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Quantitative analysis of total selenium in Se-enriched yeast products with ICP-MS using home-made closed digestion system for sample preparation

Witphon Thosaikham[1], Rossukon Sittipout[2], Anut Chantiratikul[3], Piyanete Chantiratikul[2]

[1]Department of Chemistry, Faculty of Science and Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon, 47000 Thailand

[2]Department of Chemistry and Center of Excellence for Innovation in Chemistry (PERCH-CIC), Faculty of Science, Mahasarakham University, Kantarawichai, Maha Sarakham, 44150 Thailand

[3]Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Kantarawichai, Maha Sarakham, 44150, Thailand

*Corresponding Author: piyanete.c@msu.ac.th

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Abstract

Sample digestion is an important procedure and directly affects on the reliability of quantitative analysis of total selenium in Se-enriched yeast products. The home-digestion closed digestion system was successfully developed by specially designing and constructing from the basic equipments in laboratory that consisted of 4 main devices; water bath, cover plate, vessels and wind tunnel. The optimum conditions of the home-made closed digestion system was accurately and precisely achieved by using 2 mL of nitric acid for 0.10 g Se-enriched yeast sample, 100 °C digestion temperature and 20 min digestion time. These conditions showed good validation and acceptation with the certified value of Se-enriched yeast reference material. Furthermore, the digestion efficiency of home-made closed digestion system was as well as the recognized digestion methods especially microwave assisted digestion. Consequently, the home-made closed digestion system could be efficiently utilized as an alternative digestion method for total Se determination in Se-enriched yeast products. Additionally, the home-made closed digestion system was adapted from a basic instrument in laboratory which has affordability and easier operation rather than the recognized methods.

Keywords: Selenium; closed-digestion system; Se-enriched yeast; water bath; wet acid digestion

1. Introduction

Selenium (Se) is a trace element and well known as an essential nutrient for human health [1, 2]. Deficiency of Se is the cause of Keshan and Kashin-Beck in China, which are identified as a disorder of the heart muscle and bone, respectively [3, 4]. Moreover, the problem of Se deficiency in food has been found in several parts of the world such as the North East of China, New Zealand and some parts of Europe [5]. Therefore, the addition of inorganic selenium salt such as selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) into food cultivation has been increasingly researched for providing Se-enriched food to improve the problem of Se-insufficiency in daily food [6 – 9].

Currently, Se-enriched yeast is mostly recognized as the most successful commercial Se-enriched food product, which has high concentration of organic Se species, especially selenomethionine (SeM) [10 – 12]. Consumption of Se-enriched yeast has been recognized as a beneficial and safe form of Se

for health [13, 14]. The manufacture of Se-enriched yeast has usually utilized sodium selenite as an inorganic Se source for addition into the medium of yeast fermentation [15]. Selenite is highly absorbed, accumulated and transformed to be organic Se species through the metabolism of yeast cells [16].

Even though, Se-enriched yeast has been successfully accepted as a common organic Se for the consumer, the pathway of Se-enriched production requires a powerful methodology to analyse Se contents in Se-enriched yeast product for development of cultivation process and quality control including the benefit and toxicity assay of the product. Generally, the quantity of Se in Se-enriched yeast mainly focuses on development of an analytical method for Se speciation analysis [17 – 20]. Meanwhile, the method for total Se determination in Se-enriched yeast has been less reported even though it is a primary procedure for evaluation of total Se accumulation in yeast cells. Accordingly, the analytical procedure for total Se determination should be absolutely developed to join with the Se speciation analysis.

Determination of total Se is usually performed by using a high efficiency analytical instrument such as HG-AAS, ICP-MS, ICP-OES and fluorometric technique [21 – 25]. Nevertheless, these analytical instruments require a high efficiency sample preparation method for preparation of sample material as a homogeneous solution. Therefore, the sample preparation method is the primary procedure of total Se determination that effects to the accuracy and precision of the final result.

Generally, an opened-vessel digestion method is a favourite sample preparation method for Se-enriched sample material by refluxing the sample with concentrated acid such as HNO_3 and $HClO_4$ [26]. Furthermore, H_2O_2 is an oxidizing agent that also added to increase the efficiency of digestion, and decolorize the digested sample to be a clear solution [27]. The opened-vessel digestion method could efficiently decompose Se-enriched yeast sample. However, this method uses large amount of acid and extended time to completely reflux Se-enriched yeast cells. In addition, digestion under an atmospheric system could be contaminated by environment [28].

Nowadays, closed-vessel digestion based on microwave-assisted digestion is recognized as the most powerful technique that could rapidly digest Se-enriched yeast material by reacting with concentrated acid under high pressure of a closed-vessel. Moreover, it could save the analyst from volatilization with high temperature of digestion system [29, 30]. However, this method is quite expensive and requires enough safety system.

Water bath is a general and basic heating apparatus in laboratory that has a large size, lower cost and an excellent temperature control mechanism. It has the possibility of being developed as a new digestion apparatus. Hence, this research expressed an interest in the modification of the water bath by designing and constructing it as a powerful and economics digestion apparatus. Interest also was expressed in applying the water bath digestion apparatus for preparing Se-enriched yeast prior to determination total Se by ICP-MS.

2. Materials and methods

Equipment and reagents

Se-enriched yeast reference material (Selplex®, ES-1149, certified Se content = 2125 ± 65 mg kg^{-1}) using for optimization and validation of the proposed digestion method was obtained from Alltech Biotechnology Center (KY, USA). The four Se-enriched yeast samples (SeY1 to SeY4) provided by Vet Superior Consultant Co. Ltd, Thailand were utilized for application of the proposed digestion method. Sodium selenite and Se-methyl selenocysteine were obtained from Fluka (Germany). Selenomethionine was purchased from Acros Organics (Belgium). Selenium standard solution (AAS grade) and nitric acid were obtained from Carlo Erba (Italy). Deionized water (Milli-Q Millipore 18.20 MΩ-cm resistivity) was used for preparation of all solutions.

An ICP-MS (Elan DRC-e Perkin – Elmer SCIEX, Norwalk, USA) fitted with a cross-flow nebulizer and double-pass Scott spray chamber was utilized for determination of Se. The instrumental conditions for Se detection were as follows: radio frequency (RF) forward power: 1120 W; plasma flow-rate: 15 l min^{-1}; auxiliary flow-rate: 0.80 l min^{-1}; nebulizer flow-rate: 0.85 l min^{-1}; and Ni cones. The selected isotope of Se for mass monitoring by ICP-MS was ^{82}Se to avoid interferences by polyatomic of argon [29].

A water bath (TW 12, Julabo, Germany) was used for modification as a new digestion apparatus. A microwave digestion apparatus (CEM Mars 5 XR 1500, USA) was equipped with Teflon PFA digestion vessels. A muffle furnace (CWF 1200, Carbolite, UK) was employed for the dry ashing method. A hot plate (Fisher Scientific, USA) was adapted for open-vessel digestion by equipping it with a home-made heating block for wearing glass digestion vessels.

The screw cap test tubes (15 cm length × 2.50 cm vertical) used as digestion vessels of the proposed digestion apparatus were Pyrex (UK). Black Viton® septum for sealing the inner side of the cap of the digestion vessel was purchased from Supelco (USA). All glasswears and plasticwears were soaked in 5% v v^{-1} nitric acid over night, rinsed with deionized water and dried prior to use.

Design and construction of the home-made digestion apparatus

The home-made closed digestion system (HMD) was specially designed and constructed as a closed-digestion system and shown in Fig. 1 [31]. It consisted of 4 main components; (A) heating source, (B) cover plate, (C) sample and chemical container, (D) and wind tunnel. A water bath (A-1) was adapted as a heating source for the reaction of the sample decomposition with acid. However, it produces a lot of water stream during water (A-2) is boiled at high temperature. Thus, we designed and built a special cover plate from a 20 mm thickness of polyacrylic plate (B-1). The cover plate was equipped on the water bath for preventing the vaporization of water in the water bath. Moreover, it had twenty holders (B-2) for wearing the vessels. The edge of cover plate was fixed with an elastic gasket (B-3) for sealing the joint between the cover plate and water bath.

The screw cap-glass test tube (C-1) was used as a digestion vessel (C-2). The inner of its cap (C-3) was equipped with Viton® septum (C-4) for sealing and protecting the leak of vapor phase. An elastic O-ring (C-5) was utilized to seal the joint of the side of digestion vessel and the holder of cover plate. It was worn to the center of digestion vessel prior to put the digestion vessel into the vessel-holder of the cover plate.

The wind box was applied to cool down the top part of the vessels for condensing and recovering the vapor phase in the vessel. It was put on the cover plate and functioned by using a fan (D-1) to blow the wind through the vessel and ventilator (D-2).

Optimization of digestion parameter of HMD apparatus for digesting Se-enriched yeast

There are two digestion parameters of HMD apparatus; volume of nitric acid and digestion time were optimized for digestion of Se-enriched yeast to be a clear and homogeneous solution.

The volume of HNO_3 was firstly optimized by varying in the range of 1 to 3 mL. The 0.10 g of Se-enriched yeast reference material (ES-1149) was weighed into the digestion vessel (n=4). Concentrated HNO_3 was added and the vessel was closed with its cap. The vessel was worn with an elastic O-ring and put into the holder of cover plate. Then, the digestion procedure was set as follow Fig. 1. The mixture was reacted at 100 °C which was the maximum temperature of the water bath and enough to decompose matrices of the sample [27, 32]. The digestion time for the experiment was controlled at 20 min. After the sample was completely digested, the vessels were removed and stood to cool down at the room temperature for 15 min. The digestion vessels were then refrigerated at 4 °C for 30 min, which the vapor phase in the digestion vessel would be condensed and recovered as liquid

phase. The digested solution was made up to volume with deionized water in 25 ml volumetric flask and stored in polyethylene (PE) bottle prior to Se content determination with ICP-MS technique.

The second parameter was digestion time, ranging from 10 to 40 min under the optimum HNO_3 volume. Finally, the optimum condition of HMD apparatus would be decided, validated and applied to digest the real Se-enriched samples.

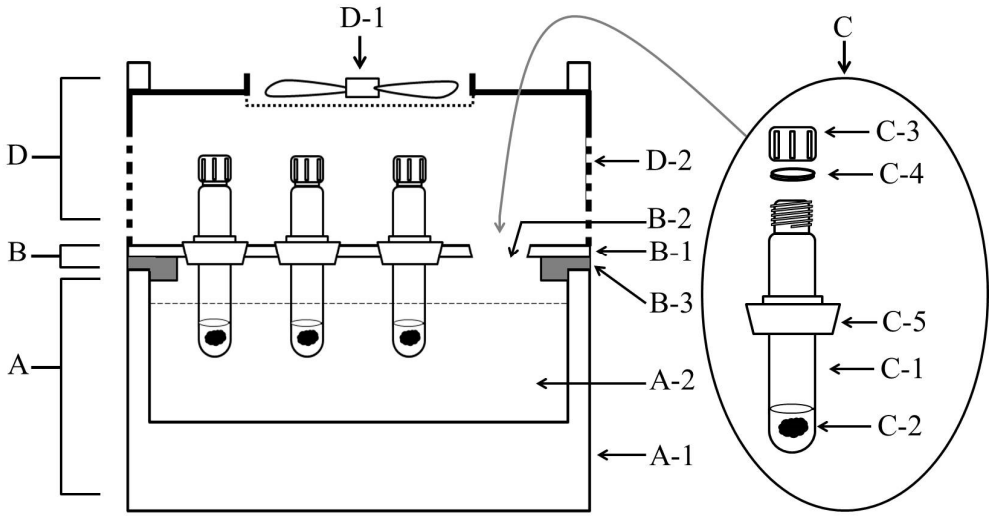

Fig. 1 Schematic of 4 main devices of the HD apparatus; heating source (A) consisted of water bath (A-1) and water (A-2); cover plate (B) consisted of thick acrylic plastic (B-1), vessel holder (B-2) and elastic gasket (B-3); sample and chemical container (C) consisted of digestion vessel (C-1), sample and chemical reagent (C-2), vessel-cap (C-3), Viton® septum (C-4) and elastic o-ring (C-5); and wind tunnel (D) consisted of fan (D-1) and ventilator (D-2).

Digestion procedures of the recognized methods

Three recognized methods were utilized for comparing with the HMD method; Microwave-assisted digestion (MWD), Opened-vessel acid digestion (OVD) and Dry ashing method (DAM).

The procedure microwave-assisted digestion method (MWD), Se-enriched yeast (0.10 g) was accurately weighted and put into the PTFE digestion vessels (n = 4), added 5 mL of concentrated HNO_3. Then, the digestion vessels were closed and placed in microwave digestion instrument. The digestion conditions were as follows: 10 min at 20 psi and 40% power, 10 min at 40 psi and 40% power, 15 min at 85 psi and 60% power, and 30 min at 120 psi and 70% power [33]. Afterward, the digestion vessels were cooled down to room temperature. The digest in the vessels were replaced, made up to volume with deionized water in 25 mL volumetric flask, and stored in PE bottle at 5 ºC before determination of total Se concentration with ICP-MS.

The demonstrated opened-vessel digestion method (OVD) by Rodushkin et al. 1999 [34] was adapted by weighing 0.10 g of Se-enriched yeast into the glasses vessel (n=4), adding 10 mL of concentrated nitric acid, placing the vessel in to the hole of heating bock which was equipped on hot plate, then the mixture was refluxed at 125 °C for 120 min. The vessel was cooled down to room temperature, brought up to volume 25 mL with deionized water, replaced into polyethylene bottle and stored at 5 °C prior to determining the concentration of Se by ICP-MS.

The dry ashing method (DAM) used was adapted from Hseu, 2004 [35]. This procedure was performed by placing 0.10 g of Se-enriched yeast into a crucible (n = 4). Then, the crucible was

covered with its cap and placed in the muffle furnace. The temperature was programmed by initially setting at lower than 100 °C, increasing the temperature to be 550 °C for heating the sample for 8 h after that the muffle furnace was cooled down to 50 °C. The ash of sample was dissolved with 5 mL of concentrated HNO_3 and adjusted to volume with deionized water in 25 mL volumetric flask, stored in PE bottle at 5 °C before total concentration of Se determination by ICP-MS.

Validation of the method

Validation of the proposed method was reported as precision and accuracy. The precision of the proposed method was presented as the repeatability and reproducibility in terms of the relative standard deviation (RSD) of the concentrations of Se. The repeatability (intra-day precision) was calculated from 5 replicates ($n = 5$) and reproducibility (inter-day precision) was calculated from three replicates a day for three consecutive days ($n = 3 \times 3$).

The accuracy test is an important analytical characteristic for guaranteeing the efficiency and practicality of HMD for digesting Se-enriched sample, when also compared with the recognized methods. Evaluation of method accuracy was defined as percentage error and percentage recovery.

The percentage error of HMD and the recognized methods were calculated with equation (1):

$$\%error = \left(\frac{|Se_{exp} - Se_{cer}|}{Se_{cer}} \right) \times 100 \quad (1)$$

Where Se_{exp} was total Se concentration in the Se-enriched yeast reference material form experiment and Se_{cer} was the certified value of Se concentration in the Se-enriched yeast reference material.

The recovery test of total Se for HMD and the recognized methods was performed by spiking different kind Se standard reagents; selenite (SeVI), selenomethionine (SeM) and Se-methylselenocysteine (SeMC). The three Se standard reagents were prepared as stock standard solution by dissolving solid reagent into deionized water. All Se standard solutions were standardized with Se standard of AAS grade. The recovery test was achieved by spiking 2 µg Se of each Se standard solutions into non Se-supplemented yeast sample prior to preparation with the digestion methods. The spiked and non-spiked digests were determined total Se concentration by ICP-MS. The percentage recovery calculation used was calculated by using equation (2):

$$\%Recovery = \left(\frac{(Se_{spiked} - Se_{unspiked})}{Se_{expected}} \right) \times 100 \quad (2)$$

Where Se_{spiked} was the total Se concentration of the spiked solution, $Se_{unspiked}$ was total Se concentration of the unspiked solution, and $Se_{expected}$ was the expected total Se concentration of the matrix solution.

The comparisons of significant differences between total Se concentrations in Se-enriched samples and percentage recovery of each Se-species from different digestion methods were tested by one-way analysis for variance (one-way ANOVA). It was performed by using the statistical program for Social Science (SPSS, Version 16.0 for windows Chicago, IL, USA).

3. Results and Discussion

Optimization of HD apparatus for digestion of Se-enriched yeast

The volume of HNO_3 optimization for digesting about 0.10 g Se-enriched yeast was presented in Table 1. The observation found that the concentration of Se in the Se-enriched yeast reference material (ES-1149) was not significantly different when the volume of HNO_3 was increased from 1 to 3 mL (p<0.01). Furthermore, the Se concentrations and percentage error of all experiments agreed with the certified value of reference material (<10% is considered to be acceptable). However, the homogeneity of the digested solutions was clearer when the volume of nitric acid was increased. This work demonstrated that digestion of 0.10 g Se-enriched yeast with 2 mL HNO_3 was enough to completely decompose as a homogenous solution at 100 °C.

The digestion time was the last condition of HMD apparatus that was optimized for Se-enriched yeast digestion. As can be seen in Table 2, the concentration of Se was not significantly different (p > 0.01) when the digestion time was increased. Furthermore, the percentage errors of each digestion time were accepted with the certified value of the Se-enriched yeast reference material (ES-1149). Although, the result indicated that increasing digestion time did not affect on the concentration of total Se in Se-enriched yeast sample, however the homogeneity of the digested solutions was clearer by using digestion time above 10 min. This work demonstrated that the optimum digestion time for Se-enriched yeast was 20 min which was enough to completely digest the Se-enriched yeast sample as clear solution.

Hence, the digestion of Se-enriched yeast sample as homogeneous solution requires enough volume of nitric acid, digestion temperature and digestion time for completely decomposing the matrices of Se-enriched yeast especially polysaccharide portions [33, 36]. In this work, the HMD was specially designed as closed-vessel digestion method that can homogenously and rapidly digest Se-enriched yeast with small volume of nitric acid rather than the recognized methods.

Table 1 The effect of volume of nitric acid on Se concentration in Se-enriched yeast

Volume of HNO_3 (mL)	Se concentration (mg kg^{-1}) (n = 4)	% Error
1.0	2090 ± 94	4.57
1.5	2121 ± 20	0.05
2.0	2237 ± 59	2.15
3.0	2217 ± 23	1.23

Table 2 The effect of digestion time on Se concentration in Se-enriched yeast

Digestion time (min)	Se concentration (mg kg^{-1}) (n = 4)	% Error
10	2205 ± 187	3.80
20	2286 ± 22	7.60
30	2279 ± 95	7.30
40	2220 ± 81	4.50

Validation of HMD apparatus

Precision of the proposed digestion apparatus for digestion of Se-enriched yeast in terms of repeatability (intra-day precision) and the reproducibility (inter-day precision) were expressed in term of relative standard deviation (RSD). The result found that the repeatability and reproducibility of the proposed digestion apparatus were 4.31% and 7.97%, respectively. Accuracy of the proposed digestion method in terms of percentage error was found to be less than 5% (Table 3). Hence, the total Se concentration in Se-enriched yeast by prepared by the HMD method was agreed with the certified value. Furthermore, the percentage error of the HMD method was very close to the MWD method but greater than that of OVD and DAM methods.

The recovery tests of total Se by spiking different kinds of Se standard reagents were a highlight of this work for testing accuracy of HMD and the recognized digestion methods. These Se standard reagents obtained in this work are indentified as important inorganic and organic Se compounds in Se-enriched yeast; Se (IV), SeM and SeMC. They were spiked into the non Se-supplemented yeast for testing the loss of them in term of total Se during they were already digested with the yeast sample by utilizing each digestion method.

The result showed that the recoveries of total Se in all Se standard reagents from HMD apparatus were approached one hundred percent and were not significantly different ($p > 0.01$) with MWD and OVD methods (Table 3). It indicated that either inorganic or organic Se standard regents did not volatilize with closed or opened-vessel wet digestion method. For the discussion of this observation, although Se in each Se compounds is presented different oxidation state; +4 presents in Se (IV) and -2 presents in SeM and SeMC [36]. However, all oxidation forms of selenium are completely oxidized by HNO_3 to be selenate (Se (VI)) which is the most stable forms of selenium in oxidative condition [27].

Therefore, the decomposition of Se-enriched yeast under pressured conditions by the closed-vessel system of HMD and MWD methods were faster than the digestion in atmospheric condition of OVD method [28]. Furthermore, the problem of contamination from the environment for Se-enriched yeast preparation could be also protected by a closed-vessel digestion system. For the sample preparation with DAM method, it showed the lowest recoveries of all Se species because these Se species could be volatilized and destroyed by combustion at high temperature and long time [32]. Consequently, the HMD apparatus could be utilized to prepare Se-enriched yeast as same as the recognized digestion methods, especially MWD and OVD methods.

Table 3 Se concentrations in Se-enriched yeast reference material and accuracy of HMD and the recognized digestion methods

Digestion methods	Se Conc. in reference material (mg kg^{-1}) (n = 4)	% Error	Percentage recoveries (%) (n=4)		
			SeIV	SeM	SeMC
HMD	2213 ± 46^{b}	4.20	96.90 ± 7.60^{b}	98.2 ± 3.10^{b}	101.10 ± 5^{b}
MWD	2208 ± 131^{b}	3.90	92.60 ± 10.20^{b}	99.9 ± 5.60^{b}	108.40 ± 10.10^{b}
OVD	2503 ± 51^{b}	17.80	104.90 ± 3.10^{b}	99.6 ± 4^{b}	104.20 ± 4.10^{b}
DAM	69 ± 14^{a}	96.70	0.63 ± 0.06^{a}	0.52 ± 0.05^{a}	1.20 ± 0.80^{c}
Certified value	2125 ± 65	-	-	-	-

* ^{a,b,c} The Se concentration is significantly difference if columns ($p < 0.01$)

Application of HD apparatus

The practicality of the HMD method was performed by applying to digest the real Se-enriched yeast samples and compared with the recognized methods. The result found that Se concentration in the Se-enriched yeast samples by digesting with HMD method were not significantly different with MWD and OVD methods ($p < 0.01$) but better than the dry ashing method ($p < 0.01$) (Table 4). It indicated that the HMD method could provide acceptable total Se concentration in all Se-enriched yeast samples when compared with MWD and OVD methods. The HMD method is an alternative, good validation and practical sample preparation for Se-enriched yeast. Moreover, the concentrations of total Se in each Se-enriched yeast samples were different. The highest and lowest total Se content were obtained in sample SY-3 and SY-4, respectively.

Even though, the Se-enriched yeast samples presented high total Se concentration but it does not indicate that the organic Se contents in the product is also related with the total Se concentration. However, the quantitative analysis of total Se could be used as a primary data for monitoring the Se-enriched yeast process, quality control of the product and the nutrition data. Therefore, the result of total Se concentration should be corroborated with Se speciation data.

Table 4 Comparison of Se concentration in Se-enriched yeast samples by using HMD apparatus and the recognized methods

Se-enriched yeast samples	Se concentration (mg kg^{-1}) (n=4)			
	HMD	MWD	OVD	DAM
SeY1	1474 ± 115^{b}	1445 ± 100^{b}	1338 ± 102^{b}	376 ± 84^{a}
SeY2	1922 ± 84^{b}	1802 ± 124^{b}	1733 ± 91^{b}	58 ± 4^{a}
SeY3	2309 ± 170^{b}	2254 ± 95^{b}	2242 ± 146^{b}	24 ± 0.10^{a}
SeY4	632 ± 14^{b}	655 ± 10^{b}	601 ± 39^{b}	53 ± 5^{a}

* ^{a,b,c} The Se concentration is significantly difference in rows (p<0.01)

4. Conclusion

The total quantitative analysis of Se-enriched yeast by ICP-MS was carried out by preparing with the home-made closed digestion system. The development of home-made closed digestion system was successfully achieved by designing and constructing from the basic equipment in laboratory. It consisted of 4 main devices; water bath, cover plate, digestion vessels and wind box. The home-made closed digestion system could homogenously digest Se-enriched yeast with good validation and approval when compared with the recognized wet acid digestion methods especially microwave assisted digestion. Consequently, it is a high efficiency and economics digestion apparatus which was developed from a very basic heating instrument in laboratory. Moreover, it could be alternatively used as a part of quality control and nutritional recommendation of Se-enriched yeast products. However, the design, material used and application range of this digestion apparatus could be further developed as a powerful digestion apparatus.

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