Bulgarian Journal of Agricultural Science, 25 (No 1) 2019, 197–203

Ultrasound-assisted and agitated enzymatic extraction of pectin from red beet (*Beta vulgaris* L. var. *conditiva*) roots

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Abstract

Velyamov, S. M., Jingilbaev, S. S., & Akterian, S. G. (2019). Ultrasound-assisted and agitated enzymatic extraction of pectin from red beet (*Beta vulgaris* L. var. c*onditiva*) roots*. Bulgarian Journal of Agricultural Science*, *25*(1), 197–203

The study demonstrates a possibility the enzymatic extraction of red beet pomace to be enhanced by mean of applying ultrasonic treatment and agitation. It was found that the highest pectin yield can be reached, if the ultrasonic treatment is conducted at a frequency of 25 kHz and the rotational speed of impeller is 1000 rpm. The latter value corresponds to the Reynolds number of impeller equal to 100. The effect of ultrasound treatment is about a decimal order higher than the effect of agitation in a case of their combine action. The ultrasonic-assisted and agitated enzymatic extraction (UAEE) at the above-pointed levels of process variables assures the pectin yield in extract to reach nearly 80% for three hours of process duration. In UAEE process the duration of extraction is 1.7 times shorter and the yield of pectin is 1.9 times higher than the values of these indicators in the case of enzymatic extraction. The applicability of UAEE extract enriched in pectin as an additive in food formulations was demonstrated by means of a positive preliminary organoleptic survey, the specified nutritional values of UAEE extract and its safety indicators evaluated.

Keywords: agitation; enzymatic extraction; pectin; red beet; ultrasound
Abbreviations:CE – Controlled experiment; Re – Reynolds number; UAEE – Ultrasonic-assisted and agitated enzymatic extraction

Introduction

As it is known, the pectin is a complex mixture of native polysaccharides in the cell wall and middle lamellae in plant tissues. The pectin content in red beetroots is 1-1.1% (Skurikhin and Volgarev, 1987; Baker, 1997; Thakur et al., 1997; Lopes da Silva and Rao, 2006). Strasser et al. (1996), Strasser and Amado (2001, 2002) revealed the fine structure of red-beet pectin. The main chain is built of D-galacturonic residues that consecutions are occasionally interrupted by L-rhamnose residues. They specified that approximately 65% of the uronic acid residues are substituted by a methyl ether group. Organic acids (acetic, ferulic, etc.) and various of neutral sugars are linked covalently to the backbone as short side chains. So, the pectin of red beet could be classified to high methoxyl pectins. Pectin is commonly extracted using hot water (60-100°C) acidified under the pH 1.5-3 using acids as hydrochloric acid, nitric acid and sulfurous for half-hour to six hours (Sriamornsak, 2003; Sharma et al., 2006; Guo et al., 2012; Wang et al., 2016). This traditional heating method, however, is time consuming and has a low efficiency with limited yield of pectin. In the recent review of Adetunji et al. (2017) the following promising non-conventional technologies for extracting pectin and polysaccharides from plant materials were listed: i) enzyme-assisted extraction (Panouille et al., 2006; Velyamov et al., 2014); ii) subcritical water extraction (Khuwijitjaru, 2016); iii) microwave-assisted extraction (Latorre et al., 2013); iv) ultrasound-assisted extraction (Panchev et al., 1994; Grassino et al., 2016; Wang et al., 2016; Maran et al., 2017; Moorthy et al., 2017). The combination of two or more above-pointed technologies amplify their effect for deriving pectin as ultrasound-assisted and enzymatic extraction (Liao et al., 2015); combined treatment by ultrasound and subcritical water (Chen et al., 2015); combined treatment by ultrasound and microwave (Peng et al., 2015; Petkova et al., 2017a). Extractions using ultrasound and mechanical agitation were applied for deriving carbohydrates (Lupatini et al., 2017) and herbal extracts (Vinatoru, 2001; Petkova et al., 2017b). There are reported other new technologies for extracting pectin, which used electromagnetic induction heating (Zouambia et al., 2017), ultrahigh pressure (Guo et al., 2012) or high-voltage electrical discharges (Almohammed et al., 2017). Several researchers reported for non-conventional treatment of red beet: by using enzymes (Fissore et al., 2011, 2012; Velyamov et al., 2014) or by means of microwaves (Latorre et al., 2013).

The health benefits of pectin are numerous and they are well known (Thakur et al., 1997; Sriamornsak, 2003; Sharma et al., 2006; Fissore et al., 2011). Researchers showed the potential of pectin-enriched products to be used as additives in food formulation (Sriamornsak, 2003; Fissore et al., 2011, 2012).

Red beetroot contains two major water-soluble pigments betalains, including the red-violet betacyanins and the yellow-orange betaxanthins (Jackman and Smith, 1996; Gasztonyi et al., 2001; Stintzing and Carle, 2004; Gengatharan et al., 2015; Mereddy et al., 2017). The health benefits of betalains are also various (Stintzing and Carle, 2004; Gengatharan et al., 2015). Betalain-rish foods (as red beetroot) and their products have great potential as a natural food colorant (Jackman and Smith, 1996; Gasztonyi et al., 2001; Celli and Brooks, 2016) and as functional foods (Gengatharan et al., 2015).

The objectives of the present study were (i) to explore the applicability of ultrasound-assisted and agitated enzymatic extraction (UAEE) for enhancing the production yield of pectin from red beet pomace; (ii) to investigate the effect of process parameters – the rotation speed of impeller and the frequency of ultrasonic waves – on the yield of pectin; (iii) to identify the convenient levels of process parameters that enable to reach high pectin yield; (iv) to assess the applicability of UAEE extract enriched in pectin as an addition in food formulations by means specifying its nutritional values, safety indicators and conducting organoleptic test.

Materials and Methods

Plant material

For the current experiments, fresh red beet (*Beta vulgaris* L. var. *conditiva*) roots from the regional Kazakh variety “Bordeaux” in technical ripeness were collected. The roots were preliminary washed and peeled as the removed waste represented 6%. Red beet juice in a quantity of 47% was extracted by a mechanical screw press. The residual press cake (pomace) in a quantity of 47% was dried thereafter. The dried pomace with a moisture content of 8% represented 22% of the mass of fresh red beetroots. The dry pomace was used as a unified batch of raw plant material for the time period of extraction trials.

Chemicals and reagents

For enzymatic extraction, a multi-component enzyme complex of the company Biotech JSC (Belgium) was used. This complex includes the enzymes pectinase and cellulases as their specific activities were 2000 U/g and 350 U/g, respectively.

The following chemicals of analytical grade were used: sodium hydroxide, acetic acid, calcium dichloride, ether and silver nitrate for determining pectin in water extracts according to the Interstate standard GOST 29059-91 (an interstate standard of the Commonwealth of Independent States – former Soviet Republics).

Lab-scale unit for ultrasound-assisted and agitated enzymatic extraction

It was designed and assembled a customized lab-scale unit for ultrasound-assisted and agitated enzymatic extraction, which design is presented on Fig. 1. The working volume was sounded by a piezoelectric transducer 3 attached to the bottom of vessel’s body 1. The producer of this ultrasound device (model “Volna” UZTA-0,2/22-OM) together with the electronic generator is the Centre of ultrasonic technologies, Biysk (Russia). Its power level was 200 W and its power intensity – 5 W/cm^2. The power density, related to 8 L working volume, was 0,025 W/cm^3.

Enzymatic extraction process

The conditions of solid-liquid extraction were as follow: (i) the solid-liquid ratio between plant raw material and water was 1:13 as 1300 mL water was added to 100 g dry red beet pomace; (ii) the temperature of extraction was 37°C; (iii) pH 7.2±0.1 of the extractant (water); (iv) the extraction duration varied up to five hours. The dry red beet pomace, used as raw plant material, was rehydrated preliminary at temperature 56°*C for 15-18 hours.*

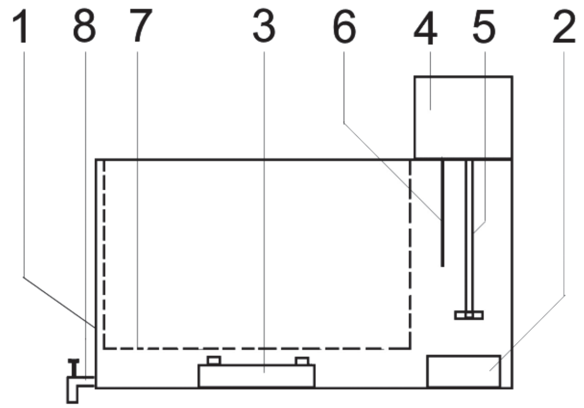

Fig. 1. Diagram of the lab-scale unit for ultrasonic-assisted and agitated enzymatic extraction:
1 – the body of process vessel; *2* – electrical heater; *3* – piezo-electric transducer generated ultrasonic mechanical vibrations; *4* – control panel; *5* – agitator with four-blade pitched impeller; *6* – temperature probe; *7* – perforated basket for plant material with working volume 8 L; *8* – valve for the outlet of extract

For enzymatic extraction, a multi-component enzyme complex was added to water-extractant – in a quantity equal to 10% of the mass of dry red beet pomace. The quantity of enzyme complex corresponds to 20000 U equivalents of the enzyme activity of pectinase. The above-pointed conditions of enzymatic extraction were specified in a previous publication (Velyamov et al., 2014). The effectiveness of extraction was evaluated by the determination of pectin content in extract derived.

Determination of pectin and dry matter

The method represented in the Interstate standard GOST 29059-91 (reissued in 2010) was used for determining the contents of pectin in water extracts. This method is based on the obtaining of insoluble calcium pectate from water soluble pectin. The quantity of soluble pectin was recalculated from the quantity of dry calcium pectate isolated. This method is also described by Ranganna (1986). Unfortunately, ISO standard for determining pectin is not available. The dry matter was determined by using the thermogravimetric method as stated in the Interstate standard GOST 33977-2016. Pursuant to this method the samples are dried at a constant temperature up to a constant weight.

Determination of nutritional values of the extract derived

The contents of protein and fat in the extract were determined as stated in the Interstate standards GOST 26889-86 and GOST 8756.21-89, respectively. The content of carbohydrate and ash, and the energy value of extract were determined by the methods represented in the handbook of Skurikhin and Volgarev (1987). The content of vitamins was established taking into account the following Interstate standards: GOST 8756.22-80, GOST 30627.3-98 and GOST 7047-55. The following Kazakh standards: ST RK ISO 12081-2010, ST RK GOST R 51301-20 05 and the Interstate standard GOST 26928-86 were used for determining the mineral composition of the extract derived.

Methodology of experiments

The enzymatic extraction without agitation and ultrasonic treatment was picked as a controlled and reference experiment. The following independent variables of ultrasonic-assisted and agitated enzymatic extraction were objects of single-variable experiments:

(i) *The rotation speed of impeller*, which varies from 600 to 1000 rpm. The speeds higher than 1000 rpm caused an excessive and undesired foaming.

(ii) *The frequency of ultrasonic waves*. According to the literature survey (Agopyan and Ershov, 2005; Vilkhu et al., 2008; Feng et al., 2011) the ultrasound with a low frequency from 18 kHz to 30 kHz at a high power intensity of 5 W/cm^2 is the most affecting on plant tissues. The ultrasound destroys cell walls. A partial depolymerization of protopectin occurs as well. The diffusion of the water in plant material and also the soluble pectin in the extract is accelerated. On the base of our preliminary investigations and available technical capabilities, it was assumed that this variable should be varied in the range from 20 kHz to 30 kHz.

(iii) *The duration of process*. The experiments were done in the range from one to five hours. The upper limit corresponds to the enzymatic extraction without agitation and ultrasonic treatment.

Really, these experiments can be referred to one-factor-at-a-time method.

The pectin yield was adopted as a response variable of the experiments in this study. The relative yield in % was calculated by the following equation:

$$Y = (P/P_0).100, \qquad (1)$$

where P is the concentration of pectin in water extract after the current extraction process, %; P_0 is the concentration of pectin in the dry red beet pomace, %.

Uncertainty of measurements

Each experiment was repeated three times. The results of experiments were expressed by the average value of the measurements together with the expanded uncertainty. The latter is based on a standard uncertainty multiplied by a coverage factor k = 2, providing a level of confidence of approximately 95%. The guide JCGM 100:2008 of the Joint committee for guides in metrology (Paris) was taken into consideration for evaluating the uncertainty in measurement.

Results and Discussion

Controlled experiment

Preliminary, the total pectin content in the raw plant material – dry red beet pomace – was determined according to GOST 29059-91 and using an extraction by ether in a Soxhlet apparatus as well. It was found that 6.89 g pectin is contained in 100 g dry red beet pomace. This corresponds to a concentration P_0 = 6.89%. A controlled experiment (CE) – enzymatic extraction with duration of five hours without agitation and ultrasonic treatment – was performed. The yield of pectin in extract reached to Y_0 = 65% calculated by Eq. 1.

Single effects of process variables

The effect of the impeller's rotational speed on the yield of pectin is presented in the Table 1. On the basis of these results, the impeller speed at 1000 rpm was assumed as more suitable. This speed level assures the highest yield of 65% to be reached for the shortest duration of extraction.

This accepted speed of impeller corresponds to the Reynolds number (Re) equal to 100. For calculating Re number the following measurements were performed and taken into consideration: (i) the outside diameter of impeller: 0.06 m; (ii) the dynamic viscosity of extract agitated: 0.612 Pa·s; (iii) the density of extract agitated: 1009 kg/m^3. The effect of ultrasonic frequency on the yield of pectin is presented in Table 2. The frequency of 25 kHz was assumed as more relevant taking into account the experimental results and the following considerations: The advantages of the ultrasound treatment at a lower frequency are: (i) the propagation of acoustic waves is broader in the process vessel as the volume of non-sounded dead zones is less; (ii) the required power level of cavitation is lower. In addition, the ultrasonic equipment at a frequency of 25 kHz is more commonly used in practice.

Comparing UAEE process and enzymatic extraction

The effects of the ultrasound-assisted and agitated enzymatic extraction (UAEE) and the controlled experiment (CE) of enzymatic extraction on the yield of pectin are presented on the Fig. 2. UAEE extraction was implemented under the following conditions: the impeller speed was 1000 rpm and the frequency of ultrasound vibration – 25 kHz.

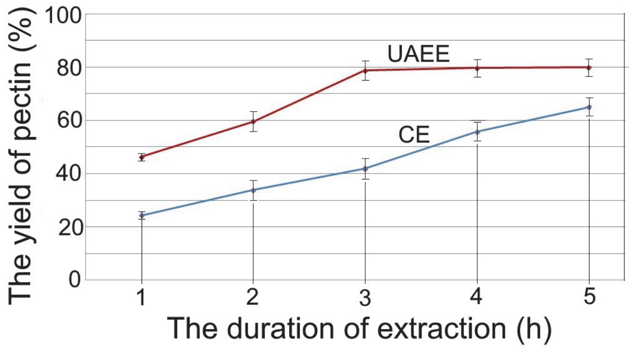

Fig. 2. Yield of pectin (%) during ultrasound-assisted and agitated enzymatic extraction (UAEE) and the controlled experiment (CE) of enzymatic extraction. The yield results are expressed by their average values together with the expanded uncertainty.

Table 1. Effect of impeller speed (rpm) on the yield (%) of pectin during agitated enzymatic extraction. The yield results are expressed by their average values together with the expanded uncertainty.

Impeller speed (rpm)	The duration of extraction (h)				
	1	2	3	4	5
600	31.3 ± 0.2	42.8 ± 0.4	54.5 ± 0.3	64.9 ± 0.2	64.6 ± 0.4
800	38.3 ± 0.4	47.5 ± 0.4	61.5 ± 0.5	65.1 ± 0.4	63.9 ± 0.3
1000	42.9 ± 0.3	49.9 ± 0.5	65.0 ± 0.6	63.9 ± 0.4	62.8 ± 0.3

Table 2. Effect of ultrasonic frequency (kHz) on the yield (%) of pectin during ultrasound-assisted enzymatic extraction. The yield results are expressed by their average values together with the expanded uncertainty.

Ultrasonic frequency (kHz)	The duration of extraction (h)				
	1	2	3	4	5
20	32.5 ± 0.3	43.5 ± 0.4	64.5 ± 0.3	70.7 ± 0.3	70.7 ± 0.3
25	45.2 ± 0.3	57.1 ± 0.4	71.9 ± 0.2	72.3 ± 0.3	72.3 ± 0.4
30	46.4 ± 0.2	58.4 ± 0.2	71.9 ± 0.2	72.4 ± 0.3	72.5 ± 0.4

The following conclusions could be brought out from these results:

* *The most suitable duration of UAEE extraction is three hours* when the pectin yield reaches 78.7% and the pectin content is 0.78-0.79% in the extract derived. This duration of UAEE extraction is 1.7 times shorter, than the duration of CE process. The pectin yield of UAEE process is 1.88 times higher, then the yield at CE experiment with the same duration.

* *The effect of ultrasound treatment is about a decimal order higher* then the effect of agitation in a case of their combine action. The difference between the yields of UAEE and CE process at the end of the third hour (see Fig. 2) is 33.1 percentage points and this value corresponds to the combine action of agitation and ultrasound. The single impact of ultrasound treatment corresponds to an increase of the yield in 30.1 percentage points at the end of the third hour in a comparison with CE process (see Table 2). Therefore, the single effect of ultrasound is 1.3 times higher, then this of agitation. The reported single impact of ultrasonic treatment (in 30.1 percentage points) is in a compliance with the results of other researches (Feng et al., 2011). They found that the ultrasound-assisted extraction increased the yield of various bioactive compounds between 11% and 35%.

The nutritional values of the extract derived

According to laboratory tests the following major nutritional values of 100 g extract were determined: (i) dry matter content 18.5 g, of which: carbohydrate 15.4 g, protein 1.9 g, ash 1.1 g and fat 0.1 g; (ii) vitamin contents: β-carotene (provitamin A) 8.0 mg, vitamin B_3 (PP) 0.12 mg and vitamin E 0.09 mg; (iii) minerals: calcium 50 mg, iron 1.5 mg, copper 0.5 mg and zinc 0.2 mg; (iv) energy value: 293 kJ (or 70 kcal).

The safety tests of the extract derived

The safety of the extract as a food additive was ascertained on the base of the following tests related to:

A. *Chemical hazards:* toxic heavy metals: lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg); radionuclides*:* caesium (Cs) and strontium (Sr); pesticides and nitrates;

B. *Microbiological hazards:* total bacterial count, total coliform count, the presence of pathogenic microorganisms, including: *Salmonella, Escherichia coli* and *Bacillus cereus;* moulds and yeasts.

All indicators tested on the above-pointed hazards were within the corresponding limit levels of the following standards and regulations:

* For toxic heavy metals: Interstate standard GOST 26930-86, Russian and Kazakh standard ST RK GOST R 51301-2005;

* For radionuclides: Method for measuring the activity of radionuclides MI No KZ 07.00.00304-2009;

* For pesticides: Kazakh standard ST RK GOST 2011-2010;

* For nitrates: Interstate standard GOST 29270-95.

Applicability of UAEE extract as a food additive in food formulations

For longer storage the UAEE extract was concentrated preliminary by evaporation under vacuum at temperature 58-60°C and absolute pressure 50-70 kPa. The content of dry matter and pectin in this concentrated UAEE extract were 24-25% ± 0,02% and 2,00-2,15% ± 0,02%, respectively. It was specified that concentrated extract can be stored safety up to a month under refrigeration conditions.

A preliminary survey was carried out for enriching juices with pectin and other biologically active components by means of adding UAEE extract concentrated. Objects of the current trials were carrot juice, a juice from carrot and apple, and a juice from apple and peach. According to a preliminary organoleptic panel test, an addition of UAEE extract concentrated in a quantity of 10% in these juices was the most suitable and acceptable with milder and sourer taste.

A high content of betalains is expected to be presented in UAEE extract as well. Jackman and Smith (1996) pointed that less than 50% of betalains can be recovered in red beet juice after the conventional press operation and up to 90% – in a case of using the traditional extraction process. Cardoso-Ugarte et al. (2014) reported that the duration of traditional process of extraction of betalains from red beet is 20 min. Therefore, UAEE extraction with duration three hours will be quite enough for extraction of residual betalains to a sufficient extent. The recovery of betalains in UAEE extract will be the object of a further study.

Conclusions

The treatment of red beetroots by means of ultrasound-assisted and agitated enzymatic extraction (UAEE) enables the process duration to be reduced nearly two times up to three hours. The pectin yield increases up to nearly 80% what is about two times higher comparing to enzymatic extraction with the same duration. It was found that the highest pectin yield can be reached at the following levels of process conditions studied: (i) 25 kHz frequency of ultrasound with a power intensity of 5 W/cm^2; (ii) 1000 rpm rotational speed of impeller, what corresponds to the Reynolds number of impeller equal to 100. The effect of ultrasound treatment is about a decimal order higher, then the effect of agitation in a case of their combine impact. The nutritional values of

UAEE extract and the safety indicators related to chemical and microbiological hazards were evaluated. These results and the positive preliminary organoleptic survey illustrates the applicability of the UAEE extract enriched with pectin as an additive in food formulations with health benefits.

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Received: 23.03.2018; ***Accepted:*** 11.05.2018

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