SCREENING THE EFFECT OF EXTRACTION CONDITIONS ON ANTIOXIDANT POTENTIAL OF POTATO PEEL EXTRACTS: A COMPARATIVE EVALUATION

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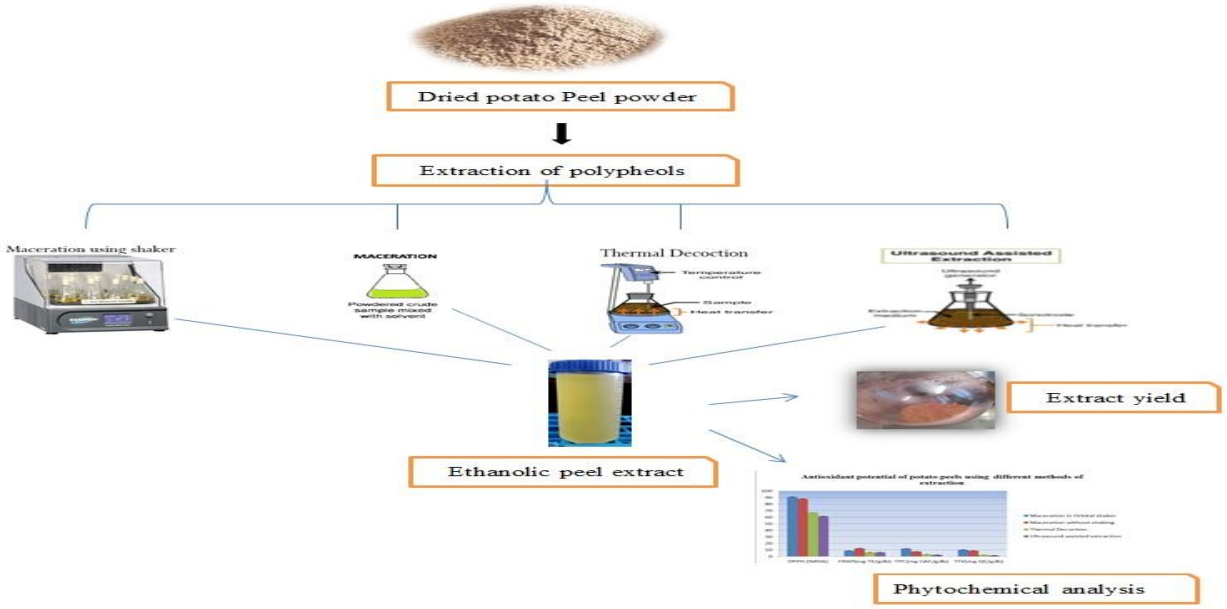
Graphical abstract



Abstract
Potato peels are reported to be a good source of phenolic compounds which are in higher concentration in peels than in potato pulp. Therefore, extraction of these bioactive compounds from peel waste should be done in an economically viable, sustainable and efficient way. Hence, this study aimed for a comparative evaluation of different techniques of extraction in order to achieve a high extraction rate of phenolic compounds. The methods compared were maceration (24 h at room temperature without agitation for 3 repeated cycles), maceration (24 h at 37°C with continuous agitation at 120 rpm for 3 repeated cycles), ultrasound-assisted extraction (15 min at 90% pulse ratio and 60 kHz frequency) and thermal decoction (80 °C for 22 minutes) using 100% ethanol with constant 1:10 solid: solvent ratio for all the four methods. The extraction efficiency was evaluated based on extraction yield, total phenolic content, total flavonoid content and antioxidant activity measured by DPPH and FRAP assay. The highest extraction yield was obtained with maceration (24 h at 37°C with continuous agitation at 120 rpm for 3 repeated cycles) and lowest extraction yield with ultrasound assisted extraction attributing to its sono-chemical effects. Phytochemical analysis i.e. total phenolic content, flavonoid content and antioxidant activity was found to be highest in extract obtained by maceration for 24 h at 37°C with continuous agitation. Strong correlation between phenolic, flavonoid contents and antioxidant activity assays and phenolics compounds identified i.e.ferulic acid, gallic acid and quercetin by HPLC confirmed the authenticity of results.

Keywords: Maceration, ultrasound-assisted extraction, phytochemical analysis, pulse ratio, correlation

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1. INTRODUCTION

Globally, potato (*Solanum tuberosum*) isrecognised asthe fourth major widely produced and consumed stapletuber crop in the world following rice, wheat and maize (Alves-Filho et al., 2018; Pathak et al., 2017; Zaheer & Akhtar, 2016).Nutritionally this crop is a good source of dietary fibre, carbohydrates, minerals and phenolic substances with a high value

protein and enriched with number of micronutrients i.e. vitamins B_1, B_3 and B_6 and minerals such as potassium, phosphorus and magnesium (Burlingame et al., 2009; Singh et al., 2011).However, it has been reported by Charmley et al. (2006) that an approximate 40-50% of potato production is unsuitable for human consumption which includes the waste generated during processing of potatoes. A large fraction of population consumes potatoes in processed forms such as French fries, chips, and puree etc. Processed potato constitutes to only 50-60% of raw material and rest is discarded as waste. The by-products of processing include cull potatoes, potato peels etc. which is estimated to be 33-35% of original potato fresh weight. Today, a large proportion of this waste is being utilized as animal feed and biofuel (Jeddou et al., 2016; Schieber and Saldana, 2009;Sepelev and Galoburda, 2015).Moreover, it's a zero-value waste accounting to 15-40% depending on peeling method i.e. steam, abrasion or lye peeling. These peels comprising the major portion of the processing waste pose a severe problem of disposal to the potato industry since wet peels are predisposed to microbial spoilage. Therefore, their valorization should be of prime interest to the food industry for sustainable environment and bearing the cost loss associated with management of waste generated (Akyol et al., 2016; Gebrechristos and Chen 2018; Hossain et al. 2014).

Considering the peel composition, the potato peel extract has been known to exhibit antioxidative and antiradical activities (Friedman et al., 2017). It has been used to prevent lipid oxidation in oil-in-water emulsions and plant oils (Habeebullah et al., 2010), minced mackerel meat (Farvin et al., 2012), and ground beef patties (Mansour, Khalil, 2000) and other health-positive effects. Therefore extraction which is the first step of the valorisation of bioactive compounds from plant matrices to recover any target compound should be economically viable, sustainable, quick and efficient (Apel et al., 2020; Giuffrè et al., 2018). As a result, to date studies have been conducted investigating the effect of different extraction techniques such as solid–liquid extraction (SLE) (Amado et al., 2014) ultrasound-assisted extraction (UAE) (Hossain et al., 2014), pressurised-liquid extraction (PLE) (Wijngaard et al., 2012), or microwave assisted extraction (Singh et al., 2011) on the recovery of phenolic compounds from potato peels.

Therefore, in this study a comparative analysis of four different extraction methods (maceration with and without shaking, ultrasonic and thermal decoction) selected on the basis of feasibility of facilities available is done to establish the best suitable method for the extraction of bioactive compounds from potato peel samples using 100% ethanol.

While SLE (maceration) and thermal decoction are conventional methodsbased on direct physical contact between solid and solvent, UAE is a non-conventional technique based on mechanical disruption of cell walls by ultrasoundthus reducing particle size of matrix and allowing greater penetration of solvent into the sample matrix which might result in higher and faster recovery of bioactive compounds**(**AL-Bukhaiti et al., 2018; Chemat et al., 2017; Vilkhu et al., 2008).

2. MATERIALS AND METHODS

Materials

Potato peels (*Solanum tuberosum* cv. Lady Rosetta (red skin)) obtained using abrasive peeling were collected from local potato chip manufacturing industry in New Delhi, India on 3rd October, 2018.

The peels were collected in plastic zip lock bags and thereafter washed thrice under running tap water to remove any debris and then rinsed with distilled water. Washed peels were weighed and sun-dried for 3-4 days until constant weight was obtained.

After complete dryness, they were ground to a powder and sieved using a standard 50 mesh size sieve to ensure symmetry of particle size. Dried powder samples were weighed and kept in aluminium zip lock pouches, at -20°C until the day of analysis.

Reagents

The analytical grade chemicals (DPPH, Folin-Ciocalteu reagent, Aluminium chloride, sodium hydroxide, hydrochloric acid), solvents (Ethanol) and phenolic acid standards (gallic acid, quercetin, and Trolox etc.) used were purchased from Hi-media Laboratories, Mumbai, India; Sisco Research Laboratories, Mumbai, India and Sigma Aldrich, Bengaluru, India. The assays were carried out using Milli-Q water (Merck Millipore; Billerica, USA).

Preparation of Potato peel extracts

Maceration at 37°C in shaker

10 g of grounded potato peel powder was dissolved in 100 mL of 100% ethanol. The solution was stirred for 24 h at 120 rpm at 37°C using an orbital shaker and centrifuged at 2800 rpm for 10 min. The extracts were filtered through Whatman No.1 filter paper and the residue was re-extracted two more times under the same conditions. The three filtrates obtained were combined and solvent was evaporated in a rotary evaporator below 45°C. The extracts obtained after evaporation of organic solvent were subjected for further analysis (Modified: Rowayshed et al., 2015).

Maceration at room temperature (18°C at the time of extraction without shaking)

10 g of powdered peel was extracted with 100 ml of 100% ethanol at room temperature (18°C at the time of experiment) overnight and centrifuged at2800 rpm for 10 min. The supernatant was collected in a separate bottle and the residue was re-extracted two more times under the same conditions. The three filtrates were combined and solvent was evaporated in a rotary evaporator below 40°C. The extract obtained after evaporation of organic solvent was used as natural antioxidant (Modified: Farvin et al., 2012).

Ultrasound assisted extraction

Ultrasound-assisted extraction was done according to the methodology proposed by Anaya-Esparza et al. (2018)and Samarin et al. (2012) with some modifications.

10 g of grounded peel powder was dissolved in 100 mL of 100% ethanol. An ultrasonic probe was immersed at 2 cm into the solution and kept in ice bath to control the rise in extraction temperature caused by exothermic reactions. The experimental conditions used for the extraction were frequency of 60 kHz with sonication amplitude of 90% and pulse cycle of 1 second (0.6 seconds of power discharge and pause of 0.4 s)for 15 min.

The solute-solvent mixture was then centrifuged for 10 min at 3000g and the supernatant was separated from the residue and filtered using filter paper Whatman No. 1. Then solvent was evaporated in a rotary evaporator below 40°C.

Thermal decoction

Thermal decoction was carried out under the conditions optimized by Wijngaard et al. (2012) for extraction of polyphenols from industrial potato peel waste generated.

10g peel powder was mixed with 100 mL of 100% ethanol into amber glass bottles, and the solution was slowly boiled at 80°C for 22 mins (optimized time-temperature).

The extracts werecooled to room temperature and centrifuged for 10mins at 995g. The supernatant obtained were filtered through Whatman No. 1 filter paper. The filtrate obtained was collected and solvent was evaporated using rotary evaporator below 45°C.

The extract obtained after solvent evaporation was stored at -20°C inamber glass bottles wrapped with aluminium foil until analysis.

Extraction yield

Extraction yield was defined as the percentage of the soluble polyphenols extracted from the total weight of the sample (g).

The extraction yield was calculated using the equation as suggested by Aydar et al. (2017).

$$\text{Yield}(\%) = \frac{\text{Extracted soluble polyphenols (g)}}{\text{Sample (g)}} \times 100$$

PHYTOCHEMICAL ANALYSIS

Determination of antioxidant (properties of extracts) activity

Radical scavenging activity (RSA %) assay: Free radical scavenging activity (RSA) of the sample was measured using the method described by Rowayshed et al. (2015),with some modifications**.** An aliquot of the sample solution (40 µL) was mixed with 2.9 ml of 0.1 mM DPPH (2, 2-diphenyl-1, 1-picrahydrazyl) in ethanol solution, incubated for 30 min at 25°C in dark; the decrease in the absorbance at 517 nm was measured. Ethanol was used as blank.
Antioxidant activity was expressed as percentage inhibition of the DPPH radical and was determined by the following equation:

Scavenging activity (%) = 1 – (As/Ao) × 100

Where: As is the Absorbance of the sample and Ao is the Absorbance of the control.
Control: 40 µL of ethanol mixed with 2.9 ml of DPPH methanol solution.

Ferric reducing antioxidant power (FRAP) assay: Antioxidant activity was measured using the ferric reducing antioxidant power (FRAP) assay with some modifications (Rowayshed et al., 2015).
The FRAP assay of compound materials in reducing ferric ion (Fe+3) to ferrous ion (Fe+2). (Fe+2/TPTZ) forms a blue complex color which increases the absorption at 593 nm. The FRAP reagent contained 2.5 ml of a 10 mmol/L TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40mmol/l HCL plus 2.5 mL of 20 mmol/l FeCl3.6H2O and 25 ml of 0.3 mol/l acetate buffer (pH 3.6) and was freshly prepared and warmed at 37°C prior to usage. Aliquots of 0.1 ml sample solution was mixed with 3ml FRAP reagent and the absorbance of reaction mixture at 593 nm was measured spectrophotometrically after incubation at 37°C for 10 min. Calibration was against a standard curve (0.02-0.1 mg/ml) using freshly prepared Trolox.

Determination of total polyphenol content (TPC)

The total phenolic content of PP ethanolic extracts was determined based on the method of Amado et al. (2014), slightly modified using the Folin–Ciocalteu Reagent (FCR) with gallic acid as a standard. 0.1 mL of sample or blank was mixed with100 µL of diluted (1:10; FCR: Water) FCR and, after 5 min, 1 mL of a Na_2CO_3 solution (7%)was added. After incubation for 1 h at room temperature, the absorbance was read at 760 nm with PerkinElmer Lambda 25 UV/Vis spectrophotometer in 1 cm cuvettes. Readings were compared with a standard curve of gallic acid and the total phenolic content was expressed as mg of gallic acid equivalent per g of freeze dried solid (mg GAE/g).

Determination of total flavonoid content (TFC)

The total flavonoid content was measured by the method described by Silva et al. (2017) with modifications. The reaction mixture contained 100 µL of the PP extract and 430 µL of 5% $NaNO_2$, which was incubated for 5 min. After incubation, 30 µL of $AlCl_3$ (10%) and 440 µL of NaOH (1 mol/l) were added to the reaction. The absorbance was read at 496 nm with Perkin Elmer Lambda 25 UV/Vis spectrophotometer, in 1 cm cuvettes. The results were expressed as mg of quercetin equivalents (QE) per gram (mg QE/g).

HPLC analysis of peel extract for identification of compounds in the extract

Potato peels: The supernatant (20 µL) obtained from the above extraction was directly injected into a Cosmosil C18-MS-II column (5 µm, 4.6 mm i.d. × 250 mm) HPLC column. The mobile phase consisted of the following linear gradient: acetonitrile (A) and 0.5% formic acid (B): (A) =5% (0–5 min), 18% (5.1–30 min), 53% (30.1–70 min), 90% (70.1–80 min), and 5% (80.1–100 min). The flow rate was 1.0 mL/min at 35 °C, peaks were monitored at 320 nm, and UV/Vis spectra were recorded.Chromatographic comparison with analytical standards, absorbance spectra, and

mass spectra, were used to identify compounds(Friedman et al., 2017).

Statistical analysis

Results are expressed as means±S.D of triplicate readings. Statistical analyses are performed using one-way ANOVA for mean comparisons and Post-hoc Tukey HSD test at a 95.0% confidence level to establish the significant difference among the means using STATA software. Correlation analyses were performed using a Pearson correlation test in Excel, 2010.

3. RESULTS AND DISCUSSION

Extraction yield

Extraction yieldof the potato peels extracts obtained using four methods of extraction are presented in Table 1 as % yield. Highest extraction yield was found to be of extract obtained by maceration at 37°C in orbital shaker with 3 repeated cycles of extraction whereas the extract obtained by ultrasound assisted extraction had the lowest yield which can be explained in terms of relation between chemical composition (dietary fibre content) of potato peels and experimental conditions used for extraction.

Potato peels are good source of dietary fibre comprising of 3.4% pectin and 2.2% cellulose with varying amount based on the peeling method but this amount remains unaffected by abrasive peeling (Javed et al., 2019)which has been used to obtain this Lady rosetta variety peels. And as reported in literature most insoluble-bound phenolics chemically form covalent bonds with cell wall substances including pectin, cellulose, arabionoxylan and structural proteins and account for relatively arge fraction (20%–60% in vegetable, fruits and legume/seeds) compared to the soluble phenolics in foods (Nayak et al., 2015).Therefore bound phenolics such as ferulic acid which is present as ester of hemicellulose and gallic acid esterified to glucose (as in hydrolysable tannins), or catechins or proanthocyanidins (as in condensed tannins) (Manach et al., 2005) require longer time of extraction for separation of bound compounds to get fully diffused into extracting solvent as conducted in conventional extraction thus giving maximum yield (Ramli et al., 2014). However the slight difference in the yields of extracts obtained using orbital shaker and without shaking is due to increase in mass transfer rate of solute by greater turbulence generated by shaker (Subramaniam et al., 2015). Moreover the high temperature used in thermal decoction resulted in higher yield comparative to UAE attributed to disruption of cell walls and solubilisation of the target compounds unlike in UAE where high temperature may cause a reduction in the sono-chemical effects, resulting in lower recovery yields of antioxidants (Chematet al., 2017). Similar results were reported by Papoutsis et al. (2018) for the recovery of polyphenols and antioxidants from lemon waste using an optimised SLE technique which was higher than those obtained from an optimised UAE.

Comparison between different extraction methods in terms of TPC, TFC and antioxidant capacity (measured by DPPH and FRAP assay)

The phytochemical analysis of the peel extracts obtained using four methods of extraction are summarised in Table 2.

Table 1: Extraction efficiency of conventional and non-conventional method of extractions

Method of Extraction	Time	Temperature	% Yield
Maceration in Orbital shaker	3 repeated cycles (24hrs. each)	37°C	2.134±0.74
Maceration without shaking	3 repeated cycles (24hrs. each)	Room temperature (18°C at the time of experiment)	1.892±0.54
Thermal Decoction	22 minutes	80 °C	1.253±1.03
Ultrasound assisted extraction	15 minutes	Ice bath	0.876±0.85

The data are the mean ± SD of three replicates

Table 2: Phytochemical analysis of the potato peel extracts obtained by four extraction methods

Method of Extraction	DPPH (%Radical scavenging activity)	FRAP assay (mg TE/gdb)	Total Phenolic Content (mg GAE/gdb)	Total Flavonoid Content (mg QE/gdb)
Maceration in Orbital shaker	91.07 ± 0.43[a]	8.76 ± 0.95	12.02 ± 0.56	10.19 ± 0.21
Maceration without shaking	88.23 ± 1.04[a]	12.35 ± 1.95	7.77 ± 0.13	8.89 ± 0.11
Thermal Decoction	66.97 ± 2.56[b]	6.21 ± 0.57[c]	3.35 ± 0.81[d]	2.51 ± 0.03
Ultrasound assisted extraction	61.48 3.89[b]	6.26± 0.59[c]	2.55 ± 0.54[d]	1.51 ± 0.25

The data are the mean ± SD of three replicates
TE: Trolox equivalent; GAE: Gallic acid equivalent; QE: Quercetin equivalent
Numbers in the same column followed by the same letter are not significantly different at P<0.05.

Among the four methods used for extraction of polyphenols keeping solid: solvent ratio constant (1:10), conventional maceration extraction at 37°C using orbital shaker was found to be most efficient, in obtaining extract with highest antioxidant potential attributing to long contact time between solid and solvent and repeated cycles of extraction while UAE was not found to be as efficient and resulted in least TPC and TFC values.

This could be explained as inefficiency of UAE to extract hydrolysable polyphenols such as ellagitannins due to the large amount of exothermic energy produced during extraction which may result in oxidation of molecules with high molecular mass. However heat treatment used in thermal decoction (80°C) could hydrolyze those high molecular weight polyphenols into smaller fractions and hence result in comparatively high antioxidant potential and phenolic contentthan UAE(Poodi et al., 2017; Sousa et al., 2016). With respect to the antioxidant capacity estimated by FRAP assay, significant differences ($p<0.05$) were observed between maceration with shaking and without shaking treatments attributing to theenhancement in diffusion of bioactive compounds in solvent caused by high particle movements by shaker. Moreover antioxidant activities values estimated by DPPH and FRAP assay were found to be significantly different for all the four treatments. The difference in the results obtained by two assays can be explained in terms of difference in types, relative amounts, and reaction of antioxidant constituents in extracts and possibly due to the fact that phenolic compounds respond differently depending on number of phenolic groups (Tamuly et al., 2013).

The lowest values of DPPH-RSA, FRAP, TPC, and TFC obtained with ultrasonic extraction (Table 1) could also be explained by free radicals generation in the fluid due to the ultrasonic power causing significant degradation of polyphenols by inducing a reaction with flavonoids owing to their high redox potential (Ignat et al., 2011; Da Porto et al., 2013). Sun et al. (2017) had reported an ultrasound induced significant degradation of flavonoid cyanidin-3-glucosylrutinoside, resulting in an alteration in antioxidant activity of red raspberry fruits. Moreover, the high frequency used (60kHz) in the extraction has also been reported to significantly affecting the intensity of the acoustic cavitation in solvent medium resulting in lower phenolic yield and antioxidant activity, a lower frequency has been found to be more favourable for higher recovery of phenolics by creating large but relatively fewer cavitationalbubbles which collapse with higher energylevel, thus resulting in a greater degree of cell disruption (Wu et al., 2013).Similar findings were reported by Altemimi et al. (2015), for polyphenol recoveryusing ultrasonication from spinach where the ultrasonic bath operating at 37

kHzwas more effective than 80 kHz at temperature–power–time combination of 40 °C, 50% and 30 min,with regard to extraction yield, total phenols and %DPPH inhibition.The other important factor affecting the TPC and TFC of the extracts obtained UAE is amplitude of ultrasonic waves generated during the extraction (Zhou et al., 2004). The high amplitude (90%) used for extraction also caused a reduction in TPC and antioxidant capacity of extracts, as reported by Lanez and Haoua (2017) a decrease in amplitude results in higher amounts of TPC and TFC.

However, the TPC of the extracts obtained by UAE and thermal decoction was not significantly different (Table 2) but high temperature used in thermal decoction resulted in comparatively high phenolic yield by increasing the solubility and diffusion of bioactive compounds from plant matrix into the solvent (Pinelo et al., 2005). Though, this yield was much lower than the TPC of the extracts obtained by maceration with and without shaking as heating above an optimum temperature may have caused a decline in TPC attributing to the degradation of cell walls by rise in temperature resulting in the release of both phenolic compounds and enzymes involved in oxidation. In addition, the activity of these oxidative enzymes i.e., peroxidase, polyphenol oxidase gets enhanced by temperature applied leading to lower polyphenol yields (Abad et al., 2007, Mizobutsi et al., 2010). The lower antioxidant capacity of extracts obtained by UAE and thermal decoction could becredited to the lower phenolic and flavonoid content in these extracts since a high correlation between TFC, TPC and antioxidant assays (DPPH and FRAP) was observed (Figure 1).

Correlation between phenolic/flavonoid content and antioxidant activity

The role of phenolic compounds as antioxidants has been recognized by means of their ability to donate electron and formed a stable radical inter mediates. The present study found that scavenging activity and reducing power were highly correlated with total phenolic and total flavonoid content of potato peel extracts (Figure 1). The variation in correlation among the FRAP, TPC and TFV the selected plant species may be due to a different reaction mechanism involved in these assays. Strong correlation of DPPH with FRAP with value 0.659, confirms the authenticity of results of antioxidant potential for the peel extracts.

Phytochemicals identification by HPLC

The major phenolic compounds and flavonoids contributing to the antioxidant activity of potato peel extracts with highest antioxidant potential i.e. extract obtained by maceration at 37°C were ferulic acid, gallic acid, quercetin, gallic acid and vanillin.

Figure 1: Pearson correlation analysis

4. CONCLUSIONS

The findings of this study suggest thatselection of a suitable extraction technique is a very crucial prerequisite in antioxidant studies by having a direct impact on the recovery and physiological functions of antioxidant compounds to be extracted from the plant materials. From the results it can be seen that influence of extraction methoddepends upon not only on the physical property of the sample but also on its chemical composition and extraction conditions. Therefore, application of any extraction method for food or dietary supplement warrant further research before being produced in large scale in order to identify the most efficient extraction technique for extracting its antioxidants.In this study, the conventional maceration at 37° in shaker proved to be a best method for the extraction of bioactive compounds from potato peels based on the indices i.e. extraction yield, total phenolic content, total flavonoid content and antioxidant activity of the extracts.

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