding Tables 2 and 3.

Table 2.The elastic properties of the leatherettes

Nr.	The yellow sample τ, μs	C_R m/s	C_U m/s	$E*10^7$, Pa	$G*10^7$ Pa	The light blue sample τ, μs	C_R, m/s	C_U, m/s	$E*10^7$, Pa	$G*10^7$ Pa
1	162	3030	1061	0.96	0.40	203	2730	1668	1.25	1.11
2	159	3700	1295	1.43	0.60	203	2780	1668	1.25	1.11
3	162	3030	1061	0.96	0.40	205	2500	1500	1.01	0.90
4	157	4350	1523	1.97	0.83					
5						201	3130	1878	1.59	1.41
6	158	4000	1400	1.67	0.70	197	4170	2502	2.81	2.50
7	159	3700	1295	1.43	0.60	197	4170	2502	2.81	2.50
8	156	4000	1400	1.67	0.70	201	3130	1878	1.59	1.41
9	157	4350	1523	1.97	0.83					
10	158	4000	1400	1.67	0.70	199	3570	2142	2.06	1.83
11						193	6250	3750	6.32	5.62
12	158	4000	1400	1.67	0.70	197	4170	2502	2.81	2.50
13	162	3030	1061	0.96	0.40	200	3330	1998	1.79	1.59
14	159	3700	1295	1.43	0.60	200	3330	1998	1.79	1.59
15	157	4350	1523	1.97	0.83	200	3330	1998	1.79	1.59

Table 3.The elastic properties of the leatherettes

The sample number	τ, s	The light yellow sample C_U, m/s	C_R, m/s	$E*10^8$, Pa	$G*10^8$ Pa
1	179	3570	1428	1.15	0.47
2	173	6250	2500	3.51	1.45
3	180	3330	1330	0.99	0.41
4	182	2940	1180	0.78	0.32
5	184	2630	1050	0.62	0.26
6	182	2780	1110	0.69	0.29
7	175	5000	2000	2.25	0.93
8	175	5000	2000	2.25	0.93
9	177	4170	1670	1.57	0.65
10	179	3570	1430	1.15	0.48
11	178	3850	1540	1.33	0.55
12	184	2630	1050	0.62	0.26
13	179	3570	1430	1.15	0.48
14	179	3570	1430	1.15	0.48
15	180	3330	1330	0.99	0.41

DISCUSSION

Results reveal the varies of the sound velocities in a wide range. Perhaps defects are revealed including delamination, inclusions, cracks and gaps, also transverse to the main direction, which are produced by bonding faults, by insufficient resin, incomplete curing or lack of interior-layer adhesion. To differentiate between possible reasons of wide ultrasound velocity dispersion further analysis is needed, including variation of test frequency, because the latter determines the depth of material responsible for velocity variation. However, experimental work shows, that the attenuation in these materials is usually very high and that limits the highest applied frequency. For better material investigation further acoustic devices should be applied, which should permit to locate the strong anisotropic regions. For these purposes manual testing methods are mostly not economical. In practice large installations with mechanized scanning and full immersion techniques should be applied /1/. Perhaps a through-transmission mode should be of more advantage. One can also observe a rear - face echo from a reflector placed in the water behind the piece. It can be

speculated that surface wave velocity can be misinterpreted for several reasons. One of the reason could be a surface coated with grease. It is not ruled out, that the smallest dirt on the inspected material can false the results. The wave can also follow a curved surface and travel with little interference over edges. Perhaps surface wave test can indicate crack-like defects lying just below the surface /1/. Therefore also other acoustic test methods should be applied including sound transmission methods /1/.

The testing problems relevant to the inspection of strip and thin sheet material differ greatly according to the fabrication and contract requirements. Perhaps the use of ultrasonic spectroscopy involves the investigation of received signal to determine its frequency content. This can be done by frequency analyst and auxiliary equipment to give an amplitude-frequency curve for the received signal. This can be compared with the corresponding curve of the original transmitted pulse. If the material concerned has little attenuation the back-wall echo is identical with the transmitted pulse but the reflection at a defect acts as a frequency filter in the overall system. The wider is the frequency band of the transmitter, the shorter is the transmitter pulse, the better information concerning the defect can be expected from analysis of the echo frequency spectrum /1/.

CONCLUSION

It is possible to determine physico-mechanical properties (elasticity module E, shear module G and Poissen coefficient) of the leatherettes by the ultrasonic testing method without structure destruction.

This method is possible to be used for operative qualitate control of various thickness and configuration leatherettes.

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