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Novel arginine deiminase-based method to assay L-arginine in beverages

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ABSTRACT

A highly selective and sensitive enzymatic method for the quantitative determination of L-arginine (Arg) has been developed. The method is based on the use of recombinant bacterial arginine deiminase (ADI) isolated from the cells of a recombinant strain *Escherichia coli* and *o*-phthalaldehyde (OPA) as a chemical reagent. Ammonia, the product of the enzymatic digestion of Arg by ADI, reacts with OPA and forms in the presence of sulfite a product, which can be detected by spectrophotometry (S) and fluorometry (F). The linear concentration range for Arg assay in the final reaction mixture varies for ADI-OPA-F variant of the method from 0.35 μM to 24 μM with the detection limit of 0.25 μM. For ADI-OPA-S variant of the assay, the linearity varies from 0.7 μM to 50 μM with the detection limit of 0.55 μM. The new method was tested on real samples of wines and juices. A high correlation ($R = 0.978$) was shown for the results obtained with the proposed and the reference enzymatic method.

1. Introduction

L-Arginine (Arg), one of the most abundant amino acids in grape juices and wines, is closely related to the ethyl carbamate (EC) level in wines (Huang & Ough, 1989; Wu et al., 2014). Arg is degraded to urea and ornithine by the yeast enzyme arginase (EC 3.5.3.1). Urea is assimilated by the yeast cells, but some amount of this metabolite is released into the fermentation medium under excess arginine in grape juice. Accumulated urea in the medium can spontaneously react with ethanol to form very toxic and carcinogenic EC (Alexander et al., 2007; Huang & Ough, 1989). Citrulline, formed from Arg metabolism *via* the arginine deiminase pathway by wine malolactic bacteria, is the second significant precursor of EC in wine (Liu, Pritchard, Hardman, & Pilone, 1996; Spayd et al., 1994; Uthurry, Lepe, Lombardero, & Del Hierro, 2006). Both urea and citrulline accumulation in the fermentation medium mainly depends on the Arg level and the type of microorganism (Liu et al., 1996). If Arg concentration in juice is higher than 1000 mg L^{-1} (5.0 mM), the potential EC concentration in wine will be above 15 μg L^{-1}, the current voluntary limit in the United States (Ough, Stevens, & Almy, 1989), while Canada and Czech Republic have legalized this limit to 30 μg L^{-1}. France, Brazil, Germany and Swiss also control EC level in some alcohol drinks (Conacher & Page, 1986; Jiao, Dong, & Chen, 2014; Mira De Orduña, Liu, Patchett, & Pilone, 2000).

Determination of EC in fermented foods and beverages involves various strategies. Usually the adapted extractive techniques and pre-concentration steps are followed by gas chromatography (GC) coupled to mass spectrometry (MS). High-performance liquid chromatography (HPLC) and semi-quantitative spectroscopic methods (infra-red) are also proposed as valuable alternatives to the classical but time-consuming GC–MS (Jiao et al., 2014).

Two types of preventive methods of decreasing the EC levels in food are described. First, adapted and optimized practices in all steps of the chain of food (or beverages) production lead, in general, to low EC level. Secondly, the abatement of EC precursors can be done by adapted enzymatic, physical–chemical or chemical methods according to the nature of raw materials and conditions of their production processes. So, to ensure the high quality of final product and to avoid potential health hazard of carcinogenic EC, quantitative analysis of its precursor, Arg in fruit juices and wines is an actual problem.

A variety of physicochemical detection procedures for Arg analysis have been developed. These procedures were based on ion exchange chromatography (Huang & Ough, 1989; Spayd et al., 1994), fluorometry (Parniak, Lange, & Viswanatha, 1983), spectrophotometry (Casadebaig, Dupin, & Mesnard, 1979; Goldschmidt & Lockhart, 1971; Khramov, Petrova, & Binova, 1980; Micklus & Stein, 1973; Sakaguchi, 1925; Sastry &

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Tummuru, 1994; Wang, Liang, Zhao, Feng, & Li, 2008; Yamasaki, Shimer, & Feeney, 1981), capillary electrophoresis (Narezhnaya, Askalepova, Nikashina, Krukier, & Pogorelova, 2010), HPLC (Chu, Huang, Pao, & Li, 2003), HPLC–MS (Aldamiz-Echevarria & Andrade, 2012; El-Khory et al., 2011).

The first attempts of the colorimetric (spectrophotometric) determination of Arg were limited only to the Sakaguchi reaction with α-naphthol and sodium hypochlorite (Sakaguchi, 1925). In further modifications of this method various chromogenic agents were applied: diacetyl-α-naphthol (Goldschmidt & Lockhart, 1971), 2-methyl-α-naphthol (Casadebaig et al., 1979), 5-chloro-7-iodo-8-hydroxy quinoline (5-chloro-7-iodo-8-HQ) (Khramov et al., 1980), α-naphthol and 2,3-butanedione monoxime in the method of Voges-Proskauera (Micklus & Stein, 1973), thymol-(2-propyl-5-methyl) phenol and sodium hypobromite in the method of Sastry (Sastry & Tummuru, 1994), 8-hydroxyquinoline (HQ) and sodium hypobromite (Wang et al., 2008); *p*-nitrophenylglyoxal and sodium ascorbate (Yamasaki et al., 1981); 1,2,4-trihydroxy anthraquinone (THAQ) and hydrogen peroxide (Mitič, Miletič, Pavlovič, Tosic, & Velimirović, 2007). The comparative characteristics of the most common methods of Arg assay are summarized in Table 1 (Gayda, Stasyuk, & Gonchar, 2014).

The construction of Arg-sensitive biosensors has already been reported. The main analytical characteristics of the potentiometric, conductometric and amperometric biosensors on Arg, based on enzymes and cells, were described in detail in our papers (Gayda, Stasyuk, Klepach, & Gonchar, 2015; Gayda et al., 2014; Stasyuk, Gayda, & Gonchar, 2014; Stasyuk, Smutok, Gayda, Kovalchuk, & Gonchar, 2011; Stasyuk, Smutok, Gayda, Vus, & Gonchar, 2012).

Thus, the known physicochemical approaches for Arg monitoring require special skills, are time-consuming and expensive, and often have poor precision, low sensitivity and selectivity. So the further development of novel highly selective and sensitive methods, including enzymatic ones, for Arg determination in food industry, in particular, in wine making, is necessary.

The enzymes of Arg metabolism, namely, arginase, arginine deiminase and arginine decarboxylase, are the promising tools for Arg assay. Due to the high cost of natural Arg-selective enzymes, the search for the alternative sources of these enzymes, including recombinant microorganisms, as well as the development of effective technologies for enzymes isolation and purification are the relevant problems.

We have reported earlier about isolation and characterization of human liver arginase I (EC 3.5.3.1; L-arginine amidinohydrolase) from the recombinant yeast cells, over-producing this enzyme (Stasyk, Boretsky, Gonchar, & Sibirny, 2015; Stasyuk et al., 2014; Zakalskiy et al., 2012). The number of arginase-based analytical approaches for quantitative determination of Arg were elaborated and characterized, namely, enzymatic methods with spectrophotometric and fluorometric detection of the reaction product (Gayda et al., 2014; Stasyuk, Bass, Gayda, Yepremyan, & Gonchar, 2015; Stasyuk, Gaida, & Gonchar, 2013; Stasyuk, Gayda, Gayda, & Gonchar, 2012); bi-enzyme (arginase/urease) and microbial electrochemical sensors (Gayda et al., 2015). All proposed arginase-based analytical approaches were tested on the real samples of Arg-containing commercial pharmaceuticals and wines.

In this paper, we describe the development of a novel enzymatic method for Arg determination, based on using recombinant ADI and *o*-phthalaldehyde (in the presence of sulfite) with the fluorometric and spectrophotometric detection of the product (ammonia) and evaluation of the proposed method on real samples of wines and juices.

2. Materials and methods

2.1. Chemicals

o-Phthalaldehyde (OPA), 2,3-butanedione monoxime (DMO), thiosemicarbazide (TSC), sulfuric acid (95–98%), phosphoric acid (85%), trichloroacetic acid, ethanol, Na_2SO_3 were purchased from Sigma–Aldrich. Amino acids and inorganic salts were obtained from Merck (Darmstadt, Germany). All buffers and standard solutions were prepared with Milli-Q system (Millipore) purified water.

2.2. Enzyme

Arginine deiminase (ADI) expression, folding *in vitro* and purification were done as reported previously with some modifications (Fayura, Boretsky, Pynyaha, Wheatley, & Sibirny, 2013). Cells of *Escherichia coli* BL21 (DE3) (Studier & Moffatt, 1986) were transformed with plasmid pET3d-ADI. Transformants were selected on Luria–Bertani medium at 37 °C supplemented with agar (18 g L^{-1}), ampicillin (100 mg L^{-1}), Arg (0.2%) and glucose (0.5%). Selected transformants were employed to produce ADI using C-750501 medium supplemented with 1.25% glycerol and 0.01% lactose following autoinduction protocol modified earlier (Fayura et al., 2013; Studier, 2005).

The induced cells were harvested by centrifugation (5000×*g* for 15 min at 4 °C), washed with 20 mM sodium phosphate buffer, pH 7.2 (PB), and stored at −70 °C. To obtain a suspension of ADI inclusion bodies, the bacterial pellet was resuspended in 50 mM Tris–HCl buffer, pH 7.8 that contained 1 mM EDTA and 0.2% lysozyme and treated as described earlier (Fayura et al., 2013). Obtained inclusion bodies were solubilized in 50 mM potassium phosphate buffer (pH 7.2), containing 1 mM EDTA, 5 M guanidine hydrochloride and 5 mM DTT. Refolding of ADI was carried out by rapid 100-fold dilution of solubilized protein with 20 mM potassium phosphate buffer, pH 7.2, containing 200 mM KCl, 1 mM DTT and 1 mM EDTA followed by incubation at 15 °C for 72 h.

Table 1
Comparison of different chemical and physicochemical methods of L-Arg assay.

Method/reference	λ_{max}	ε	Sensitivity		Linear range		Time
	nm	$mM^{-1}\ cm^{-1}$	mg L^{-1}	mM	mg L^{-1}	mM	min
Sakaguchi (Sakaguchi, 1925)	525	15.7	0.7	$4.0 \cdot 10^{-3}$	1.0–45	0.006–0.26	40
5-chloro-7-iodo-HQ (Khramov et al., 1980)	440	57.4	0.9	$5.2 \cdot 10^{-3}$	1.0–100	0.006–0.57	10
Voges-Proskauera (Micklus & Stein, 1973)	535	3.07	0.8	$4.6 \cdot 10^{-3}$	1.0–100	0.006–0.57	30
Sastry (Sastry & Tummuru, 1994)	440	13.0	0.3	$1.7 \cdot 10^{-3}$	–	–	40
HQ (Wang et al., 2008)	500	66.0	1.2	$6.9 \cdot 10^{-3}$	1.5–12	0.009–0.07	10
p-nitrophenylglyoxal (Yamasaki et al., 1981)	475	–	–	–	0.03–0.33	$(0.2–2.0) \cdot 10^{-3}$	30
THAQ (Mitič et al., 2007)	540	–	1.5	$8.6 \cdot 10^{-3}$	4.4–87.5	0.03–0.50	5
Sodium hypobromite (Gange, Francis, Costin, Barnett, & Lewis, 2005)	–	–	0.02	$0.1 \cdot 10^{-3}$	0.52–21.1	0.003–0.12	40
Tris (2,2-bipyridyl)ruthenium(III) (Costin, Paul, & Lewis, 2003)	–	–	$0.6 \cdot 10^{-3}$	$3.4 \cdot 10^{-6}$	$0.8–1.5 \cdot 10^{-3}$	$(4.5–8.6) \cdot 10^{-6}$	30
Zone electrophoresis (Narezhnaya et al., 2010)	–	–	3	0.02	6.0–1000	0.03–5.74	60
HPLC (Chu et al., 2003)	–	–	0.3	$2.0 \cdot 10^{-3}$	1.0–500	0.006–2.87	40

The renatured enzyme was diafiltered and purified by anion-exchange chromatography using Q-Sepharose column and a linear gradient of 0–1 M NaCl in PB. The active fractions were pooled and NaCl was adjusted to 2 M. The enzyme was applied to a Phenyl-Sepharose column, washed with PB, containing 2 M NaCl, and eluated with the buffer containing 1 M NaCl. As a result, stable, almost homogenous enzyme preparations with a specific activity of 30–35 U/mg of protein were obtained.

Solution of the purified enzyme was concentrated using ultrafiltration spin column Vivaspin-Turbo 15 (Sartorius Stedim Biotech GmbH, Germany), filter-sterilized using 0.22 μM filters (Ce 0459 Millex-Gv, Millipore, USA) and stored at 4 °C.

The protein concentration was determined by the Lowry method. ADI activity was determined as described (Misawa, Aoshima, Takaku, Matsumoto, & Hayashi, 1994). One unit (U) of activity was defined as the amount of enzyme that converted 1 μmol Arg to L-citrulline per min at 37 °C.

2.3. The development of ADI-OPA-based methods

2.3.1. Preparation of OPA reagent

2 g OPA was dissolved in 5 mL 95% ethanol and mixed with 100 mL 0.1 M borate buffer, pH 10. The final reagent (14.3 mM OPA) was supplemented by sodium sulfite to the final concentration of 0.16 mM (Goyal, Rains, & Huffaker, 1988) and stored in darkness at room temperature until usage.

2.3.2. Ammonium ions calibration

An aliquot of 0.15 mL ammonium chloride solution with concentration from 0.005 mM to 1.25 mM in PB was mixed in plastic tube with 3 mL OPA reagent, closed carefully and heated at 60 °C for 15 min. As a blank, OPA reagent with PB (without ammonia) was used. Fluorescence intensity and absorbance for the resulted OPA-ammonium product were determined and the corresponding calibration graphs, for fluorometric (OPA-F) and spectrophotometric (OPA-S) modes of registration, were built. Fluorescence emission value of the tested sample was registered at 415 nm (under excitation at 360 nm) using a Quantech digital filter fluorometer (Thermo Scientific, United States). Optical density was registered at 350 nm with Shimadzu UV1650 PC spectrophotometer (Japan). Each experiment was repeated for 3 times.

2.3.3. Arg calibration

0.1 mL Arg solution with concentration from 0.0012 mM to 10 mM in 0.2 M PB, pH 6.5 was mixed in plastic tubes with 0.025 mL ADI (300 U/mL) solution in PB and incubated at 37 °C during 20 min for complete hydrolyses of Arg. The resulted ammonia was detected after heating in closed tubes with 2.5 mL OPA using OPA-S and OPA-F methods, as described in Section 2.3.2. As a blank, reaction mixture without Arg was used.

2.3.4. Arg assay in real samples of wines and juices

The samples of the following wines were tested in this study: dry white "Chardonnay", dry white "Sauvignon Blanc" and semisweet pink "Syrah Rose" (La belle Angele, France), dry red "Moution Cadet" (Baron Philippe de Rothschild, France), semisweet red "Bastardo" (Massandra, Ukraine), semi-sweet pink "Tokan" (Israel). Two samples of juices "Sadochok" (LtD Sandora, Ukraine) – multifruit, grape with apple – were tested too. All samples were analyzed using a standard addition test (SAT). Before assay, all samples were step-wise diluted by 0.02 M PB, pH 6.5 containing 1 mM EDTA: for chemical method – 125 and 400-fold; for enzymatic method – 50 and 100-fold. Each assay was performed for two dilutions of the sample and repeated 3 times.

2.3.5. The algorithm of calculation of endogenous Arg content

Arg assay by graphical method was done according to the following scheme:

1st step: Estimation of endogenous ammonium ions in the initial sample from the parameters of linear regression of the corresponding OPA-S and OPA-F graphs, taking into account factors of dilution (Eq. (1)); calculation of the average concentration from two values:

$$C_a = \frac{A}{B} \cdot N \quad (1)$$

where C_a – concentration of ammonia, A and B – parameters of linear regression; N – dilution factor of the tested sample, shown in graph.

2nd step: Treatment of the tested sample with ADI followed by estimation of total ammonia content (Eq. (1)) in the resulted sample by graphical method, taking into account factors of dilution, calculation of an average value of ammonia content.

3rd step: Calculation of endogenous Arg content in the tested sample according to Eq. (2).

$$C_{\mathrm{Arg}} = \left(C_a^t - C_a^e\right) \quad (2)$$

where C_{Arg}, C_a^t and C_a^e – concentrations of endogenous Arg, average contents of total and endogenous ammonia in the tested sample, respectively.

2.4. Arg assay with reference enzymatic method

As a reference, an enzymatic method of Arg assay based on two enzymes – commercial urease and recombinant human liver arginase I (arginase) was used (Stasyuk et al., 2013).

Urea, the product of the enzymatic hydrolysis of Arg by arginase, reacts with 2,3-butanedione monoxime (DMO) and forms a yellow product which can be determined photometrically at 480 nm or fluorometrically at 510 nm (Rho, 1972). Arginase was isolated from the cells of the recombinant yeast *Hansenula polymorpha*, over-producer of the target enzyme as described (Stasyuk et al., 2014).

The analytical results were statistically processed using the OriginPro 8.5 software.

3. Results and discussion

3.1. The general principle and characteristics of the ADI-OPA method

A schematic representation of the reactions, on which the developed method of Arg assay is based, is shown in Fig. 1. Analytical signal is created due to the one-step enzymatic conversion of Arg to ammonium ions (stage I, enzymatic reactions). Ammonium ions react selectively with OPA in an alkali medium in the presence of sulfite, that permits to avoid reaction of OPA with any amino acids (stage II, chemical reaction).

To optimize the conditions of the chemical reaction, OPA concentration (from 7.5 to 15 mM), heating time (10–30 min) and temperature (25–65 °C) were tested (data not shown). Finally, the next conditions of chemical reaction were chosen: 14 mM OPA in the reaction mixture, 15 min of heating in water bath at 60 °C. To optimize the conditions of the enzymatic reaction, concentration of ADI was varied from 0.04 to 0.48 U/mL in the incubation mixture. As the result, the optimal ADI concentration in the incubation mixture has been estimated as 0.24 U/mL required for a complete conversion of Arg (data not shown).

The dependences of fluorescence intensity and optical density of the resulted product (OPA-ammonium) on Arg concentration, as well as the corresponding calibration graphs (F-OPA and S-OPA) for Arg assay under experimentally chosen optimal conditions are demonstrated in the Fig. 2.

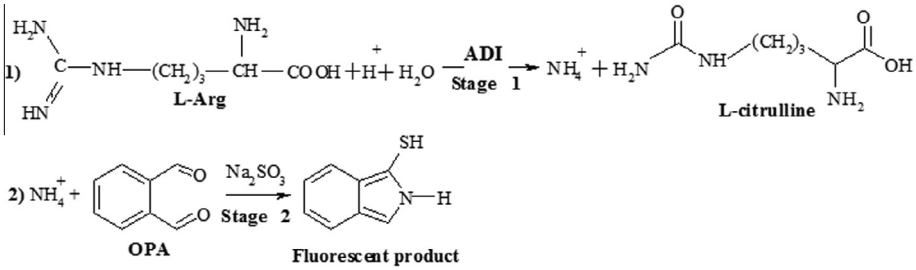

Fig. 1. The reactions for Arg detection using ADI-OPA-based method.

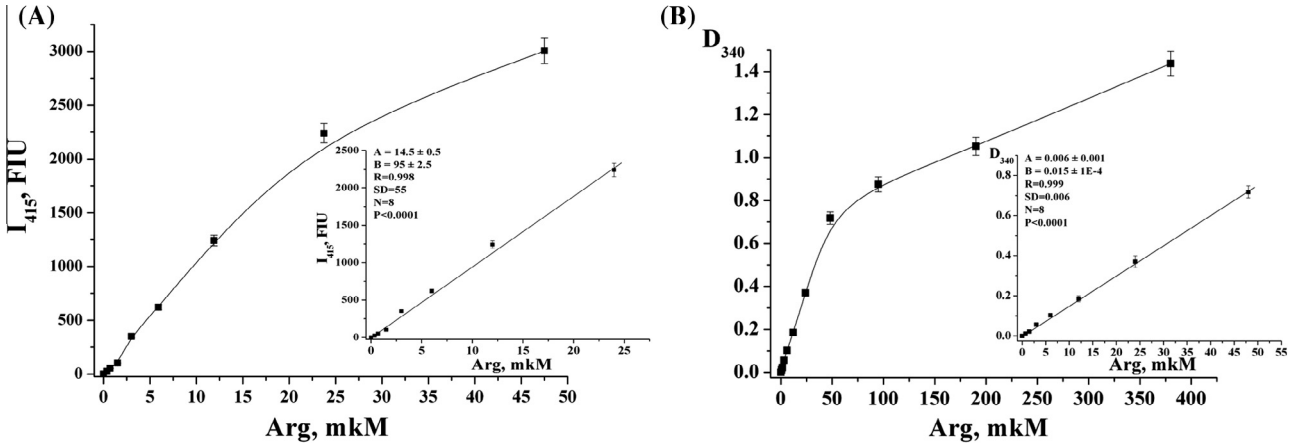

Fig. 2. The dependence of the fluorescence intensities F-OPA (A) and absorbance S-OPA (B) of final reaction product on the concentrations of Arg in reaction mixture. In sets: the correspondent calibration graphs for Arg assay.

Table 2
Characteristics of the current ADI-OPA-based and the reference enzymatic methods of Arg assay.

Method	Enzyme	Linearity range, μM	Detection limit, μM	References
OPA-F λ_{ex} = 364 nm, λ_{em} = 415 nm	ADI	0.35–24	0.25	Current paper
OPA-S λ = 340 nm	ADI	0.7–50	0.55	Current paper
DMO-S λ = 480 nm	Arginase	7–100	5	Stasyuk et al. (2012)
DMO-F λ_{ex} = 380 nm, λ_{em} = 510 nm	Arginase	0.06–200	0.045	Stasyuk et al. (2013)
OPA-S λ = 362 nm	Arginase–urease	0.9–60.0	0.85	Stasyuk et al. (2015)
OPA-F λ_{ex} = 364 nm, λ_{em} = 425 nm	Arginase–urease	0.09–6.0	0.08	Unpublished data
Nzytech kit λ = 340 nm	Arginase–urease–glutamate dehydrogenase	2.9–100	2.1	https://www. nzytech products
Multi-enzymatic pyrophosphate detection, λ = 555 nm	ADI, [a]ASS, [b]PPDK, [c]POX, [d]HRP	Till 100	–	Kameya and Asano (2014)

[a] ASS – argininosuccinate synthetase.
[b] PPDK – pyruvate phosphate dikinase.
[c] POX – pyruvate oxidase.
[d] HRP – horseradish peroxidase.

The analytical characteristics of ADI-OPA-based methods for Arg assay, obtained from Fig. 2, are summarized in Table 2 in relation to the analytical results obtained by commercial spectrophotometric enzymatic Nzytech kit, multi-enzymatic method of pyrophosphate detection and the developed earlier by us arginase-based methods.

The high selectivity is the necessary demand for each analytic method. To study the selectivity of the developed ADI-OPA-based approach, fluorescence and spectrophotometric signals on various 0.5 mM L-amino acids were tested (Fig. 3).

The analytical signal on Arg was taken as 100% and Asn, Lys and Gln gave low outputs (less than 9%). The absence of signals for other tested amino acids (ornithine, canavanine, proline, citrulline, tryptophane, glutamic acid) approved the high selectivity of ADI to Arg and the high purity of the enzyme preparation.

Thus, the developed ADI-based methods (OPA-S and OPA-F) for the quantitative Arg analysis are satisfactory sensitive and highly selective (see Table 2 and Fig. 3). The next advantages of the method, based on the usage of only one enzyme – ADI, are the simplicity of assay' procedure and low cost of the reagents. The

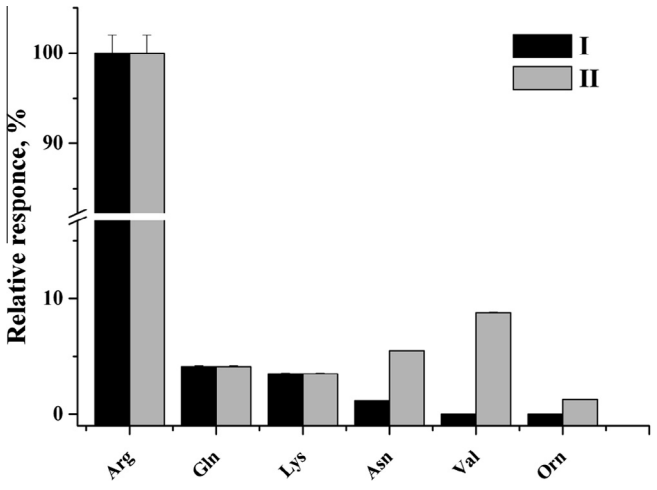

Fig. 3. Dependence of the absorption (I) and fluorescence intensity (II) of the products for various 0.5 mM amino acids.

reaction between ammonia and OPA can also proceed at room temperature, though it needs longer incubation time, more than 3 h (data not shown). This feature of the suggested ADI-based methods is promising for the development of portable test system with automatic variant of assay as well as for screening study or semi-quantitative appreciation of Arg level. It is worth mentioning, that in case of long-time incubation, the reaction mixture in microtiter plates should be closed carefully to avoid contacts with air, that contains the trace quantities of gaseous ammonia. Thus, the proposed analytical ADI-based methods being sensitive, low-cost, highly selective and suitable for both routine and micro-volume formats, will be promising in laboratories of food industry as well as in clinical diagnostics for Arg assay in blood.

3.2. Assay of Arg in real samples

Wines and juices usually contain ammonia and Arg (Austin & Butzke, 2000; Mira De Orduña et al., 2000; Ough et al., 1989; Wu et al., 2014). The developed ADI-OPA method was tested on real samples of wines and juices. To estimate the content of Arg, it was converted by ADI to ammonium ions (see Fig. 1). As a result of enzymatic reaction, 1 mol of Arg generates 1 mol of ammonia, so the content of endogenous Arg can be calculated as the difference of the concentrations of ammonia in the tested samples: total (after enzymatic hydrolysis) and endogenous (without ADI addition). To evaluate the impact of the wine's components on the results of the assay, a standard addition test (SAT) was applied. SAT is a type of quantitative analysis approach often used in analytical chemistry, whereby the standard is added directly to the

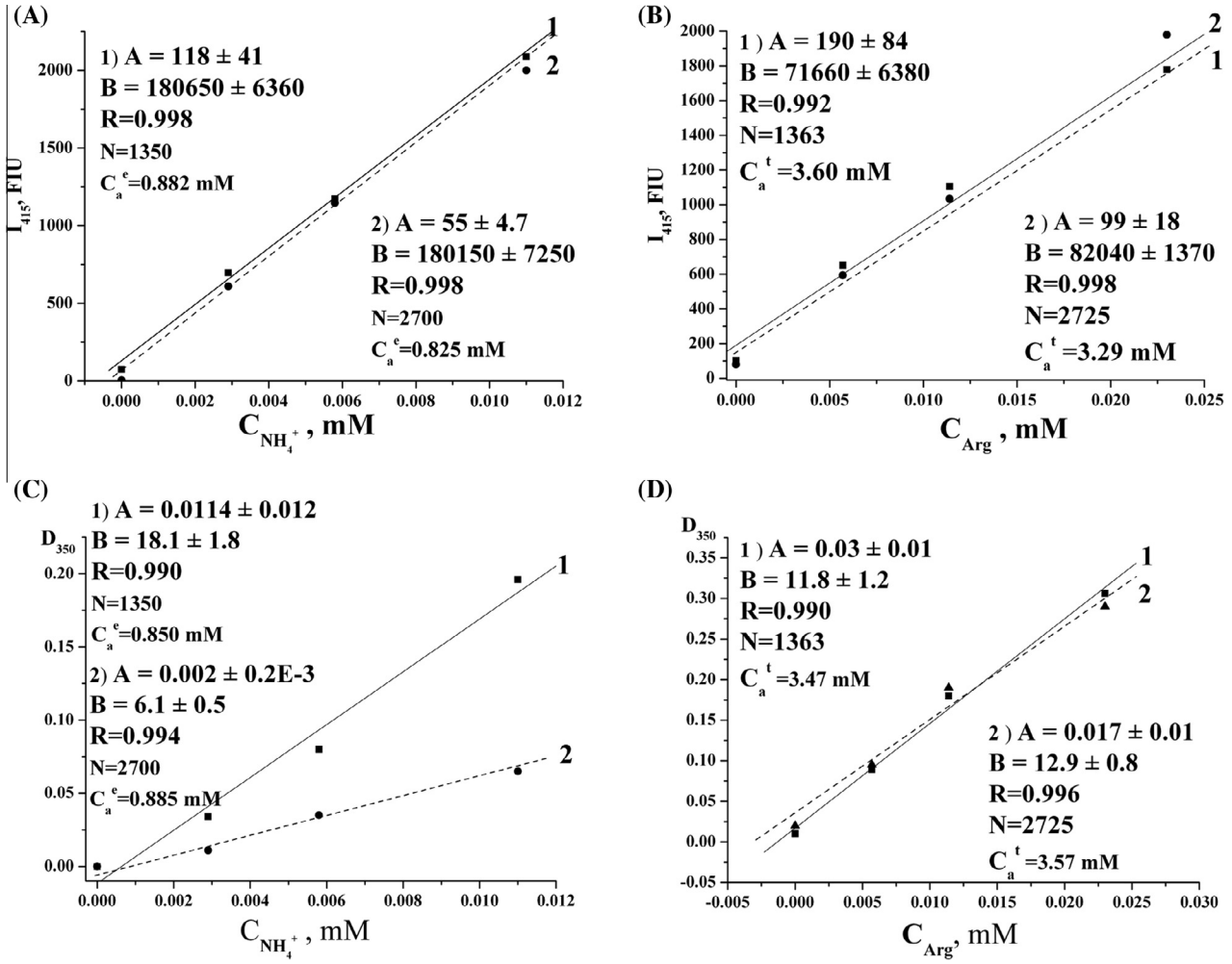

Fig. 4. Assay of total and endogenous ammonia by OPA-F (A and B) and OPA-S (C and D) graphical methods for two dilutions of the juice "Sadochok" (multifruit). $C^{+}_{NH_4}$ and C_{Arg} – concentrations of added standards to the samples – ammonium ions and Arg, respectively; C^{t}_{a} and C^{e}_{a} – calculated concentrations of ammonium ions, endogenous (A and C) and total (B and D), in the tested sample.

Table 3
Concentration of Arg (C_{Arg}) in food samples, determined by different enzymatic methods, mM.

Method sample	ADI-based (current paper)				Arginase-based (Stasyuk et al., 2013)			
	OPA-F		OPA-S		DMO-F		DMO-S	
	C_{Arg}	CV, %	C_{Arg}	CV, %	C_{Arg}	CV, %	C_{Arg}	CV, %
Wine "Moution Cadet"	2.05 ± 0.04	1.90	1.85 ± 0.040	2.2	1.97 ± 0.01	1.01	1.96 ± 0.05	2.01
Wine "Bastardo"	2.50 ± 0.04	1.80	ND	–	2.55 ± 0.04	1.20	2.36 ± 0.06	1.70
Juice "Sadochok" grape and apple	2.16 ± 0.01	0.50	2.20 ± 0.03	1.40	2.06 ± 0.04	2.43	1.99 ± 0.03	2.01
Juice "Sadochok" multifruit	*2.59 ± 0.03	1.20	*2.65 ± 0.03	1.10	2.50 ± 0.04	1.70	ND	–

ND – not determined; CV – coefficient of variation. Values are expressed as mean ± SD. * – these results were obtained from graphs, demonstrated in Fig. 4.

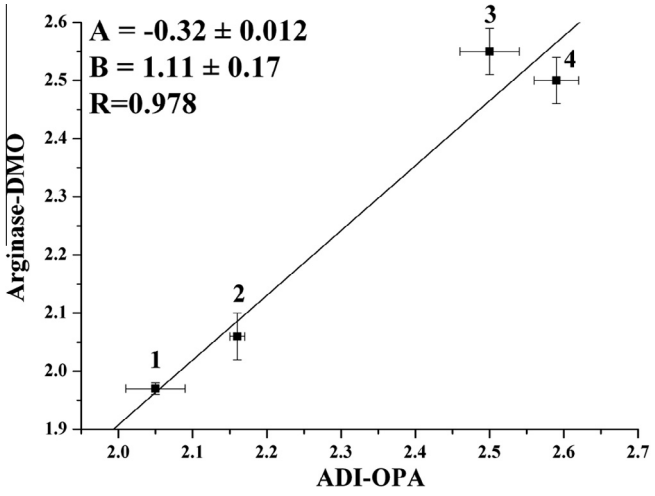

Fig. 5. Correlation between the results of Arg estimation by two enzymatic fluorometric methods: ADI-OPA and Arginase-DMO. Tested samples: Wine "Moution Cadet" (1), Juice "Sadochok" grape and apple (2), Wine "Bastardo" (3), Juice "Sadochok" multifruit (4).

aliquots of analyzed sample. SAT is used in situations where sample components also contribute to the analytical signal, thus making it impossible to compare the analytical signal (Harris, 2003).

The procedure of Arg assay by graphical method was demonstrated in detail (see Fig. 4 and description in Section 2.3.5) for the juice "Sadochok", multifruit.

All real samples were tested by the proposed methods in the same manner. Contents of Arg (mM), estimated by OPA-S method in wines "Souvignon Blanc", "Syrah Rose", Chardonnay and "Tokan", are 3.54 ± 0.02, 1.88 ± 0.40, 3.2 ± 0.05 and 2.18 ± 0.03, respectively. The other tested samples of wines and juices were analyzed using ADI-OPA-based methods in comparison with the arginase-based ones as the reference method (Table 3).

The reproducibility of the proposed analytical methods is satisfactory: the coefficients of variation are less than 2.5%. It is worthwhile mentioning that obtained Arg contents are in range published by another authors (0–9.5 mM) (Li, Liang, Feng, Liu, & Wang, 2008; Stines et al., 2000). The values of Arg contents in the tested samples, obtained by two enzymatic approaches – ADI-OPA-F and Arginase-DMO-F, demonstrated strong correlation: $R = 0.978$ (Fig. 5). Thus, the developed ADI-OPA enzymatic methods can be used for a monitoring of Arg in the samples of wines and juices.

4. Conclusions

A quantitative enzymatic assay for Arg determination based on the usage of recombinant bacterial arginine deiminase (ADI) and *o*-phthalaldehyde (OPA) in the presence of sulfite was developed and characterized. The final product of enzymatic reaction, the ammonium ion, in the presence of sulfite, forms a fluorescent product with OPA, that can be detected with spectrophotometric and fluorometric modes. An effectiveness of the ADI-based analytical method was proved on the real samples of wines and juices. A high correlation ($R = 0.978$) was shown to be between the results obtained with the proposed method and the reference Arginase-based approach. The advantage of the novel enzymatic ADI-OPA method is the possibility of simultaneous assay of Arg and ammonia, although the most attractive characteristic of this method is a simplicity of analytic procedure and economic effect due to the usage of only one enzyme, ADI.

Thus, the proposed low-cost ADI-OPA method of Arg assay being very selective and sensitive, fast, valid and simple can be promising for food quality control in food industry to predict a potential risk of EC generation in wines.

Ethical statement

The authors declare that there is no conflict of interests regarding the publication of this paper.

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