Agronomy Research **14**(S2), 1271–1283, 2016

Extraction of biologically active compounds from fruit, berry and grain Grist using ViscoStar 150L enzyme complex

N.V. Barakova[1,*], N.N. Skvortsova[1] and N.Y. Sharova[2]

[1]International Research Centre 'Biotechnologies of the Third Millennium', ITMO University, St. Lomonosova 9, RU 191002 Saint-Petersburg, Russian Federation
[2]Federal State-Funded Scientific Establishment 'All-Russia Research Institute for Food Additives' (FGBNU VNIIPD), Chair of Food Microingredients' Processing, 55 Liteinii ave., RU 191014 Saint-Petersburg, Russian Federation
[*]Correspondence: n.barakova@mail.ru

Abstract. This work aimed to evaluate the efficiency of treating plant tissue with enzymatic agent ViscoStar 150L for the extraction of biologically active compounds. In the current study the screening of extraction methods from citrus fruit peels (grapefruit, lemon, orange) was performed. The samples treated with enzymatic agent ViscoStar 150L showed better extraction results than the traditional ethanol/water extraction method. Citrus peels' extracts assayed for antioxidant activity (determined as ferric reducing antioxidant power – FRAP) decreased in the following order: grapefruit > orange > lemon. The enzymatic agent ViscoStar 150L proved to have a positive synergic effect on juice yield from cowberry previously treated with a complex pectolytic enzymatic agent. The enzymatic agent ViscoStar 150L proved to have a synergic effect on grain grist mashes previously treated with an amylolytic enzymatic agent, the inhibitor activity of the compounds produced by actinomycetic microorganisms grown on substrates based on these mashes being higher than that of previously known inhibitors.

Key words: ViscoStar 150L, cowberry, citrus peel, grain grist, antioxidants.

INTRODUCTION

Enzymes are *in vivo* process catalysts characterized by selectivity and specificity. An enzyme with absolute specificity would catalyze the reaction of one substrate only, while group-specific enzymes would catalyze similar process of a group of related compounds. For polyvalent substrates, or systems comprising multiple substrates, the optimal strategy would be using sets of enzymes targeted at different compound types.

Although the source of many enzymes is plant and animal tissue, controlled condition growth on various substrates makes use of the enzyme producing capacity of microorganisms. In bioengineering, enzymatic agents that contain multiple enzymes produced by sole controlled condition grown microorganisms are often used. ViscoStar 150L is one such enzymatic agent, produced by controlled condition grown *Trichoderma longibrachiatum* fungi, containing various cellulases and proteases.

Over the course of several decades the stable tendencies in food engineering have been reclaiming raw materials from by-products (Okwu & Emerike, 2007) and utilizing wildberry mass (Baraboï, 1984) due to their high biologically active compound content

(Ivanova, 1995; Rumiantseva et al., 2006; Zhilova, 2006; Bazarnova, 2007; Ovsiannikova et al., 2012; Liutikova, 2013). There is an increasing awareness of foodstuffs' antioxidant capacity, certain components of said foodstuffs being able to prevent biopolymer oxidation *in vivo* by scavenging active oxygen forms (Meliauskas et al., 2004; Temerdashev et al., 2006, Pantelidis et al., 2007). The addition of antioxidant components of plant origin to the food products is one of the ways to prolong the products' shelf-life due to the inhibition of the oxidation processes (Bazarnova, 2010). Citrus fruit and berry processing is known to yield significant amounts of antioxidants (Baraboi, 1984; Okwu & Emerike, 2007).

Citrus fruits are among the top consumer choices for fresh fruit. For processed citrus products, juices are in the lead. The peel, being the by-product of juice production, can still be recycled into dried dice, marshmallow, and citrus oils. Long shelf life products such as dried dice and jams retain significant P vitamin activity, the value usually associated with citrus juice (Grandall, 1977; Braddock, 1995). Citrus peel contains versatile biological agents such as flavonoids that improve metabolism, prevent atherosclerosis by improving blood vessel elasticity and exhibit antioxidant activity (ChongDe Sun et al., 2005; Zia-ur-Rehman, 2006; Jayaprakasha et al., 2008; Boshtam, 2011; Jabri karoui & Marzouk, 2013).

Cowberry is a valuable wildlife resource for the Northwest Russia and the Baltic states. Cowberry's nutritional and immunity modulating values are caused by its high content of carbohydrates, organic acids, vitamins, tannins, pectins and trace elements. The phenolic compounds contained in cowberry contribute to capillary wall strength, inflammation and atherosclerosis control and antioxidant capacity (Menshhikova et al., 2012; Liutikova, 2013).

Using starchy raw materials in the non-traditional role of nutrient suppliers for various biologically active compounds producing microorganisms (Akulova & Selezneva, 1995)

Claiming, or extracting, said biologically active compounds can be carried out in various ways (maceration, digestion, percolation, and others) usually involving: aggressive physical, such as ultrasonic, electro-thermic and electro-flotative, and chemical destructive techniques and reagents; prolonged exposure to same, resulting in time consumption; and unsatisfactory extraction specificity.

Enzyme treatment, however, does not carry any of these negative effects, the sole disadvantage being limited affordability (Schobinger, 2004).

Taking into account the diverse chemical nature of citrus peel, wildberry and grain grist components, extraction of said components should be aided by a complex enzyme solution or several solutions (Ovsyannikova et al., 2012; Pekhtereva et al., 2012). The extracts that would result, rich in various biologically active compounds, could be applied to multiple tasks related to food production.

By using biocatalysis it is possible to break down grain raw materials to the carbohydrates which are substrata for microorganisms that are biologically active substances' producers (Sharova, 2015).

MATERIALS AND METHODS

The following enzymes and enzyme solutions were used in the current study.

ViscoStar 150 L (ENMEX, S. A. de C. V. 'Tarchomin Pharmaceutical Works 'POLFA' S.A.', Poland) enzyme complex contained several hemicellulases derived from controlled condition grown micromycetic *Trichoderma longibrachiatum* fungi.

Fructozym MA (ERBSLÖH Geisenheim AG, Germany) enzyme complex contained several pectinases, as well as other glycosidases, derived from controlled condition grown ascomycetic *Aspergillus niger* fungi.

Aspergillus awamori glucoamylase (EC 3.1.2.3, 1,4-α-D-glucan glycohydrolase) (Shandong Longda Bioproducts Co., Ltd., China).

Thermozyme 1000 L (ENMEX, S. A. de C. V.) enzyme solution contained α-amylase derived from controlled condition grown *Bacillus subtilis*.

Amylosubtilin (Sibbiofarm, Russia) contained α-amylase derived from controlled condition grown *Bacillus subtilis*.

Pancreatin (Biosintez, Russia) contained α-amylase derived from pig pancreas.

Saccharomyces cerevisiæ invertase (EC 3.2.1.26, β-D-fructofuranesidase) (Biolar, Russia).

The citrus fruits used in the experiments were commercially available in St.-Petersburg, Russia, and devoid of sort specificity. Ester oils were purchased from a retail drug vendor in St.-Petersburg, Russia.

Citrus extract preparation**.** Peels were crushed in a worm-type crusher. Extraction liquid was fed into weighed citrus peel samples and left for 30 ± 5 minutes; then the extract was separated by centrifugation**.** The following conditions were used for the experiment: t = **(**20 ± 2) °C; pH 7.0; dilution 1:10 (9 parts of liquid to 1 part of raw product mass, $^{v}/_{w}$).

The spectral characteristics of the extracts were examined through a Shimadzu UV-1800 spectrophotometer. All the spectroscopy sample solutions were prepared by diluting 1 ml extract by 24 ml distilled water in calibrated 25 ml flasks.

The concentration of ascorbic acid in the resulting liquid was assessed using the Murry titration method based on the ability of ascorbic acid to reduce 2,6-dichlorophenyl indophenol (DCIP) in an acidic medium (Chupakhina, 2000). Crushed peels were ground in a mortar with an addition of quartz sand while 5% hydrochloric acid was being gradually added until the consistency became paste-like. The mortar and the pestle were rinsed with 5% HCl and the rinse-offs and the ground peels diluted with 5% HCl in calibrated 100 ml flasks. Upon filtration, 10 ml of the solutions were then pipetted into Erlenmeyer flasks and titrated with a 0.001n DCIP solution until a lasting (no less than 30 seconds) rose coloration was obtained. The concentration of ascorbic acid was then calculated using the formula

$$C = a \cdot 0.088 \cdot V_1 \cdot 100 \cdot b^{-1} \cdot (V_2)^{-1} = a \cdot 8.8, \quad (1)$$

where: C – ascorbate concentration, mg dm^{-3}; a – amount of DCIP spent on titration, ml; 0.088 – coefficient of resolution for pure ascorbate; b – peel sample mass, g; V_1 – extract volume (100 ml); V_2 – clarified solution volume titrated (10 ml).

Content of phenolics in the extracts was assessed by the Folin-Ciocalteu colorimetric method using Folin reagent produced by Fluka (Dienisienko, 2015). Calibrated 25 ml flasks

were used for mixing 12.5 ml of sample liquid, 2.5 ml of Folin reagent, 7.5 ml of 20% (%$_w$) sodium carbonate and sufficient amount of distilled water to raise the resulting volume to 25 ml. The optical density values (D) were read on a Shimadzu UV-1800 (Shimadzu, Japan) spectrophotometer at 760 nm after a settling time of 20 minutes.

Determination of the total antioxidant activity (AOA) of extracted compounds. Total AOA of the extracts was determined using a modified ferric ion reducing antioxidant power method – FRAP with an indicator system of Fe(III)/Fe(II) – *o*-phenantroline (Temerdashev et al., 2006). Phenanthroline (NPF Ural Invest) and chloric iron (Rexant) were used. Optical density was assessed with a PEC N-57 photoelectric colorimeter with a 507 nm transmission peak light filter. After a cuvette (10 mm thick liquid slate) was used for mixing 0.3 ± 0.01 ml extract, 0.3 ± 0.01 ml 0.045M o-phenantroline solution, 0.3 ± 0.01 ml 0.025M $FeCl_3$ and 1.5 ± 0.01 ml 96% (%$_v$) ethanol, the optical density values were read after a settling time of 20 minutes. The readings were then resolved for pure ascorbate using a standard calibration curve.

Preparation of cowberry juice. Cowberry was puréed in a LMT-1 lab mill and 0.05% (%$_w$ wet mass) Fructozym MA enzyme solution was added to the purée with a 1 hour reaction time at 30 °C, then 0.01% (%$_w$ wet mass) ViscoStar 150L enzyme solution was added with a 1.5 hours reaction time at 50 °C. The juice was then drained by a lab press.

Cowberry and cowberry juice analysis was carried out according to respective Russian national standards unified with the AIJN Code of Practice.

The anthocyanin content was assessed by colorimetry using a PEC N-57 photoelectric colorimeter at $\lambda = 510$ nm. The colorimeter readings were then resolved for cyanidin-3 glycoside content 100 g^{-1} raw material wet mass using a calibration curve.

Liquids' gravity was assessed with a PTR46 refractometer (Index Instruments) using methodology described in (Yermakov, 1972) and in a manner according to the user manual.

Rice flour and rye grist mashing technique was derived from (Sharova et al., 2012).

Examination of rice flour and rye grist mashes involved growing such strains as *Streptomyces lucensis* VKPM AS-1,743 (Sharova & Hodkievich, 2009) and *Streptomyces violaceus* VKPM AS-1,734 (Sharova et al., 2009) on substrates based on the mashes. Spectroscopy was used to compare corresponding inhibitory activity towards pancreatic α-amylase (EC 3.2.1.1; 1,4-α-D-glucan glycohydrolase) as described in (Sharova, 2015), Activity towards glucoamylase (EC 3.2.1.3) of *Aspergillus* was measured by the glucose oxidase method (Xiao et al., 2006). The inhibitors were prepared with a method previously described in (Sharova & Hodkievich, 2009).

The pH stability of the inhibitors was tested by comparing inhibitory activity before and after exposing 0.1% solutions of the inhibitors to a 0.1M universal buffer solution (pH 2–12) at (25 ± 1) °C for 180 minutes.

The temperature stability of the inhibitors was assayed based on the difference in inhibitory activity before and after exposing 0.1% solution of the inhibitors to 25–200 °C in distilled water for 180 minutes.

Inhibitor IR spectrums were examined with a Specord 75R spectrograph (Specord, Germany) in transmission mode: resolution 4,000, amplification 8.0×, mirror speed 0.6329, diaphragm 100.00, DTGSKBr detector, KBr beam splitter.

All experiments were performed with at least three replications. Statistical analysis was performed using Microsoft Office Excel tools with an assumed 95% confidence level.

RESULTS AND DISCUSSION

Citrus peel extraction and analysis

The principal value of citrus peel lies in high flavonoid content, resulting in an equally significant antioxidant value of the peel extracts (Devis, 1947; Bacco, 1998). Plant tissue extraction is a complex physical and chemical process. Plant cell wall is a lipid and carbohydrate complex layered on a rigid cellulose fibre underlay, resulting in structural strength as well as selective permeability. For the better effectiveness of biologically active compounds' extraction from vegetable raw material, enzyme treatment is commonly used to facilitate cell wall destruction. Qualitative assay of the biocatalysis efficiency in screening experiments was based on spectroscopic analysis. The flavonoid content assessment technique makes use of a short wave maximum in the UV absorption spectrums of flavonoid-rich solutions at 286 nm (ethanol/dimethyl sulphoxide 10:2). The prominence of the maximum in the spectrums of extracts derived from all the peel types allowed skipping $AlCl_3$ chelation before direct spectroscopy (Yevseieva, 2013). The absorption spectrums observed all had a maximum at $\lambda = 280$ nm (Fig. 1). The hypsochromic shift of the absorption maximum can be attributed to polarity alteration and solubilizing capacity of the solvent.

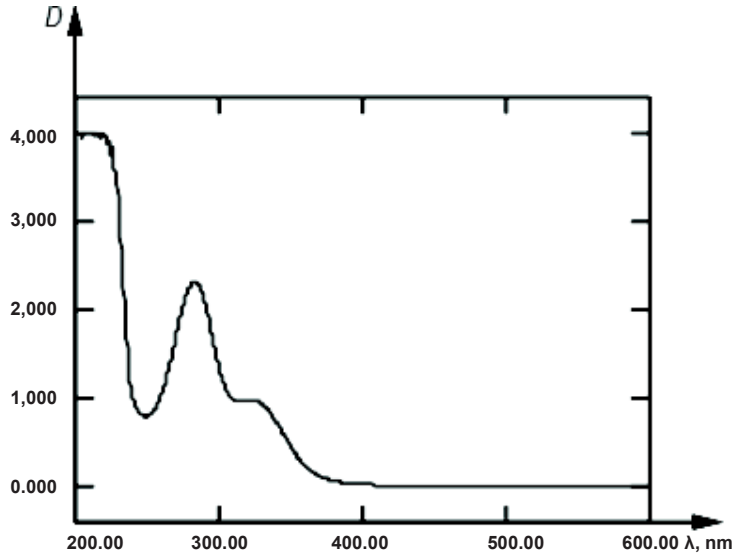

Figure 1. Grapefruit peel extract absorption spectrum.

Optical density (D) values in the respective UV absorption spectra were effectively used to compare extraction efficiencies of 60% ethanol/water (%v) solution to solutions with enzyme at different concentrations (Fig. 2).

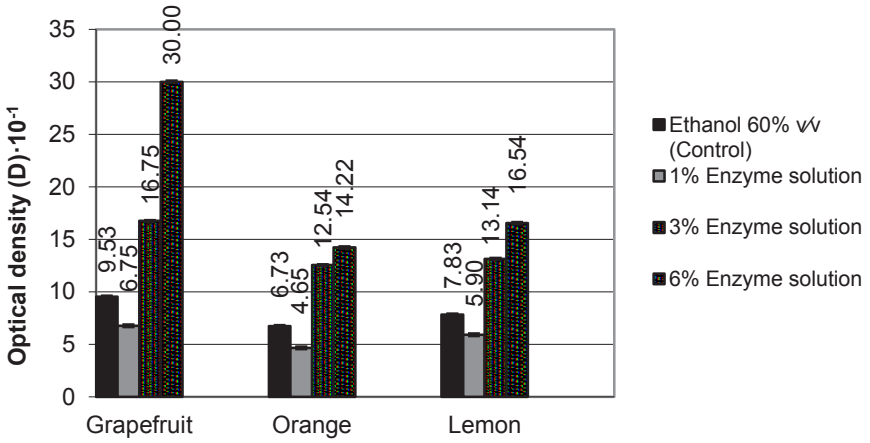

Figure 2. The effect of enzyme solutions on citrus peel extraction efficiency.

As shown in Fig. 1, the overall efficiency of optically active ingredients' extraction by means of enzyme treatment is better than that of the common ethanol extraction method. Additionally, as the concentration of the enzyme solutions gets higher, the extraction effectiveness gets better. One can see that the effect of enzymes is particularly noticeable in grapefruit peel extracts.

Further analysis of various peel extracts with 6% ViscoStar 150L concentration was carried out to determine the biologically active compounds' content and total antioxidant activity (AOA). AOA assay methods often involve significant reagent and time expenditure (Hasanov et al., 2004). The modified FRAP-based ferric ion reducing antioxidant power method allows direct assessment of low molecular antioxidant content (Benzie & Strain, 1996). The Fe(III)/Fe(II)−*o*-phenantroline indicator system performs express examination of liquids' reduction capacity which is an effective antioxidant potential indicator (Temerdashev et al., 2006).

The modified FRAP method was used to examine total AOA of fresh citrus fruit juices, the corresponding peel extracts and ester oils (Table 1). As one can see, the least AOA is demonstrated by the fruit juices, followed by the ester oils, and the most AOA is demonstrated by the peels, especially the grapefruit peel.

Table 1. Assessment of total AOA of extracts (E), citrus oils (EO) and juices

	Grapefruit			Orange			Lemon		
	E	EO	Juice	E	EO	Juice	E	EO	Juice
Ascorbate equivalent, mg ml^{-1}	0.40 ± 0.01	0.19 ± 0.02	0.09 ± 0.01	0.20 ± 0.01	0.12 ± 0.01	0.02 ± 0.00	0.18 ± 0.01	0.16 ± 0.01	0.04 ± 0.00

The data obtained contradict the results of a previously published study (Gorinstein et al., 2005) where orange peel was found to have more total AOA as per the modified FRAP method, but the contradiction may be attributed to sort-to-sort differences (Izosimova, 2004). A correlation has been found between TAA and the content of native oxidation protectors of the plant, such as ascorbic acid and phenols (Makarova et al., 2010).

Citrus fruits are characterized by high ascorbic acid retention in storage due to the absence of the ascorbate lyasing enzyme, ascorbate oxidase (EC 1.10.3.3.). According to ascorbic acid content assessment, the richest in ascorbic acid is the grapefruit peel extract, followed by the lemon peel (Fig. 3).

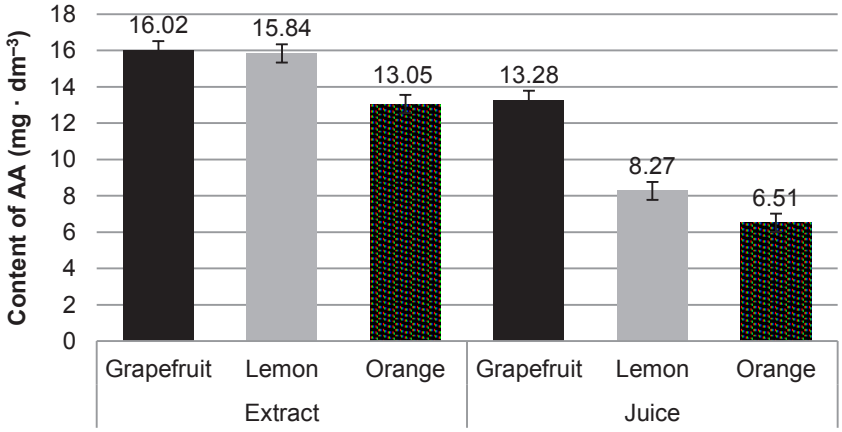

Figure 3. Ascorbic acid content of citrus peel extracts and citrus juices, mg^{-1}.

Total phenolic compound content was assessed via optical density (*D*) measurement after colouration by Folin reagent. The method is based on the ability of phenolics to reduce phosphotungstenate and phosphomolybdate contained in Folin reagent to indigo coloured oxides and reading the optical density values with a colorimeter.

Grapefruit and lemon peel extracts proved to retain more light at the given wavelength, which means they contain the most phenolic compounds (Fig. 4).

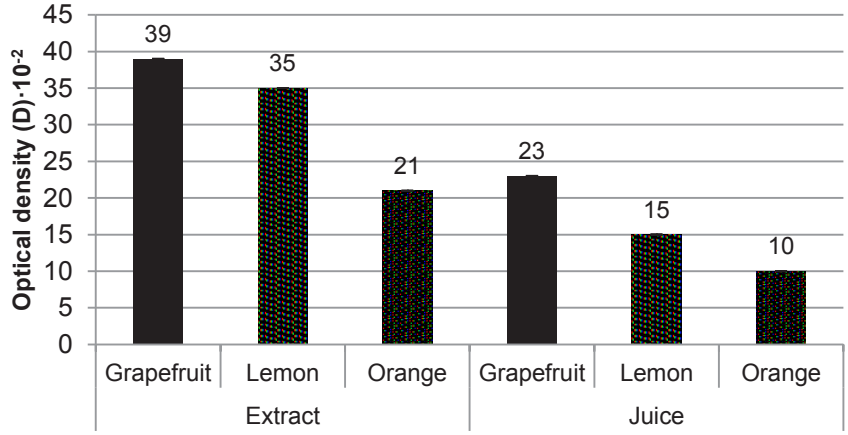

Figure 4. Optical density (*D*) of peel extracts and citrus juices with Folin reagent at 760 nm.

The results are theoretically predictable because an increase in the content of both ascorbic acid and phenolics must cause an AOA increase, but a clear correlation is not always found (Fedoseeva et al., 2008). This is believed to be the result of not only the

polyvalence of antioxidants in vegetative raw materials, but also of the difference in AOA assessment techniques. For instance, in one study (Gorinstein et al, 2005) ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) method tested TAC varied little between red and white grapefruit, while in another study (Anagnostopoulou, 2006) DPPH (2,2-diphenyl 1-picrylhydrazyl) method tested AOA proved to be much higher in red grapefruit. Additionally, some researchers are inclined to attribute TAC more to the ascorbate content and less to the phenolic content (Del Caro et al., 2004).

Biocatalysis in cowberry juice preparation

Analysis of lingonberries grown in Karelia (Table 2) and comparison of their indicators with the data presented in the literature (Izosimova, 2004) leads to the conclusion about the high nutritional and medicinal properties of cranberries growing in Karelia.

Table 2. Characteristics of the cowberry used in the experiment

Material (place of growth)	Characteristics (wet mass all)				
	Solids, Plato	Sugars, % $^{w}/_{w}$	Organic acids, % $^{w}/_{w}$	Ascorbate, mg dm^{-3}	Anthocyans, mg dm^{-3}
Cowberry (Karelia)	9.9 ± 0.1	7.7 ± 0.3	1.8 ± 0.1	14.8 ± 1.2	264.0 ± 0.8

Various doses and types of enzyme solutions were tried out on cranberry purée to study the effect on juice yield (Table 3). As shown in Table 3, the use of Fructozym pectolytic enzyme complex allowed for 10% ($^{w}/_{w}$) more juice to be collected, while the addition of ViscoStar 150L solution as well as Fructozym increased the yield by further 10%.

Table 3. The effect of enzyme solutions on cranberry juice yield

Sample number	Enzyme solution	Enzyme solution dosage, % $^{v}/_{w}$ wet mass	Treatment conditions Temperature, °C	Time, h	Juice yield, % $^{v}/_{w}$ wet mass
1	None (Control)	–			65
2	Fructozym MA	0.05	30	0.5	72
3	Fructozym MA ViscoStar 150 L	0.05 0.01	50	1.5	78

The various effects of enzyme solutions on the basic characteristics of cranberry juice are shown in Table 4.

Table 4. The effects of enzyme solutions on cranberry juice basic characteristics

Sample number	Enzyme solution	Juice yield, % $^{v}/_{w}$	Gravity, Plato	Anthocyans, % $^{w}/_{v}$	Ascorbic acid, % $^{w}/_{v}$
1	None (Control)	65	10.2 ± 0.3	23.8 ± 0.6	1.0 ± 0.1
2	Fructozym MA	72	13.6 ± 0.2	24.6 ± 0.3	1.2 ± 0.1
3	Fructozym MA ViscoStar 150 L	78	14.8 ± 0.2	25.4 ± 0.3	1.4 ± 0.2

Extraction of biologically active compounds from cranberries with the use of enzyme solutions allows for increase of juice yield, anthocyans and vitamin C content and can, therefore, be recommended for industrial-scale cranberry juice manufacturers.

Biocatalysis in grain grist mashing

According to various examinations (Akulova & Selezniova, 1995; Sharova & Hodkievich, 2009; Sharova et al., 2012; Sharova, 2015), actinomycetic bacteria grown on polysaccharide-based mediums produce inhibitors that show more activity towards amylases (α-amylase and glucoamylase) specific to macromolecular carbohydrates that are glycosidases, meaning that they hydrolyse glycoside bonds in starch molecules. α-amylase cleaves alpha bonds that are deep inside the molecule, effectively destructing the starch before glucoamylase hydrolysis may start that breaks individual glucose links off the terminus. α-amylase and glucoamylase thus both regulate the supply of glucose to the bloodstream that, if left uncontrolled, may lead to diabetes, obesity and other carbohydrate metabolism issues. At least one of the sources of these enzymes in animal (including human) bodies is believed to be the pancreas. Other known amylase sources are microorganisms. Therefore, it is important to test the inhibitors produced on common source amylases, not least on α-amylase derived from the body of an animal.

Glycosidase inhibitors are commonly produced by strains of Streptomyces, such as *Streptomyces violaceus* VKPM AS-1,734 and Streptomyces lucensis VKPM AS-1,743.

To study further the prospective applications of ViscoStar 150L for the extraction of biologically active compounds from vegetative raw material, the following experiment was conducted.

In the experiment rice flour and milled rye (with various pulverization ratios) were treated with ViscoStar 150L and Thermozyme 1,000L enzyme solutions during mashing to produce suitable substrates for microbial amylase inhibitor production. The effect of ViscoStar 150L on the composition of these substrates and subsequent changes in inhibitor yield from bacteria grown on these substrates was then examined. Pancreatic α-amylase derived from the pancreas of a pig as well as various microbial amylases were used as test samples for inhibitory activity assessment. The choice of pig pancreas amylase is based on the similarity between the biochemical processes in the body of a pig with said processes in the human body.

It has been shown that in the case of rice flour mash peak accumulation of inhibitors showing maximum activity towards pancreatic α-amylase requires the medium to contain 75–80% $^{w}/_{w}$ dextrins and 20–25% $^{w}/_{w}$ oligosaccharides (glucose, maltose).

In the mash of rye grist pancreatic α-amylase inhibitory activity proved to be 1.5–2 times lower (Table 5).

It has been shown that amylase inhibitors produced on rice flour mash substrate possess inhibitory activity towards other amylases, such as *B. subtilis* α-amylase, *Aspergillus awamori* glucoamylase and *S. cerevisiæ* invertase, even if 3–4 times lower than the 'target' activity.

Amylase inhibitors produced on rye mash substrate do not show inhibitory activity towards microbial glucoamylases. It may be attributed to a significantly different inhibitor producing microorganisms' substrate composition.

Table 5. Starchy raw materials hydrolyzing inhibitory activity

Strain name	Grist mashed	Inhibitory activity in mash, cm^{-3}, towards			
		pig pancreas α-amylase	*B. subtilis* α-amylase	*Aspergillus awamori* glucoamylase	*S. cerevisiæ* invertase
Streptomyces lucensis VKPM AS-1,743	Rice flour	1,200 ± 50	400 ± 15	250 ± 80	450 ± 25
	Rye grist	600 ± 50	240 ± 20	–	380 ± 10
Streptomyces violaceus VKPM AS-1,734	Rice flour	1,100 ± 50	350 ± 15	250 ± 80	410 ± 12
	Rye grist	600 ± 50	130 ± 20	–	180 ± 10

The inhibitory activity towards pancreatic α-amylase assessed after rice flour and rye grist centrifugate fermentation was 1.5 times higher in comparison to the results of non-centrifuged hydrolysates (1,600–1,800 units of inhibitory activity per cm^{-3}). The centrifugation removes high molecular mass compounds of protein and carbohydrate-protein nature that slow down the growth, development and biosynthetic activity of *Streptomyces*. Membrane filtration was used to clarify the cultural liquids of *Streptomyces* and to produce isolated solutions of pancreatic α-amylase inhibitors.

According to IR-spectroscopy, the inhibitors produced are carbohydrates in nature and contain α-1,2- and α-1,4-glycoside bonds, double bonds, carbonyl, hydroxyl, =NH and $–NH_2$ prosthetic groups (Fig. 5).

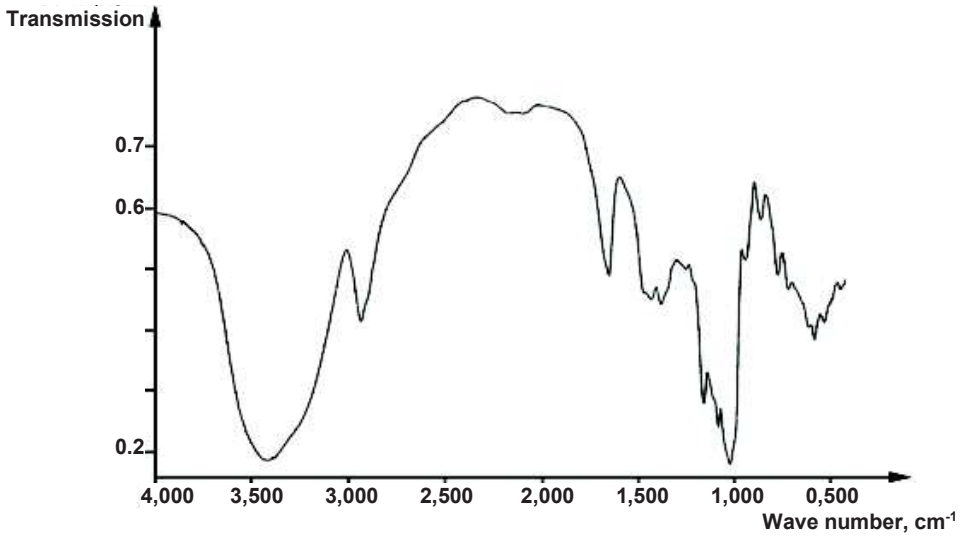

Figure 5. Pancreatic α-amylase inhibitor spectrogram.

The IR spectra of the inhibitor from Streptomyces lucensis VKPM AS-1,743 and inhibitor from *Streptomyces violaceus* VKPM AS-1,734 contained intensive bands characteristic of stretching and deformation vibrations and vibrations of the double bonds. Oscillations in intervals of change of the wave number (ν) were observed: 3,450–3,400 cm^{-1} (hydroxyl group, imines group and amino group associated); 3,100–2,900 cm^{-1} (methyl group); 1,750–1,600 cm^{-1} (aldehyde group, a double bond); 1,400–1,200 cm^{-1} (hydroxyl group, deformation); 1,200–1,000 cm^{-1} (acetylene group),

850–700 cm^{-1} (amino group, deformation). Deformation and skeletal vibrations of polyatomic systems are in the region of spectrum below 1,500 cm^{-1}. Oscillations of α-1,4 and α-1,2 glycoside bonds (ν = 756, 934 and 938 cm^{-1}) were observed. The data of IR spectra testify to the similarity of the structures of the inhibitor from *Streptomyces lucensis* VKPM AS-1,743 and inhibitor from *Streptomyces violaceus* VKPM AS-1,734.

The inhibitors produced supposedly retain their activity throughout a wide temperature (20–200 °C) and pH (2–12) spectrum. The inhibitors may serve as the substance for medicinal forms and food additives for prevention and treatment of diabetes, obesity and other carbohydrate metabolism issues.

CONCLUSIONS

Biocatalysis by ViscoStar 150L enzyme solution allows for a 2–3-fold increase in the effectiveness of biologically active compounds' extraction from citrus fruit peels. Peel extracts demonstrate higher total antioxidant capacity compared to fresh juices and ester oils. Extracted ascorbic acid and flavonoid content is also higher than that of juices, both the highest in grapefruit.

The use of Fructozym and ViscoStar 150L solutions in cranberry juice preparation allowed for a 20% ($^{w}/_{w}$) increase in juice yield, a 14.5% increase in gravity, a 10% increase in anthocyan and a 13.6% increase in vitamin C content.

The activity of inhibitors produced by microorganisms cultivated on mediums derived from grain grists retain almost 100% of their activity throughout a wide temperature and pH spectrum. The data obtained do not contradict previously known microbial pseudo-saccharide enzyme inhibitor properties and show possibilities for use of biologically active compounds derived from grain mashes as substance for food additives.

ACKNOWLEGMENTS. This work was partially financially supported by Government of Russian Federation, Grant 074-U01 and Federal State-Funded Scientific Establishment All-Russia Research Institute for Food Additives (FBGNU VNIIPD).

REFERENCES

Akûlova, N.Y. & Sielezniova, A.A. 1995. Microbic α-glycosidase inhibitors of pseudo-carbohydrate nature: a review. *Appl. Biochem. and Microbiol*. **31**(4), 371–380. (in Russian).

Anagnostopoulou, M.A, Kefalas, P., Papageorgiou, V.P., Assimopoulou, A.N., Boskou, D. 2006. Radical scavenging activity of various extracts and fractions of sweet orange peel (*Citrus sinensis*). *Food Chem*. **94**(1), 19–25.

Bacco, A., Culvelier, M.-T., Richard, H. & Berset, C., 1998. *J. Agric. Food Chem*. **46**(6), 2123–2129.

Baraboï, V.A. 1984. Plant Phenols and Health. Moscow, Naûka, 160 p. (in Russian).

Bazarnova, Y.G. 2010. Plant extracts as natural food spoilage inhibitors. *ITMO Univ. Scient. J., Food Engineering Series*. Available: http://www.processes.ihbt.ifmo.ru/ru/journal/308 (in Russian).

Bazarnova, Y.G. 2007. On the content of some biologically active agents possessing antioxidant activity in wildlife fruits and herbs. *Problems in Nutrition* **76**(1), 22–26. (in Russian).

Benzie, I.F. & Strain, I.I. 1996. The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power' – the FRAP assay. *Anal. Biochem*. **239**, 70–78.

Boshtam, M., Moshtaghian, J., Naderi, G., Asgary, S., and Nayeri, H. 2011. Antioxidant effects of *Citrus aurantifolia* (*Christm*) juice and peel extract on LDL oxidation. *J. Res. Med. Sci*. **16**(7), 951–955.

Braddock, R.J. 1995. By-products of citrus fruit. *Food Technol*. **49**(9), 74–77.

Chong De Sun, Kun Song Chen, Yang Chen, Qing Jun Chen, 2005. Content and antioxidant capacity of limonene and nomiline in different tissues of citrus fruits of four cultivars during fruit growth and maturation. *Food Chem*. **93**(4), 599–605.

Chûkhchin, D.G. & Tûpin, P.A. 2013. A Way of Quantitative Assessment of Dehydrogenase Activity of Microorganisms⁄Patent №2476598 (RU), IPC C12Q1⁄32 (2006.01) C12N9⁄02 (2006.01). (in Russian).

Chûpakhina, G.N. 2000. Physical and Biochemical Techniques for Plant Analysis. Kaliningrad, KSU. 59 p. (in Russian).

Del Caro, F., Piga, F., Vacca, V. & Agabbio, M. 2004 Changes of flavonoids, vitamin C and antioxidant capacity in minimal processed citrus segments and juices during storage. *Food Chem*. **84**(1), 99–105.

Denisienko, T.A., Vishnikin, A.B. & Tsiiganok, L.P. 2015. The effect of molybdenum diphosphate and Folin-Ciocâlteu reagent on phenolic compounds. *Analytics and Control* **19**(3), 242–251. (in Russian). DOI: http://dx.doi.org/10.15826/analitika.2015.19.3.001

Devis, W. B. 1947 Determination of flavonones in citrus fruits. *Industrial and Engineering Chem. Covering the field of analytical chemistry* **7**, 476.

Fedoseeva, A.A., Lebedkova, O.S., Kanibolockaja, L.V., Shendrik, A.N. 2008. Antioksidant activity of tee extracts. *Himiâ rastitel'nogo syr'â* **3**, 123–128. (in Russian) URL: http://cyberleninka.ru/article/n/antioksidantnaya-aktivnost-nastoev-chaya#ixzz46XmeNaTr

Gorinstein, Sh, Drzewiecki J., Sivan, A., Trakhtenberg, S., Katrich, E., Giner, J., Soliva-Fortun, R., Elez-Martiinez, P. & Martin-Belloso, O. 2005. Effect of antioxidants and proteins on the quality of Izraeli Jaffa red and blond grapefruits. *Eur. Food Res. and Technol*. **22**(1–2), 119–124. DOI 10.1007/s00217-004-1124-x

Grandall, P.G. 1977. Practical aspects of food manufacturing: citrus flavonoids, specially citrus products. *Proc. of 17th Ann. Short Course.* Matthews, R. F, Ed. Univ. of Florida. p. 99–125.

Hasanov, I.I. Ryžova, G.Â. & Mal'ceva, E.V. 2004. Methods of antioxidants research. *Himiâ rastitel'nogo syr'â* **3**, 63–75. (in Russian). http://www.chem.asu.ru/chemwood/volume8/2004_03/0403_063.pdf

Jabrikaroui, I. & Marzouk, B. 2013. Characterization of bioactive compounds in Tunisian bitter orange (*Citrus aurantium L.*) peel and juice and determination of their antioxidant activities. *BioMed Research International*, pp. 1–12. DOI: 10.1155/2013/345415.

Ivanova, E.A. 1995. A chemical and technological assessment of the quality of honeysuckle berries during freezing, storage and processing. (In partial fulfillment of Ph. D. research programme (Abstract)). (in Russian).

Izosimova, I.V. 2004. Scientific and practical basis of cowberry (*Vaccinium vitas-idæa*) and cranberry (*Oxycoccus palustris*) utilization. (In partial fulfillment of Ph. D. research programme (Abstract)). 26 p. (in Russian).

Jayaprakasha, G.K., Chidambara, K.N. & Bhimanagouda, S. Patil, 2008. Antioxidant activities of polyphenol containing extracts from *Citrus*. *Functional Food and Health* **993**(24), 264–276.

Liutikova, M.N., 2013. The effect of maturity and storage conditions on biologically active compounds in *Vaccinium vitas-idæa* and *Oxycoccus palustris*. (In partial fulfillment of Ph. D. research programme (Abstract)). Surgut, 26 pp. (in Russian).

Makarova, I.V., Ziuzina, A.V. & Miroshkina, Y.I., 2010. Antioxidant activity of citrus fruits. *The University Newsletter*, *Food Engineering Series*, p. 5–8. (in Russian).

Meliauskas, G., Venskutonis, P.R. & van Beek, T.A., 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem*. **85**, 231–237.
Menshhikova, Y.B., Lankin, V.Z. & Kandalintseva, N.V. 2012. Phenolic antioxidants in biology and medicine. Lap Lambert Academic Publishing, 495 pp. (in Russian).
Okwu, D.E. & Emenike, I.N. 2007. Nutritive Value and Mineral Content of Different Varieties of Citrus Fruits. *Journal of Food Technology* **5**, 105–108.
Ovsiannikova, E.A., Kiseliova, T.F., Potapov, A.N. & Dyuzhev, A.V., 2012. The effect of biological catalysis on the process of extraction of nutrients from wildlife berries of Siberia. *Equipment and techniques of food production* **4**, 1–4. (in Russian).
Ovsyannikova, E.A., Kiseliova, T.F., Potapov, A.N. & Dyuzhev, A.V., 2012. The effect of biological catalysis on the process of extraction of nutrients from wildlife berries of Siberia. *Equipment and techniques of food production* **4**, 1–4. (in Russian).
Pantelidis, G.E., Vasilakakis, M., Manganaris, G.A. & Diamantidis, G. 2007. Antioxidant capacity phenil, anthocyanin and ascorbic acid contents in raspberries blackberryes, red currants, gooseberryes and Cornelian cherries. *Food Chem*. **102**, 777–783.
Pekhtereva, N.T., Amelchenko, V.V. & Kononova, E.I. 2012. A Way of Obtaining Plant Extracts⁄Patent № 2466554 (RU). (in Russian).
Rumiantseva, G.N., Varfolomeieva, O.A. & Kindra, N.A, 2006. Preserving the gelating ability of pectin in the process of its extraction from leftover apple tissue. *Storage and Processing of Vegetative Raw Materials* **7**, 25–28. (in Russian).
Sharova, N.Y. & Hodkievich, O.A. 2009. Extraction and analysis of pseudo-carbohydrate glycosidase inhibitors. *The RAAS Newsletter* **1**, 65–67. (in Russian).
Sharova, N.Y., Hodkievich, O.A. & Pozdniakova, T.A. 2009. Cultural, morphological, physiological and biochemical properties of new glycosidase inhibitor producing actinomycetic strains. *The RAAS Newsletter* **3**, 74–76. (in Russian).
Sharova, N.Y., Kameňkova, N.V. & Hodkievich, O.A. 2012. Novel enzyme solutions for citric acid production from destroyed rye. *Storage and Processing of Vegetative Raw Materials* **6**, 37–39 (in Russian).
Sharova, N.Y. 2015. Inhibitors of amylases from *Streptomyces lucensis* VKPM AS-1743 and *Streptomyces violaceus* VKPM AS-1734. *Appl. Biochem. and Microbiol*. **51**(1), 46–52. (in Russian).
Shobinger, U. 2004. Technology of fruit and vegetable juice: scientific basis and technologies - St. Petersburg: Professija, 2004. 750 pp
Temerdashev, Z.A. Chrapko, N.V., Tsyupko, T.G., Voronova, O.B. & Balaba, A.N. 2006. Determination of antioxidant activity of food products using indicator system Fe (III) / Fe (II) - Organic reagent. *Zavodskaja laboratorija. Diagnostika materialov* **72**(11), 15−19. (in Russian).
Xiao, Z. A., Storms, A. & Tsang, R. 2006. Quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities. *Anal. Biochem*. **351**(1), 146–148.
Yermakov, A.I. (ed.), 1972. *Biochemical Techniques for Plant Analysis*. 2nd (revised) ed. Leningrad, Kolos, 456 pp. (in Russian).
Yevseieva, O.S. 2013. Development and validation of a technique for the flavonoid content assessment in certain species of *Citrus*. *The Science Newsletter*, *Medicine Series*: *Pharmacy* **24**(1), 55–60 (in Russian).
Zhilova, R. M. 2006. Development of a production technique of pastries fortified with wildlife blackberry biologically active compounds (In partial fulfillment of Ph. D. research programme (Abstract)). St.-Petersburg, St.-PSRFEA, 17 p. (in Russian).
Zia-ur-Rehman. 2006. Citrus peel extract – a natural source of antioxidants. *Food Chem*. **99**(3), 450–454.