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THE YEAST CULTURE *SACCHAROMYCES CEREVISIAE* IN THE BIOTECHNOLOGY OF ETHANOL PRODUCTION FOR USE IN THE PHARMACEUTICAL INDUSTRY

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The yeast *Saccharomyces cerevisiae*, isolated from different grape cultivars, was used for Ethanol production. The biotechnological properties of yeast culture for Ethanol production were determined in wine yeast. Grape pomace was used as the raw material for fermentation. After fermentation completion, the resultant product was distilled (rectified), then used preparation of medicinal herbal spirituous extracts. This article presents the results of analysis of the Ethanol concentration for medicinal spirituous herbs. For Eucalyptus spirituous extract, Ethanol concentration should not be less than 65% for complete extraction of the polyphenols and essential oils. It was determined that the Ethanol concentration for Wormwood should not be less than 65 v/v% to extract glycosides. The Ethanol concentration for Echinacea spirituous extracts should not be less than 41% for complete extraction of pentosans, polyphenols, polysaccharides, and organic acids (caffeic and cichoric acids) from the medicinal herb. The Ethanol concentration for Peppermint extracts should not be less than 81 v/v% to extract essential oils. Ethanol concentration for Calendula extracts should be not less than 65 v/v%, to extract flavonoids, in terms of hyperoside, not less than 0.04%. It was found that the medicinal herbal spirituous extracts preparation using Ethanol produced from grape pomace (*spiritus vini*) is a new perspective and prospective direction for the pharmaceutical industry.

At present, production of Ethanol for the pharmaceutical industry takes place in one of two ways: fermentation of food raw material by hydrolysis of plant materials: and synthetic – hydration of ethylene. Production of Ethanol by fermentation usually proceeds with the participation of some enzymes [1, 2, 9]. As raw materials, cheaper materials such as starch of potatoes and cereal, are usually used. Another more readily available mechanism for Ethanol production is connected with the technology of sugar fermentation by yeast [3, 4]. A large amount of Ethanol is used in pharmacy settings for the preparation of spirituous extracts of medicinal herbs. It is very important, therefore, to have yeast cultures with high enzymatic activity and power, which will produce high levels of Ethanol into fermented substrate during fermentation. Before yeast culture can

be added to the fermentation substrate, it is necessary to grow yeast in a separate vessel in a sterile medium over a period of 15–19 hours. The most suitable materials for culture media are: green barley malt, rye flour, and sugar production waste [7, 8]. It is necessary to bear in mind that, to support stable enzymatic activity, yeast culture usually requires more organic nitrogen then is present in the raw materials. By the third day of fermentation, yeast will eliminate most of the nitrogen in the fermented substrate; therefore, the addition of nitrogen from mineral substances in the form of ammonium and phosphorous salts such as: ammonium chloride, ammonium sulphate, and diammonium phosphate, is required. If, in the fermented substrate, there is less than the necessary amount of nitrogen, enzymatic activity of the yeast will spontaneously decrease. After ten days, the fermentation processes decrease, and fermentation achieves completion. The substrate resulting from fermentation should be purified by distillation (rectification). Primary Ethanol concentration is usually 40% v/v. In Ethanol rectification, the general goal is to process from raw alcohol – 40% v/v, and to increase Ethanol concentration using distillation and rectification of Ethanol to 96% v/v with minimal impurities content. To this, a crude alcohol rectification process is carried out on specialized rectification equipment. This equipment allows the separation of the water-alcohol mixture to separate azeotropic fractions, which differ from each other by boiling temperature. One of these fractions is a food rectified Ethanol, which could be used in the pharmaceutical industry for production of medicinal spirituous herbal extracts.

The aim of this research was to study the yeast culture *Saccharomyces cerevisiae* at grape pomace fermentation for Ethanol production, and its use in the pharmaceutical industry for the production of spirituous medicinal herbal extracts.

To achieve this goal, the following tasks were undertaken. The yeast culture *Saccharomyces cerevisiae,* with a high level of Ethanol production, was used. The fermentation process proceeded using grape pomace. To support yeast culture with nitrogen, on the third day of fermentation, diammonium phosphate was added to the fermentation substrate as a source of nitrogen and phosphorus. The fermentation process continued for ten days, and then the fermented grape pomace substrate was rectified to produce concentrated Ethanol (*spiritus vini*), up to 92–95% v/v. As the ethanol was purified and analyzed, it was used for spirituous medicinal herbal extracts. Most grape pomace rich in raw material is mainly found in vineyard area. These include the southern regions of the Ukraine such as: Crimea, Kherson, Nikolaev, Odessa region, and Transcarpathia region (Uzhgorod). During the vintage season, a grape pomace remains which is from 15 to 25% of the total weight of the harvested grapes. This amount of grape pomace should, therefore, be processed into high quality Ethanol, called *spiritus vini*, for use in the pharmaceutical industry. The medicinal herbal spirituous extracts from grape pomace have remarkable antioxidant and radical

scavenging properties in correlation with their content of total polyphenol substances [5, 6]. Such a natural product as Ethanol (*spiritus vini*), produced using the biotechnology of grape pomace processing and production of the spirituous medicinal herbal extracts will, therefore, be valuable for use in the pharmacy setting [12, 14].

MATERIALS AND METHODS

Saccharomyces cerevisiae yeast cultures, with a high level of Ethanol production, were isolated during the vintage season from the vineyards of the Agrocultural Company "Koblevo", located in the Nikolaev region and Tairov Research Institute, in the Odessa region of the Ukraine. The following yeast cultures were selected for the research.

I. Laboratory yeast cultures isolated from fermented grape must from the "Koblevo" Agricultural Company:

Yeast cultures obtained from white grape varieties:

- Y-3450; MAFF-230112 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Rkatsiteli.
- Y-3480; MAFF-230119; NRRL Y-63636 – *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Chardonnay.
- Y-3481; MAFF-230120; NRRL Y-63637 – *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Riesling Rhenish.
- Y-3484; MAFF-230123; NRRL Y-63640 – *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Traminer.
- Y-3491; MAFF-230130; NRRL Y-63645 – *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Muscat Ottonel.
- Y-3488; MAFF-230127; NRRL Y-63643 – *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Irshai Oliver.

Yeast cultures obtained from red grape varieties:

- Y-3454; MAFF-230116 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Cabernet-Sauvignon.
- Y-3453; MAFF-230115 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Merlot.
- Y-3451; MAFF-230113 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Odessa Black.
- Y-3486; MAFF-230125; NRRL Y- 63641 – *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Bastardo.

II. Laboratory yeast cultures isolated from fermented grape must of Tairov Research Institute:

Yeast cultures obtained from white grape varieties:

- *Y-3439; **MAFF-230101 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Selena.
- Y-3440; MAFF-230102. *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Sukholimansky.

- Y-3441; MAFF-230103 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Opalovy (Opaline).
- Y-3444; MAFF- 230106 *Saccharomycescerevisiae* isolated from fermented grape must of the variety Aromatic.
- Y-3445; MAFF- 230107 *Saccharomycescerevisiae* isolated from fermented grape must of the variety Odessa Muscat.

Yeast cultures obtained from red grape varieties:

- Y-3438; MAFF-230100. *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Charivny (Magic).
- Y-3436; MAFF-230098. *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Tairov Ruby.

The designated yeast culture numbers were derived from those deposited in the:

* USRCB – Local Culture Collection.

** MAFF – Culture Collection of Microorganisms, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki, Japan. National Institute of Agrobiological Sciences, Genebank of Japan.

*** NRRL – National Research Regional Laboratory, ARS, Peoria, USA.

Primary yeast isolation was carried out using Inhibitory Mold Agar medium and then cultivated in Wort Agar (Becton Dickinson Company, USA). The morphological properties of yeast cultures were analyzed after their primary isolation from fermented grape musts. Yeasts were identified by polymerase chain reaction (PCR) using universal yeast primers. After identification, each isolated yeast culture was deposited in the Culture Collection of National Regional Research Laboratory (NRRL), ARS, Peoria, USA; in the British National Collection of Yeast Culture (NCYC), Norwich, UK and in the Genebank of Japan (MAFF), National Institute of Agrobiological Science (NIAS).

All biochemical parameters were tested in the fermented grape pomace samples obtained from white and red grape cultivars. The volume fraction of Ethanol, total sugar (glucose/fructose), and polyphenols were determined by liquid chromatography (Ultimate 3000, Dionex Company, Germany), and other parameters by a spectroscopy method using the Bacchus-II spectrometer (Microdom Company, France). It is important that *Saccharomyces cerevisiae* yeast cultures are resistant to the presence of high levels of Ethanol, up to 15%v/v. As fermentation proceeds, Ethanol production increases. It is, therefore, necessary for yeast culture to be resistant to the Ethanol present. Some yeast culture can grow in the presence of 16% v/v, however they will require special nutrient supplements with a rich amount of amono acids and nitrogen content. Fresh grape pomace from red and white grape cultivars fermentation was obtained from the winery Company "Koblevo", in the Nickolaev region of the Ukraine. Grape pomace contains pulp of the grape pomace, grape skins, and grape seeds.

For Ethanol production it is necessary to use the following method: it is necessary to add 10 kgs fresh grape pomace to the fermentation vessel, it is also necessary to add 10 liters of water, in the ratio (1:1), and 500 g of sugar (sucrose), to the 10 kgs of grape pomace liquid (5%); then it is necessary to mix thoroughly. After this, it is necessary to add a supplement to the mixture called "Cardifit" – potassium bisulphite ($K_2S_2O_5$), – the concentration of which is necessary to add to the grape pomace is 0.5–1.5 g to 100 kgs of grape pomace to achieve 25–75 mg SO_2 to the liter. In these conditions, potassium bisulphite ($K_2S_2O_5$) decomposes to form sulphur dioxide (SO_2) in amounts equivalent to 50% of the introduced weight. Sulphur dioxide, introduced via potassium bisulphite, acts to protect from oxidation and aids in resolving microbiological contamination with bacteria and mold. After thorough mixing of the grape pomace, and stabilization over a – 24 hours period, it is then necessary to add pure *Saccharomyces cerevisiae* yeast culture, which has been cultivated for 48 hours at +26 – degrees Celsius. On the third day of the fermentation process is necessary to add 1,0 g diammonium phosphate for every 10 kgs of grape pomace mixture, as a nitrogen supplement for the yeast. On the fourth day, it is necessary to add another amino acid nutrient supplement for the yeast into the fermenting mixture at a concentration of 2,0 g for every 10 kgs of grape pomace liquid. Fermentation bioprocessing will continue over period of 10 days. Then, following fermentation competion, it is necessary to check the Ethanol concentation in the grape pomace fermented mixture, and then to extract the Ethanol by distillation and rectification to obtain an Ethanol concentrate of up to 92–96% v/v.

Medicinal herbs were obtained from the pharmaceutical Company and "Lubnyfarm", Poltava region, "Viola", in the Zaporozhe region of Ukraine, and from the Botanical Garden of the Odessa Mechnikov National University. The following medicinal herbs were investigated: Echinacea leaves and/or roots with dry rhizome (Echinacea purpurea L.), Ginseng root (Panax ginseng L.), Restharrow root (Ononis arvensis L.), Eleuterococcus root (Eleutherococcus Senticosus Maxim L. (Siberian ginseng)), Hawthorn fruit (Crataegus oxyacantha L.), Peppermint leaves (Menthae piperitae folia L.), Wormwood leaves and stems (Artemisia absinthium L.), Calendula flowers (Calendula officinalis L.), and Eucalyptus leaves (Eucalyptus globules L.). Pure Ethanol, obtained following fermentation from grape pomace was used as diluted spirituous solution with distilled water in different concentrations for medicinal herbs. It was made with for the purpose of optimal extraction of medicinal ingredients from the herbs [10, 11, 13].

Statistical deviation and significance were evaluated by the Student`s t-test with P – value: $P < 0.1$; $P < 0.05$; $P < 0.01$. Each test was repeated three times to confirm the exact result.

RESULTS AND DISCUSSION

Ethanol obtained from grape pomace must correspond to the Ukrainian State Standard (DSTU) 4221–2003. Rectified Ethanol 96% must be checked for

the following parameters: The density of the liquid (at 20°C) – 812 kg/m^3 (approximately – 0.8 kg/L). The Vapor Density (at 760 mm of mercury column) should be 1,601 kgs/m^3. Boiling temperature (at 760 mm of mercury column) should be 78,15 °C. Specific heat of vaporization should be 925 kJ/kg.

Table 1. Ethanol production volume fraction in prospective *Saccharomyces cerevisiae* yeast culture

Source (grape variety)	MAFF collection number, Genebank of Japan	USRCB collection number	Ethanol production volume fraction, % v/v
Yeast cultures obtained from white grape varieties (Koblevo):			
Rkatsiteli	230112	Y-3450	14,08
Chardonnay	230119	Y-3480	14,17
Riesling Rhenish	230120	Y-3481	12,66
Traminer	230123	Y-3484	12,11
Muscat Ottonel	230130	Y-3491	12,42
Irshai Oliver	230127	Y-3488	12,98
Yeast cultures obtained from red grape varieties (Koblevo):			
Cabernet-Sauvignon	230116	Y-3454	15,45
Merlot	230115	Y-3453	13,52
Odessa`s Black	230113	Y-3451	12,06
Bastardo	230125	Y-3486	15,17
Yeast cultures obtained from white grape varieties: (Tairov`s Research Insitute)			
Selena	230101	Y-3439	14,15
Sukholimansky	230102	Y-3440	13,40
Opalovy (Opaline)	230103	Y-3441	14,94
Aromatic	230106	Y-3444	13,79
Odessa`s Muscat	230107	Y-3445	15,55
Yeast cultures obtained from red grape varieties: (Tairov`s Research Insitute)			
Charivny (Magic).	230100	Y-3438	12,27
Tairov`s Ruby	230098	Y-3436	12,45

Note: MAFF – Culture Collection of microorganisms Ministry of Agriculture, Forestry and Fisheries. National Institute of Agrobiological Science (NIAS), Gene Bank, Tsukuba, Ibaraki, Japan.

The morphology of *Saccharomyces cerevisiae* yeast presented in fig. 1–2. Microscopically, under immersion investigated and illustrate that some cells of yeast are large and either rounded or oval shaped.

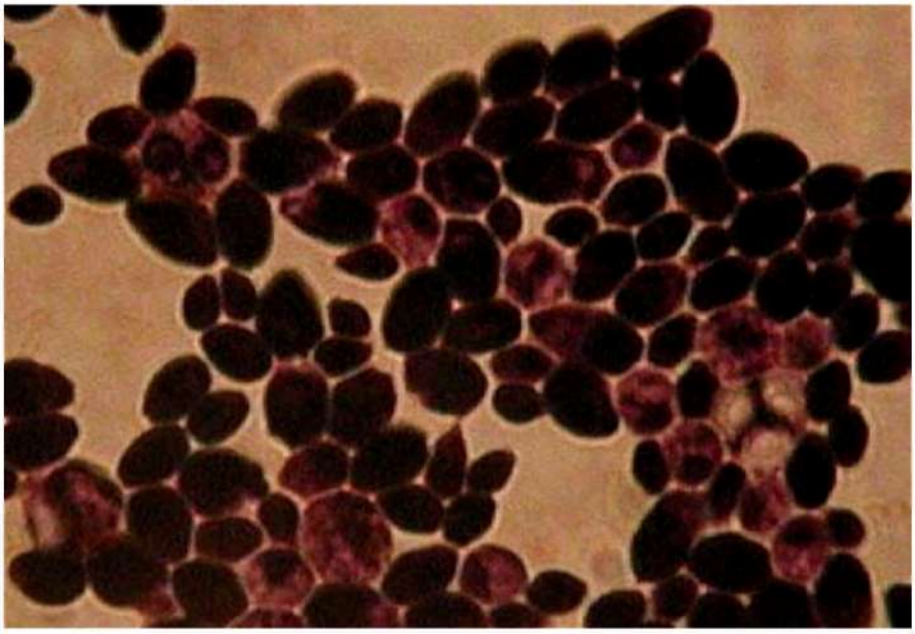

Fig. 1. Morphology of *Saccharomyces cerevisiae* yeast culture isolated from red grape variety « Bastardo » MAFF-230125; NRRL Y-63641.
the volume fraction of alcohol (ethanol) production – 15.17 v/v%; stained by Gram method using Safranine; magnification – x900.

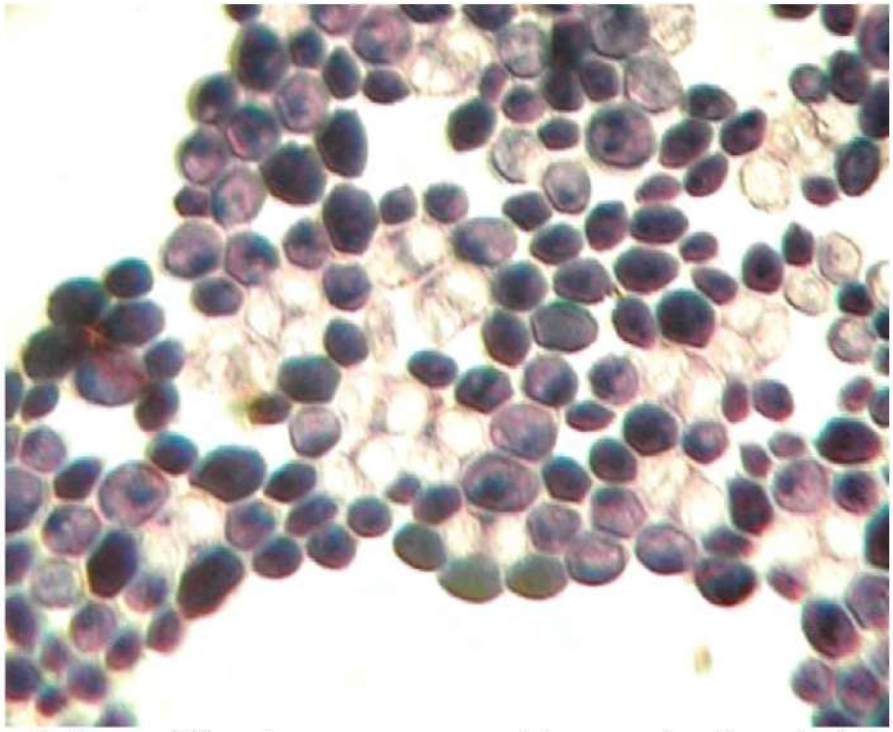

Fig. 2. Morphology of *Saccharomyces cerevisiae* yeast culture isolated from red grape variety « Cabernet-Sauvignon » MAFF-230126; NRRL Y-63642.
the volume fraction of alcohol (ethanol) production – 15.45 v/v%; stained by Gram method; magnification – x900.

Table 2. Criteria for Ethanol identification and product requirements.

Evaluation parameters	Required standard	Ethanol from grape pomace	Pharmasept
Description	Transparent, colorless, volatile, flammable, hygroscopic liquid	corresponds	corresponds
Identification	**A)** Compliance with the requirements of the relative density. **B)** Compliance the infrared spectrum to the reference. **C)** The intensity of the blue color of the solution. **D)** Formation of yellow precipitate after addition of diluted NaOH and 0,05M iodine solution.	corresponds	corresponds
Transparency	Transparent	corresponds	corresponds
Chromaticity	Colorless	corresponds	corresponds
Acidity or alkalinity	No more 0,003%	corresponds	corresponds
The relative density	From 0,805 g/cm^3 up to 0,812 g/cm^3	0,80924 g/cm^3	0,80706 g/cm^3
Optical density	240 nm – 0,40 250–260 nm – 0,30 270–340 nm – 0,10	corresponds	corresponds
Dry residue	No more 0,0025% m/v	corresponds	corresponds
Iron	No more 0,0001%	corresponds	corresponds
Heavy metals	No more 0,0002%	corresponds	corresponds
Microbiological purity	Non-availability of Staphylococcus aureus and Pseudomonas aeruginosa. Total number of aerobic microorganisms (TAMC) – 10^2 quantitative conventional units per gram and total number of yeast and mold (TYMC) – 10^2 quantitative conventional units per gram	corresponds	corresponds

From the results presented in Table 1, comparing standard Ethanol and Ethanol obtained from grape pomace, there are no major differences in the physical and chemical components of both types of Ethanol. Both types of Ethanol meet the requirements of the Ukrainian State Standard (DSTU) 4221-2003. Rectified Ethanol 96% corresponds to the standard requirements.

Table 3. Spirituous herbal extracts prepared from ethanol produced from grape pomace and from standard Ethanol "Pharmasept"

Evaluation parameters	Echinacea	Ginseng	Restharrow	Eleuthero coccus	Hawthorn fruit
	Echinacea purpurea L.	Panax ginseng L.	Ononis arvensis L.	Eleutherococcus Senticosus Maxim L.	Crataegus oxyacantha L.
Ethanol produced from Grape pomace					
% of Ethanol concentration	42 ± 2.0	70 ± 2.5	70 ± 2.5	42 ± 1.5	70 ± 1.5
Dry solids, %	0.5 ± 0.1	*0.6 ± 0.05	*0.38 ± 0.05	*0.62 ± 0.02	*0.47 ± 0.02
Ethanol produced from potato and grain – "Pharmasept"					
% of Ethanol concentration	42 ± 2.0	70 ± 2.5	70 ± 2.0	42 ± 3.6	70 ± 1.5
Dry solids, %	*0.43 ± 0.02	*0.53 ± 0.02	*0.28 ± 0.02	*0.54 ± 0.02	**0.38 ± 0.01

Note: standard deviation was calculated, statistical significance of difference was evaluated using Student's t-test; *P*-value * $P \leq 0.05$; ** $P \leq 0.01$.

Spirituous liquid extract of Echinacea 45% of Ethanol and dry aerial parts or dry root rhizome of the Echinacea purpurea. The active ingredients constitute a complex of biologically active substances, containing polysaccharides echinacoside, esters of cinnamic acid, essential oils and micro-elements. Echinacea liquid extract prevents excessive activation of free radical oxidation reactions and restores the functional activity of the natural antioxidant system. With regular use, it increases the body's resistance to adverse environmental factors (to ionizing radiation, nutritional deficiency of natural bio-regulators, etc.). Echinacea extract is effective in the treatment of diseases of the oral mucosa and periodontal and integument, providing anti-inflammatory, hemostatic, and regenerative effects. The spirituous extract of Ginseng roots (*Panax ginseng* L.) is a transparent, yellowish liquid. Ginseng tincture contains triterpene glycosides – panaksozides (A-G), pectins, saponins, fatty acids and essential oils, vitamin C, B_1, B_2, the macro- and micro-elements and other substances. The spirituous extract of Ginseng roots relates to the acting on the central nervous system, and is reffered to as a "tonic" adaptogenic agent. Ginseng tincture strengthens the processes of excitation in the neurons of the cerebral cortex and in the latter parts of the stem, improves reflex activity, activates metabolism, and improves the ability to work. The spirituous liquid extract of Rest-harrow (*Ononidis arventis* L.), roots in the ratio (1:5), is a reddish brown colour liquid, with a distinctive odor, and bitter taste. Rest-harrow (*O. arvensis*) tincture contains isoflavones (ononin, onospin), tannins, triterpene saponins, resins, citric acid, starch, and essential oil. The isoflavones and saponins stabilize cell membranes, and reduce blood vessels permeability reduces. The spirituous liquid extract of the Eleuterococcus roots in the 40%

Ethanol, in the ratio (1:1) extract of rhizomes and roots of Eleutherococcus senticosus. The liquid is dark-brown, transparent in thin-layer, and has an distinct, peculiar smell. The rhizomes and roots of Eleutherococcus eleutherosides contain eleutherosides: A, B_1, C, D, E, F and G, coumarin derivatives, flavonoids, volatile oil, vegetable wax, resin, starch, etc. Eleutherosides Eleutherococcus extract improves physical and mental performance, resistance to adverse environmental factors, increases visual acuity, improves metabolism, and has some stimulating gonadotropic and hypoglycemic action. The spirituous extract of Hawthorn (*Crataegus oxyacantha* L.). Biologically active substances in hawthorn fruits (flavonoids, choline, acetylcholine, tannins, phytosterols, triterpene acids) increase blood circulation in the coronary vessels and vessels of the brain, increase myocardial sensitivity to cardiac glycosides and reduce its excitability, leading to certain strengthening the heart muscle. Hawthorn tincture is used in functional disorders of the cardiovascular system (cardiopsychoneurosis, etc.), as an auxiliary remedy in mild forms of cardiac arrhythmias (e.g. atrial fibrillation, and paroxysmal tachycardia).

Table 4. Spirituous herbal extracts prepared from ethanol produced from grape pomace and from standard Ethanol "Pharmasept"

Evaluation parameters	**Peppermint leaves**	**Wormwood leaves**	**Calendula flowers**	**Eucaliptus Leaves**	**Rotokan (ratio 2:1:1)**
	Menthae piperitae L.	*Artemisia absinthium* L.	*Calendula officinalis* L.	*Eucalyptus globules* L.	*Matricaria chamomilla* L; *Calendula officinalis* L; *Achillea millefolium* L.
Ethanol produced from Grape pomace					
% of Grape pomace Ethanol concentration	82 ± 2.0	65 ± 1.5	65 ± 2.0	65 ± 1.5	42 ± 2.0
Dry solids, %	2.6 ± 0.1	3.0 ± 0.1	3.2 ± 0.2	4.6 ± 0.4	*0.7 ± 0.05
% of Eitheric oils	*0.5 ± 0.04	–	–	*0.48 ± 0.02	
Ethanol produced from potato and grain "Pharmasept"					
% of Ethanol "Pharmasept"	82 ± 2.0	65 ± 1.5	65 ± 1.5	65 ± 2.0	42 ± 2.7
Dry solids, %	2.8 ± 0.1	2.4 ± 0.2	2.6 ± 0.1	3.2 ± 0.2	*0.58 ± 0.03
Eitheric oils	*0.38 ± 0.02	–	–	*0.42 ± 0.03	–

Note: standard deviation was calculated, statistical significance of difference was evaluated using Student's t-test;
P-value * $P \leq 0.05$.

The spirituous extract of Peppermint leaves (1:20) in 80% Ethanol (contains menthol and its esters), and other biologically active substances contained in the leaves of peppermint (ketone-menton, oleanolic and ursolic acids, and flavonoid glycosides), stimulate the sensory receptors of oral and gastric mucous membranes, which results in a reduction in nausea and prevention of vomiting. Peppermint tincture is used for functional disorders of the cardiovascular system, as a sedative, antispasmodic, and cholagogue actions. It also used at nausea and vomiting, and as an anti-emetic.

The spirituous extract of Wormwood herb (*Artemisia absinthium* L.) (1:5) in 70% Ethanol. Wormwood tincture – herbal remedy, which has choleretic action and stimulates the secretion of gastric juice (stimulates the production of HCl in the stomach). The mechanism of action of this remedy is due to irritation of sensory nerve endings – taste receptors of the mucous membranes of the mouth, tongue reflex that causes increased secretion of gastric juice, increased appetite, and improves the digestive process.

The spirituous extract of Calendula flowers (*Calendula officinalis* L.) is a transparent liquid, with a yellow to brownish tint color with a yellowish tinge. It contains tincture of calendula flowers (1:10) in 70% Ethanol. Biologically active substances, which are contained in the flowers of calendula (flavonoids and carotenoid glycosides, saponins, tannins, essential oils, and organic acids) have antimicrobial, anti-inflammatory and moderate choleretic action. Spirituous extract of

Calendula flowers is used topically as an antiseptic in infected (pus) wounds, burns, cuts and abrasions to the skin and inflammation of the mucous membranes of the mouth, gums (stomatitis, gingivitis), pharynx, tonsils (tonsillitis, pharyngitis). For oral use – as cholagogue in biliary dyskinesia ducts, cholangitis, and cholecystitis (as a part of combined therapy).

The spirituous extract of Eucalyptus (Eucalyptus globules L.) is a transperent greenish-brown liquid in ratio (1:5), with a distinctive smell. Extractant is 70% Ethanol. Tincture of Eucalyptus contains structure tannins, ellagic acid, flavonoids, resin, wax, and essential oil, which is composed of various terpene compounds, which are especially active in gram-positive, and gram-negative microorganisms, with harmful effects on fungi and protozoa. Eucalyptus spirituous extract inhibits the growth of Staphylococcus aureus, Escherichia coli, Mycobacterium tuberculosis, Trichomonas, and dysentery amoeba. The main component of the essential oil is a cineole monocyclic terpene. Eucalyptus tincture has antiseptic, anti-inflammatory and disinfectant action due to the oxidative effect of cineole monocyclic terpene and the anti-inflammatory effect associated with almost all of its physiologically active substances.

Rotokan is a product of herbal origin. It has local anti-inflammatory, antiseptic, and haemostatic effects. Rotokan promotes regeneration of damaged mucosa. It is a mixture of liquid medicinal herbal spirituous extracts:

chamomile, calendula, and yarrow in a ratio of 2:1:1. It is a dark brown liquid with an orange tint, and a distinctive smell. During storage there can be some sedimentation, which is normal for this remedy. It is indicated in dental practice in adults with oral inflammatory diseases of the mucous membranes of the mouth (e.g. aphthous stomatitis, periodontal disease, and necrotizing ulcerative gingivostomatitis). Rotokan can be used to treat periodontitis after the removal of dental plaque and curettage of pathological gums. It is usually administered in the gingival pockets for 20 minutes, in a turunda abundantly moistened in the rotokan spirituous herbal extract. This procedure is performed 4–6 times during the day. For diseases of the oral mucosa, 15–20 minutes applications are usually used, or Rotocan oral rinses for 1–2 minutes, 2–3 times per day, for 2–5 days. Before using Rotocan, it is necessary to dilute one teaspoonfull of the remedy in 250 mls of warm water. Rotokan should be kept in a cool, dark place to preserve its medicinal properties. Rotokan can also be used in the treatment of stomach and intestines as an anti-inflammatory, and anti-ulcer remedy in the following conditions: gastritis, gastroduodenitis, chronic enteritis and colitis, gastric and duodenal ulcers, chronic diseases of the large and small intestines, chronic cholecystitis, and after surgical removal of gallbladder. In these indications, it is necessary to prepare Rotocan solution to use orally, drinking 50–60 mls (quarter cup) or 125 mls (half cup) 30 minutes before meals, 3–4 times per day, or within 40–60 minutes following a meal. Such a course of treatment should continue for 2–3 weeks.

Just one Experimental Agricultural Company "Tairovskiy" Tairov Research Institiute growing grapes contains great potential for grape pomace, amounting to 115.88 tonnes, during grape harvesting season. Thus, from this amount of grape pomace, it is possible to produce ethanol (*spiritus vini*), amounting to 13.8 tonnes of pure ethanol, for pharmaceutical use.

Table 5. Harvesting season grape varieties and amount of grape pomace obtained for ethanol (spiritus vini) production from the experimental Agriculture Company "Tairovskoe"

Grape variety	**Harvested area (hectares)**	**Grape yield (hwt/ha) 2013 year**	**Amount of grapes (in tonnes)**	**Amount of grape pomace (in tonnes)**
1	**2**	**3**	**4**	**5**
Table grape varieties for juice production				
Odessa Souvenir	2.19	59.00	12.92	2.58
Arcadia	16.58	50.40	83.56	16.71
Delight (Vostorg)	1.53	50.00	8.76	1.75
Queen of vineyards	4.18	45.00	18.81	3.76
Moldova	10.54	13.00	14.70	2.94
Pleven	0.53	10.00	0.60	0.12
Strasheni (Strashenskiy)	1.74	10.50	1.82	0.36
Total for table grape varieties	37.29	237,90	141.17	**28.22**

1	2	3	4	5
Industrial grape varieties for winemaking				
Odessa Black	33.15	77.00	256.10	51.22
Sukholimansky	17.51	43.00	75.30	15.06
Aligote	5.80	12.00	6.80	1.36
Muscat Odessa	9.42	67.00	63.10	12.62
Rubin Tairovsky	2.30	6.00	1.40	0.28
Cabernet – Sauvignon	2.34	37.00	8.80	1.76
Goldish Early	2.44	22.00	5.40	1.08
Bluish (Golubok)	3.96	45.00	17.90	3.58
Traminer (pink)	0.49	16.00	0.70	0.14
Chardonnay	2.26	12.00	2.80	0.56
Total for industrial varieties	79.67	337.00	438.30	**87.66**
Total for table and industrial grape varieties	116.96	574.90	579.47	**115.88**

Table 6. Harvesting season grape varieties and amount of grape pomace obtained for ethanol (*spiritus vini*) production from the Agriculture Company "Koblevo"

Grape variety	**Harvested area (hectares)**	**Grape yield (hwt/ha) 2013 year**	**Amount of grapes (in tonnes)**	**Amount of grape pomace (in tonnes)**
Industrial grape varieties for winemaking				
Aligote	49.70	460.70	779.10	155.82
Bastardo	14.94	115.40	172.50	34.50
Chardonnay	108.43	357.00	817.25	163.45
Cabernet – Sauvignon	83.87	204.70	340.50	68.10
Isabella	75.64	102.30	197.05	39.41
Irshai Oliver	56.00	85.50	478.90	95.78
Merlot	70.00	235.50	389.95	77.99
Muscat Hamburg	28.20	133.80	377.30	75.46
Muscat Ottonel	40.80	92.90	379.20	75.84
Odessa Black	35.00	129.20	229.55	45.91
Odessa Muscat	3.50	96.60	33.80	6.76
Rkatsiteli	77.77	512.70	1103.30	220.66
Rhein Riesling	74.55	216.80	565.55	113.11
Sauvignon	72.27	191.10	436.25	87.25
Traminer (pink)	36.04	172.00	310.50	62.1
Total for industrial varieties	826.74	3106.20	6610.70	**1322.14**

The Agricultural Company "Koblevo"growing grapes contains great potential for grape pomace, amounting to 1322.14 tonnes, during grape harvesting season. Thus, from this amount of grape pomace, it is possible to produce ethanol (*spiritus vini*), amounting to 158.02 tonnes of pure ethanol, for pharmaceutical use.

CONCLUSIONS

In order to produce of Ethanol from grape pomace, prospective *Saccharomyces cerevisiae* yeast cultures, with a high level of Ethanol production were used. On completion of fermentation, the fermented grape pomace mixture obtained was distilled, rectified, purified and concentrated. For the preparation of spirituous medicinal herbal extracts, we used different concentrations of Ethanol (*spiritus vini*). The study demonstrated that Ethanol produced from grape pomace is a good extragent for medicinal leaves and roots. Prepared spirituous herbal extracts showed good extraction, with a healthy amount of dry solids. We can conclude that production of Ethanol from grape pomace is a prospective direction for its use in the pharmaceutical industry for the production of spirituous medicinal herbal extracts and tinctures.

REFERENCES

1. Arzani K., Koushesh-Saba M. Enhancement of 'Sultana' grape (Vitis vinifera)maturity by pre-veraison ethanol and methanol spray // Indian Journal of Agricuitural Sciences. – 2005. – Vol. 75. – No.10. – P. 670–672.
2. Bayraktar V.N. Organic acids concentration in wine stocks after *Saccharomyces cerevisiae* fermentation // J.Biotechnologia Acta. – 2013. –Vol.6. – No.2. – P. 97–106.
3. Buescher W.A., Siler C.E., Morris J.R., et al. High Alcohol Wine Production from Grape Juice Concentrates // Amer. J. Enol.Vitic. – 2001. –Vol. 52. – No4. – P. 345–351.
4. Chervin C., Kereamy A. E., Renouf V. Changes in Grape Maturity Induced by Spraying Ethanol //Acta Horticulturae. XXVI International Horticultural Congress: Viticulture – Living with Limitations. Toronto, Canada. – 2004. – P. 305–311.
5. Hernández-Jiménez A., Kennedy J.A., Bautista-Ortín A.B., et al. Effect of Ethanol on Grape Seed Proanthocyanidin Extraction //Amer. J. Enol.Vitic. – 2012. – Vol. 63. – No.1. – P. 57–61.
6. Guerrero M.S., Torres J.S., Nuñez M.J. Extraction of polyphenols from white distilled grape pomace: optimization and modeling // Bioresour. Technol. – 2008. – Vol.99. – No.5. – P. 1311–1318.
7. Karabulut O.A., Romanazzib G., Smilanickc J.L., Lichterd A. Postharvest Ethanol and potassium sorbate treatments of table grapes to control gray mold // Postharvest Biology and Technology. – 2005. – Vol.37. – P. 129–134.
8. Korkiel L.J., Janse B.J., Viljoen-Blooml M. Utilising Grape Pomace for Ethanol Production // S.Afr. J. Enol. Vitic. – 2002. – Vol. 23. – No.1. – P. 31–36.
9. Kroyer G. Antioxidant properties of grape pomace extracts // Institute of Chemical Engineering Department of Natural Products and Food Chemistry Vienna University of Technology.Austria. – P. 1–7.
10. López E.F., Gómez E.F. Simultaneous Determination of the Major Organic Acids, Sugars, Glycerol, and Ethanol by HPLC in Grape Musts and White Wines //Journal of Chromatographic Science. – 1996. – Vol. 34. – P. 254–257.
11. Luque-Rodríguez J.M., Luque de Castro M.D., Pérez-Juan P. Dynamic superheated liquid extraction of anthocyanins and other phenolics from red grape skins of winemaking residues // Bioresour Technol. – 2007. – Vol. 98. – Iss. № 14. – P. 2705–2713.
12. Peralbo-Molina Á, Priego-Capote F, Dolores Luque de Castro M. Comparison of extraction methods for exploitation of grape skin residues from ethanol distillation // Talanta. – 2012. – Vol.101. – P. 292–298.

13. Sandhu A.K., Gu L. Adsorbtion/desorbtion characteristics and separation of anthocyanins from muscadine (Vitis rotundiflora) juice pomace by use of macroporous adsorbent resins // J. Agric. Food Chem. – 2013. – Vol. 61. – No.7. – P. 1441–1448.
14. Zheng Y., Lee C., Yu C., et al. Ensilage and Bioconversion of Grape Pomace into Fuel Ethanol // Journal of Agricultural and Food Chemistry. – 2012. – Vol. 60. – Iss. № 44. – P. 128–134.

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КУЛЬТУРЫ ДРОЖЖЕЙ ВИДА *SACCHAROMYCES CEREVISIAE* В БИОТЕХНОЛОГИИ ПОЛУЧЕНИЯ ВИННОГО СПИРТА ДЛЯ ИСПОЛЬЗОВАНИЯ В ФАРМАЦЕВТИЧЕСКОЙ ПРОМЫШЛЕННОСТИ

Ключевые слова: *винные дрожжи, виноградные выжимки, этанол, ферментация,* Saccharomyces cerevisiae, *spiritus vini.*

Культуры дрожжей *Saccharomyces cerevisiae*, выделенные из различных сортов винограда были использованы для производства этанола. Были установлены биотехнологические свойства дрожжевых культур для производства этанола. Для брожения в качестве сырьевого материала были использованы виноградные выжимки. После завершения брожения, полученный продукт дистиллировали (ректифицировали), затем использовался для приготовления спиртовых экстрактов из лекарственных трав. Установлено, что оптимальная концентрация спирта для производства спиртовых экстрактов из листьев Эвкалипта должно быть не менее 65% для полной экстракции полифенолов и эфирного масла. Для полыни была установлена оптимальная концентрация этанола для экстракции гликозидов не менее 65%. Для спиртовых экстрактов Эхинацеи концентрации этанола должна быть не менее 41% для полного извлечения из лекарственных трав пентозанов, полифенолов, полисахаридов, органических кислот (кофейная и цикориева кислоты). Для экстракта Мяты перечной оптимальная концентрация этанола должна быть не менее 81% для полной экстракции эфирного масла. Для экстракта Календулы оптимальной концентрацией этанола должно быть не менее 65%, для наиболее полной экстракции флавоноидов в пересчете на гиперозид не менее 0,04%.

Было показано, что изготавливаемые лекарственные травяные спиртовые экстракты с использованием этанола (*siritus vini*), произведенного из виноградных выжимок является перспективным направлением для использования в фармацевтической промышленности.

В. М. Байрактар

КУЛЬТУРИ ДРІЖДЖІВ ВИДУ *SACCHAROMYCES CEREVISIAE* У БІОТЕХНОЛОГІЇ ОТРИМАННЯ ВИННОГО СПИРУ ДЛЯ ВИКОРИСТАННЯ У ФАРМАЦЕВТИЧНІЙ ПРОМИСЛОВОСТІ

Ключові слова: *винні дріжджі, виноградні вичавки, етанол, ферментація,* Saccharomyces cerevisiae, *spiritus vini.*

Культури дріжджів *Saccharomyces cerevisiae*, виділених з різних сортів винограду були використані для виробництва етанолу. Були встановлені біотехнологічні властивості дріжджових культур для виробництва етанолу. Для бродіння в якості сировинного матеріалу були використані виноградні вичавки. Після завершення бродіння, отриманий продукт дистилювали (ректифікували), який потім використовувався для приготування спиртових екстрактів з лікарських трав.

Встановлено, що оптимальна концентрація спирту для виробництва спиртових екстрактів з листя Евкаліпту повинно бути не менш як 65% для повної екстракції поліфенолів і ефірної олії. Для Полину була встановлена оптимальна концентрація етанолу для екстракції глікозидів не менш як 65%. Для спиртових екстрактів Ехінацеї концентрація етанолу повинна бути не менш як 41% для повного вилучення з лікарських трав пентозанов, поліфенолів, полісахаридів, органічних кислот (кавова і цікоріева кислоти). Для екстракту М'яти перцевої оптимальна концентрація етанолу повинна бути не менш як 81% для повної екстракції ефірного масла. Для екстракту Календули оптимальною концентрацією етанолу має бути не менш як 65%, для найбільш повної екстракції флавоноїдів у перерахунку на гіперозид не менш як 0,04%.

Було показано, що виготовлені лікарські трав'яні спиртові екстракти з використанням етанолу (siritus vini), виробленого з виноградних вичавок є перспективним напрямком для застосування у фармацевтичної промисловості.