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RESEARCH

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GC–MS of essential oil, metal profile and physicochemical properties of fruits of *Citrus macrophylla* Wester from Sudan

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Abstract

Background: The study investigated the essential oil composition, metal profile and physiochemical properties of *Citrus macrophylla* Wester fruits collected from Agricultural Research Corporation, Shambat area, Khartoum North, Sudan. The species was introduced by one of the authors Dr. Moawia E. Mohamed from the National Repository of *Citrus* and Dates of Riverside, California, USA. This species is known as universal rootstock.

Results: The graphite furnace–atomic absorption spectrometry determined the presence of sodium (6.38 ppm), potassium (701.93 ppm), calcium (371.28 ppm), magnesium (54.78 ppm), cadmium (0.013 ppm), zinc (0.256 ppm) and lead (0.200 ppm). The content of peel, moisture and ash of *C. macrophylla* was measured as 18.35%, 23.75% and 2.04%, respectively. Among thirty compounds identified using gas chromatography–mass spectrometry, limonene is the major compound (72.85%). The vitamin C (ascorbic acid) content of *C. macrophylla* juice was determined as 55.0 mg/100 g. The fruit diameter, fruit weight and percentages of juice, peel and seed were determined as 91.4 mm, 276.5 g, 47.92%, 26.37% and 19.20%, respectively. The pH value, total soluble solids (TSS), titratable acidity (TA) and ripening index (RI) of *C. macrophylla* are found to be 3.50, 8.00%, 7.44% and 10.81, respectively.

Conclusion: The results in this study suggested that *C. macrophylla* could be beneficial in food as healthy juice.

Keywords: Essential oils, GC–MS, GF-AAS, *Citrus macrophylla*, Rutaceae

Background

The genus *Citrus* L. is belonging to the family Rutaceae. This family comprises 140 genera and 1300 species (Anwar et al. 2008). The genus, *Citrus*, has a variety of limes, lemons, oranges, grape fruits and mandarins. The *Citrus* species is widely cultivated as a popular fruit worldwide (Abbasi et al. 2009) and indigenous to the tropical and subtropical regions of Asia as well as Malaysian Islands (Singh and Singh 2002). As the world's most widely grown fruit crop, *Citrus* fruits made mainly for their juice, one of their most important products, and their essential oils.

The essential oils of *Citrus* are mainly utilized as flavoring by a variety of food industries (Dharmawan et al. 2008). The essential oils of *Citrus* fruits are widely used in the perfume and fragrance industry, food industries such as sweet and juice flavorings. In addition, they are used as components of pharmaceuticals, antiseptics and aromatherapy products. *Citrus* oils are utilized in beauty products, deodorants, soaps and detergents (El-Adawy et al. 1999; Silalahi 2002; Saïdani et al. 2004). The recent study analyzed the oil of *Citrus sinensis* L. using GC–MS showing that ninety-six compounds identified from eight plant species and representing 99% of the total oil. Limonene was the major component in all samples (59.53–95.34%) (Zouaghi et al. 2019). Normally the *Citrus* fruits accumulate abundant of very important bioactive compounds that may affect human health. These bioactive compounds include essential oils, vitamin C (ascorbic

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acid), terpenes, flavonoids, alkaloids, fibers and minerals (Hamdan et al. 2011). Therefore, the efficacy of medicinal plants for curative purpose is due to the presence of bioactive components. But it is noticed that over-dose or prolonged injection of medicinal plants leads to accumulation of different elements which in turns cause various health problems (Sharma et al., 2009). The *Citrus* peel, often treated as agricultural pollutant, can be a potent source of plant metabolites and essential oils (Mohammed et al. 2013).

In Sudan, *Citrus* fruits are most important tree crops. The Sudanese peoples cultivated *Citrus* species for their edible fruits which include *C. aurantifolia* Swingle (lime), *C. sinensis* L. (sweet orange), *C. aurantium* L. (sour orange), *C. reticulata* Blanco (mandarin) and *C. paradise* Macf (grape fruit). In addition, *C. macrophylla* Webster. (Alemow) and *C. jambhiri* Lush (rough lemon) were introduced and cultivated recently in the field of Agricultural Research Corporation, Shambat area, Khartoum North, Sudan, by one author of the current study (M.E. Mohamed). The major component of essential oil of *Citrus jambhiri* and *Citrus macrophylla* is limonene which was determined previously in high amounts 55.4% and 60.7%, respectively (Robbins et al. 2012; Aguilar-Hernández et al. 2020).

Although macroelements are essential for plants, they may produce toxins that affect human health if taken in high concentrations, while microelements are toxic even in low concentrations. Therefore, the metal content of medicinal plants should be tested in order to control their qualities and nutritional values. Nowadays, numerous studies around the world have highlighted the importance of herbs for herbal medicines that have raised awareness of trace elements in these plants (Gebrelibanos et al. 2016).

In traditional or herbal medicine, using medicinal plants for long periods or in over-dose will accumulate elements that have serious health problems. Therefore, the authentication and quality control of medicinal plants should be investigated in order to link the chemistry of these plants with their medicinal uses. Therefore, researchers run after finding the right dose of supplement in the proper form at the right time (Sharma et al. 2009; Arceusz et al. 2010).

Citrus macrophylla, commonly known as Alemow, is a universal rootstock (Castle et al. 2009). Since this species is known to be a rootstock, most of the researches done on it from horticultural aspects such as cultural practices and physical features. The more recent study on the peel oil of *Citrus macrophylla* using GC–MS technique showed that twenty-six compounds were identified. Limonene was found to be the major component (Aguilar-Hernández et al. 2020).

To the best of our knowledge, the current research is the first study on the fruit of *Citrus macrophylla* as edible and to evaluate its nutritional value. The presence of toxic heavy metals such as Pb has detrimental effect on human health even in low concentration. The permissible limit of Pb in *Citrus* fruit is 0.10 ppm according to FAO/WHO (FAO/WHO 2011). Therefore, elemental analysis of fruit is an important part to investigate, in addition to vitamin C content. Surveying the literature and to the best of our knowledge, none of the macro- or microelement has been determined quantitatively in *Citrus macrophylla* except sodium and potassium in a single study (Levy and Lifshitz 1995). Accordingly, this study will add novel knowledge in evaluation of nutritional value of this species as an edible plant. Therefore, the main objective of this study is to investigate the macroelements (K, Na, Ca and Mg) and microelements (Zn, Co, Ni, Cd, Pb, Cr and Fe) levels in *C. macrophylla*, in addition to the physiochemical properties and the chemistry of peel essential oil.

Methods

Plant material

The fruits of *C. macrophylla* are collected from Agricultural Research Corporation, Shambat area, Khartoum North, Sudan, in May 2016 at 35 °C and humidity of 8% (Fig. 1). The species is introduced by one of the authors Dr. Moawia E. Mohamed from the National Repository of *Citrus* and Dates of Riverside, California, USA. This collection is kept at Shambat Research Station (Mohamed and ElTayeb 2009). The voucher specimen is deposited in Herbarium of Botany Department, Faculty

Fig. 1 Photograph of *Citrus macrophylla* fruit (Photo by First Author)

of Science, University of Khartoum, Sudan, for the purpose of verification.

Physiochemical properties of fruit

The granular size of fresh fruits of *C. macrophylla* is recorded using Vernier Calipers (model: EHB Stainless, Hardened, Germany). In addition, the weight of fruit, juice, peel and seed are measured using a top loading balance (model: D0001-H R 120, A & D Company Ltd EC). Vitamin C content of *C. macrophylla* juice was determined by titrimetry using 2,6-dichlorophenolindophenol (DCPIP) assay (Nielsen 2010).

Extraction of essential oil and fruit juice

The maceration of fresh peel of *C. macrophylla* using *n*-hexane (3 × 2 L) for 48 h at room temperature leads to extraction of essential oil after evaporating *n*-hexane by a rotator evaporator at 30 °C, then followed by evaporation of solvent under reduced pressure (Maimulyanti and Anton 2016). The crude oil was used for GC–MS analysis. The traditional method of juice extraction was carried out to extract fruit juice (Beveridge and Rao 1997).

GC–MS analysis of the essential oil

The GC–MS analysis of the peel essential oil of *C. macrophylla* was carried out using gas chromatography–mass spectrometry instrument at the Department of Medicinal and Aromatic Plants Research, National Research Centre, Egypt, with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC–MS system was equipped with a TG-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 ml/min and a split ratio of 1:10 using the following temperature program: 60 °C for 1 min; rising at 3.0 °C/min to 240 °C and held for 1 min. The injector and detector were held at 240 °C. Diluted samples (1:10 hexane, v/v) of 0.2 μL of the mixtures were always inject. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of *m/z* 40–450. Most of the compounds were identified using the analytical method: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

Metal profile

The determination of metal profile was achieved using Varian Spectra AA 220FS Atomic Absorption Spectrometer (AAS) at the laboratories of Ministry of Mining, Sudan. Exactly 17.01 g of the fresh fruit of *C. macrophylla* in a crucible was heated in an oven at 105 °C for 3 h until constant weight. Then, the moisture content was obtained by weight loss difference (AOAC 1990). For ash content, the dried material was heated in the muffle furnace at 450 °C for 3 h until constant weight obtained and the ash content was calculated. For elemental analysis, an accurate weight of ash was digested using concentrated nitric acid and filtered through Whatman filter paper and the filtrate was completed to a certain volume for chemical analysis using atomic absorption spectrometer (AAS) (AOAC 1990).

Using 2,6-dichlorophenolindophenol (DCPIP) assay, vitamin C (ascorbic acid) content of *C. macrophylla* juice was determined (Nielsen 2010). The total soluble solids (TSS) of fruits were measured with a handheld Refractometer (0–50% Brix) at 20 °C and were expressed as percentage (%) or degree Brix (°Bx) (AOAC 1990).

The pH value was measured with a glass electrode pH-meter (model: HANNA Instrument 8521 Portugal) at ambient temperature. The titratable acidity (TA) was determined on neutralization of 0.1 NaOH to end (pink color) with citric acid. The ripening index (RI) was calculated as ratio of TSS/TA (Ranganna 1986; Legua et al. 2011).

Results

Physiochemical properties

The physiochemical parameters of fruit of *C. macrophylla* were determined, and the results are presented in Table 1. These parameters include the juice, peel and seed percentages, in addition to size, weight, dimension, diameter, moisture, ash content, vitamin

Table 1 Physiochemical properties of *C. macrophylla* and fruit juice ($n = 3$)

Parameter (unit)	Mean	SD	Reference
Fruit length/height (mm)	85.9	0.2000	(76.76)[a]
Fruit width (diameter, mm) (mm)	91.4	0.2750	(79.92)[a]
Fruit thickness (mm)	92.2	0.3750	
Fruit weight (g)	276.5	0.5000	(235.09)[a]
Juice weight	132.5 g (47.92%)	0.2658	(44.24%)[a] (54.3%)[b]
Peel	72.9 g (26.37%)	0.0020	
Seed	53.1 g (19.20%)	0.2020	
Moisture (%)	26.4	0.1230	
Ash (%)	3.08	0.0100	
Vitamin C (mg/100 g)	55.0	0.2500	(47.0)[a]
pH-value	3.50	0.0100	(4.00)[a]
Total soluble solids (TSS) (%)	9.30	0.0800	(10.6)[a,b]
Titratable acidity (TA) (%)	0.74	0.0050	(0.66)[a] (1.23)[b]
Ripeness index (RI)	14.81	0.0780	(16.0)[a]

[a] Legua et al. (2011)

[b] Levy and Lifshitz (1995)

C content, of the fruit. In addition, the average values of pH, TSS, TA and RI of fruit juice were determined experimentally.

The juice, peel and seed percentages were determined as 47.92%, 26.37% and 19.20%, respectively. The dimensions of the fruit of *C. macrophylla* are found to be 85.9 mm, 91.4 mm and 92.2 mm for length, width (diameter) and thickness, respectively. The weight of fruit of *C. macrophylla* was experimentally determined as 276.5 g compared to 235.09 g reported before for *C. macrophylla* (Legua et al. 2011).

The moisture content of fruit of *C. macrophylla* was recorded as 26.4%. The ash content was determined as 3.08%. There are no previous studies on *C. macrophylla* for moisture and ash contents, but the current results are reasonable when compared to those of other species of *Citrus* (Azad et al. 2014; Ani and Abel 2018). Vitamin C (ascorbic acid) content was found to be 55.0 mg/100 g, which is in a good agreement with reported results 0.047% (modified to 47.0 mg/100 g) (Legua et al. 2011).

Table 1 shows the average values of pH, TSS, TA and RI of fruit juice of *C. macrophylla* as 3.50, 9.30%, 0.74% and 14.81, respectively. These results were in a good agreement with the previous study by Legua et al. (2011). Titratable acidity (TA) refers to the total concentration of titratable acids in a sample (Berezin et al. 1995). In this study, TA was found as 0.74%.

Chemical composition of peel essential oil

The chemical composition of essential oil of *C. macrophylla* peel was determined by using gas chromatography–mass spectrometry (GC–MS). The results presented in Table 2 revealed that the essential oil consists of a mixture of thirty compounds in which limonene is the major and most abundant compound (72.85%), followed by γ-terpinene (13.44%) in addition to many other

Table 2 Percentages of volatile compounds in *C. macrophylla* peel oils

No	Compound	Rt (min.)	Compound class	Rel. (%)
1	α-Phellandrene	4.57	Monoterpene	0.42
2	α-Pinene	4.76	"	1.94
3	Sabinene	4.79	"	0.28
4	β-Pinene	5.96	"	1.05
5	β-Myrcene	6.22	"	1.57
6	*p*-Cymene	7.44	Aromatic hydrocarbon	0.23
7	Limonene	7.55	Monoterpene	72.85
8	*cis*-Ocimene	7.72	"	1.94
9	α-Ocimene	8.08	"	1.00
10	γ-Terpinene	8.54	"	13.44
11	Linalool oxide	9.07	"	0.17
12	Hexachoroethane	9.20	Aliphatic alkane	0.10
13	Linalool	10.22	Monoterpene	0.29
14	1,3-Dimethyladamantane	10.88	Cyclolkane	0.58
15	1,3-Dimethyl-5-ethyladamantane	11.18	"	0.10
16	Decahydro-2,3-dimethylnaphthalene	12.82	Aromatic hydrocarbon	0.29
17	1,4-Dimethyladamantane	13.56	Cyclolkane	0.22
18	α-Terpineol	14.22	Monoterpene	0.32
19	Linalyl propionate	14.22	Ester	0.32
20	1,2-Dimethyl-5-nitroadamantane	16.26	Cyclolkane	0.19
21	σ-Elemene	19.65	Sesquiterpene	0.08
22	*trans*-Caryophyllene	23.18	"	0.63
23	*trans*-α-Bergamotene	23.75	"	0.78
24	α-Humulene	24.70	"	0.10
25	Germacrene D	25.76	"	1.01
26	γ-Elemene	26.33	"	0.24
27	β-Bisabolene	26.94	"	0.49
28	Ledene (Veridiflorol)	30.03	"	0.07
29	Junipene (Longifolene)	30.03	"	0.08
30	Ledol (α-Santalene)	32.93	"	0.07

compounds. The essential oil was characterized by the presence of monoterpene as a main class (96.0%), in addition to other classes of components such as sesquiterpenes (1.32%), hydrocarbons (1.09%), esters (0.32%) and aromatics (0.52%).

Metal profile

The results of metal profile of *C. macrophylla* fruit are presented in Table 3. The concentration of potassium (K) was found as 701.93 ppm compared to 1567.91 ppm (modified unit) in only single previous study. The concentration of sodium (Na) in fruit of *C. macrophylla* was found to be 6.38 ppm compared to 7.35 ppm in previous study which is in good agreement (Levy and Lifshitz 1995). The concentration of calcium (Ca) in fruit of *C. macrophylla* was found to be 371.28 ppm.

The concentration of magnesium (Mg) in fruit of *C. macrophylla* was found to be 54.78 ppm. The concentrations of cadmium (Cd) and lead (Pb) in fruit of *C. macrophylla* were found to be 0.013 ppm and 0.200 ppm, respectively. The concentration of zinc (Zn) in fruit of *C. macrophylla* was found to be 0.256 ppm. Although iron (Fe), nickel (Ni), cobalt (Co) and chromium (Cr) are known as essential elements for plant growth, in the current study, none of these elements were detected.

Discussion

The physiochemical parameters of fruit of *C. macrophylla* were determined including the juice, peel and seed percentages, in addition to size, weight, dimension, diameter, moisture, ash content, vitamin C content, of the fruit. In addition, the average values of pH, TSS, TA and RI of fruit juice were determined experimentally.

Table 3 Metal profile of *C. macrophylla* fruit in (ppm) ($n = 3$)

Element	Mean	SD	Reference
Macroelements			
K	701.93	0.3600	1567.91[a]
Na	6.38	0.8200	7.35[a]
Ca	371.28	0.5000	
Mg	54.78	0.2100	
Microelements			
Cd	0.013	0.0684	
Zn	0.256	0.0090	
Pb	0.200	0.0010	
Fe	ND	ND	
Co	ND	ND	
Ni	ND	ND	
Cr	ND	ND	

ND, not detected

[a] Levy and Lifshitz (1995) (modified units)

The juice percentage obtained in the current study is in a good agreement with the published work (Levy and Lifshitz 1995; Legua et al. 2011). The length, width (diameter) and thickness of the fruit of *C. macrophylla* were determined. The diameter of fruit of *C. macrophylla* is to some extent larger than for fruit of the same species studied by Legua et al. (2011). The weight of fruit of *C. macrophylla* is more or less the same as that reported before (Legua et al. 2011). The moisture and ash contents of fruit of *C. macrophylla* were determined, but there are no previous studies on *C. macrophylla* to compare with. The current results are reasonable when compared to those of other species of *Citrus* (Azad et al. 2014; Ani and Abel 2018).

Vitamin C (ascorbic acid) content was found to be higher the reported study. So, *Citrus macrophylla* is a rich in vitamin C (Legua et al. 2011). The average values of pH, TSS, TA and RI of fruit juice of *C. macrophylla* were in a good agreement with the previous study by Legua et al. (2011). The importance of RI is normally been used as a marker of maturation of fruits (Levy and Lifshitz 1995; Legua et al. 2011). Titratable acidity (TA) refers to the total concentration of titratable acids in a sample, and it is an important characteristic of the quality of numerous products such as vegetable oils, juices, wines, petroleum, motor oils and others (Berezin et al. 1995). The parameters fruit size, juice content, TSS (total soluble solids) and TA (titratable acidity) are important in establishing marketing standards for fruits (Forner-Giner et al. 2003).

The chemical composition of essential oil of *C. macrophylla* peel was performed by using gas chromatography–mass spectrometry (GC–MS) technique. The essential oil consists of a mixture of thirty compounds in which limonene is the major and most abundant compound, followed by γ-terpinene, in addition to many other compounds. The essential oil was characterized by the presence of monoterpenes as a main component in this plant species in addition to sesquiterpenes, hydrocarbons, esters and aromatics. Comparison to the literature, these results were to some extent are similar to previous studies (Verzera et al. 2003; Robbins et al. 2012; Navarra et al. 2015; González-Mas et al. 2019; Zouaghi et al. 2019; Aguilar-Hernández et al. 2020). The presence of high percentage of monoterpenes and high concentration of vitamin C encourages people to use this species as a medicinal herb.

Using graphite furnace–atomic absorption spectrometry (GF-AAS) technique determines metal profile of *C. macrophylla* fruit. Because of its diuretic nature, potassium (K) is one of the most important elements for living organism and this plant species contain considerable amount of it. The concentration of sodium (Na) in fruit of *C. macrophylla* was found to be in good agreement compared to previous study (Levy and Lifshitz 1995). In general, high concentration of sodium in edible fruits is not recommended although it has a role in the transport of metabolites. High sodium intake has been proven that it increases hypertension (Paul and Shaha 2004).

To the best of knowledge, no previous study on the concentration of calcium (Ca) in fruit of *C. macrophylla* was carried out, but when comparing our result to other *Citrus* species it is in a good agreement. The presence of Ca in high amounts is very important in bones, teeth, muscles and heart functions (Czech et al. 2020). Magnesium (Mg) is an essential element in biological systems and found as an ion (Mg^{2+}) that reacts with adenosine triphosphate (ATP) in order to be biologically active. Magnesium intake has an important role in strengthening muscles central nervous system (CNS) (Czech et al. 2020). Fortunately, the concentration of magnesium (Mg) in fruit of *C. macrophylla* was found to be reasonable compared to other *Citrus* species since there is no previous study of magnesium in *C. macrophylla*. There is no previous study on concentration of cadmium (Cd) and lead (Pb) in fruit of *C. macrophylla* to the best of our knowledge. Both cadmium and lead are known for their toxicity in food and in turn cause serious effects on kidney, liver and immune system of human (Sheded et al., 2006). This is the first time to determine the concentration of zinc (Zn) in fruit of *C. macrophylla*. Zinc is an important nutritional element. The zinc deficiency is a main reason for health problem especially in children resulting in stunted children in developing countries (FAO/WHO 1984Sian et al. 2002; Osendarp et al., 2003). Although iron (Fe), nickel (Ni), cobalt (Co) and chromium (Cr) are known as essential elements for plant growth, in the present study none of these elements were detected. The permissible limit of iron (Fe) set by FAO/WHO for edible plants is 20.0 ppm (FAO/WHO 1984). Iron is required for the formation of hemoglobin and its role in the transfer of oxygen and electron to the human body, the normal functioning of the CNS. In addition, it has a role in oxidation of carbohydrates, proteins and fats. Anemia recognition in Fe secretion may be related to its role in facilitating iron uptake and iron uptake into hemoglobin (FAO/WHO 1984).

Conclusions

Citrus macrophylla, commonly known as Alemow, is a universal rootstock. Since this species is a rootstock, most of the researches done on it were came from the horticultural aspects such as cultural practices and the physical features. To the best of our knowledge, the current research is the first study on the fruit of *Citrus macrophylla* to evaluate its nutritional values. Therefore, elemental analysis of fruit is an important part to investigate in addition to determine the vitamin C content. Surveying literature, no element has been determined quantitatively in *Citrus macrophylla* except sodium and potassium in a single study and hence this result for metal analysis is to the best of our knowledge is new knowledge.

The obtained data are useful in evaluation of nutritional value of this species as an edible plant. The fruit of *Citrus macrophylla* is rich of vitamin C, which favors its nutritional value. The current investigated plant species is a good source of Na, K, Ca and Mg. Fortunately, the concentrations of heavy metals such as Cd and Pb were found to be within the permissible limits for food to recommend as an edible plant. Accordingly, it is recommended using *Citrus macrophylla* the Alemow's fruit as an edible food although this species known only as a universal rootstock.

Abbreviations

GC–MS: Gas chromatography–mass spectrometry; GF-AAS: Graphite furnace–atomic absorption spectrometry; FAO/WHO: Food Agriculture Organization/World Health Organization; DCPIP: 2,6-Dichlorophenolindophenol.

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Authors' contributions

All authors certify that they have participated sufficiently in contributing to the intellectual content, concept, design of this work and writing the manuscript. AMAM (corresponding author) confirms that all listed authors have approved the manuscript before submission, including the names and order of authors, and that all authors receive the submission and all substantive correspondence with editors, as well as the full reviews. All authors read and approved the final manuscript. AMAM is the supervisor on the work and designed the experiments. MEM performed collection and authentication of plant materials. ASA performed extraction of essential oil and preparing all samples for analysis. NAA performed the experiments of physical properties. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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