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EVALUATION OF ANTIOXIDATIVE POTENTIAL OF TEA INFUSIONS SUPPLEMENTED WITH SOME FLAVOUR PRODUCING HERBS

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ABSTRACT
Tea bags have been proved to be a blessing for modern people who desire quick tea in between their busy schedules. Invention of tea bags solved two problems i.e. preparation of a single serving tea-brewing more conveniently and to reduce hazards of disposal after preparation of the infusion. The present study delved into characteristics of tea bags prepared using three different types of tea, viz. black tea, oolong tea and green tea, separately combining with different herbal additives, viz. mint (*Mentha spicata*), basil (*Ocimum basilicum*) and ginger (*Zingiber officinale*) that provide flavour and fragrance to adjudicate their antioxidant potential as well as contents of bioactives. The results indicated that there was no difference in the organoleptic acceptability to the consumers before and after addition of the herbs, clearly indicating the commercial potential of the prepared tea bags. Antioxidant assays like total phenolic content determination or DPPH assay indicated no substantial difference between the samples. Improvement of flavonoid contents with mint supplemented tea bags not only increased the chances of imparting better antioxidant profile to the samples, but also improved amount of tannins in the infusions, probably by inducing condensation of the biomolecules. Overall, mint supplemented tea bags have a better chance to produce positive effects systemically.

Keywords – Tea bag, Mint, Basil, Ginger, Antioxidant, Flavours

INTRODUCTION
Tea (*Camellia sinensis*) is an important commercial crop that is being cultivated in the tropical and subtropical regions of the world. It is known to be one of the most popular beverages in the world and is believed to be the most widely consumed non-alcoholic drink after water. It is produced in more than 40 countries, mainly in Asia, Africa and South America. China, India, Sri Lanka, Kenya and Indonesia account for 80% of worldwide production with China being the largest tea producer. India is the second largest producer, next to China. The polyphenolic compounds contained in tea mainly include flavonoids, flavonols, phenolic acids, tannins, and most importantly, caffeine. Catechins, the most common of the tea polyphenols are flavonoid compounds with a basic structure of □-phenyl-benzopyran (Yan *et al.*, 2020). Because of its high content of catechins and tea flavonoids, tea consumption protect against the development of cardiovascular diseases (Teshome, 2019). Tannin constituents such as thearubigins and theaflavins present mainly in black tea are formed by the enzymatic oxidation of catechins followed by condensation, and this causes the characteristic astringency. Flavonols are mainly constituted with myricetin, campherol, quercetin, chlorogenic acid, coumarylquinic acid and theogallin all of which have potent therapeutic potentials (Ratnani and Malik, 2022). Green tea hence is considered to be principal source of catechin and its derivatives among various dietary sources (Cabrera *et al.*, 2006). Several types, viz. black tea, green tea, oolong tea, white tea, dark tea and yellow tea with different flavor and aroma profiles are being

prepared through different processing techniques (Wang *et al.*, 2022). Green tea is prepared from unfermented leaves, the leaves of oolong tea are partially fermented, and the black tea is fully fermented (Jolvis Pou, 2016). The more the leaves are fermented, the lower the polyphenol content and the higher the caffeine content. Green tea was the first tea to be discovered, which is a non-fermented tea thus retains more natural substances in fresh leaves and has less vitamin loss. It produces a clear soup with strong flavor convergence thus making it most preferable (Zhao *et al.*, 2022). People in Asian countries more commonly consume green and oolong tea while black tea is the most popular drink in the United States.

Tea beverage is an infusion of the dried, semi-dried or fresh leaves of tea plant, prepared in water by heating for a stipulated period of time. In western countries, the infusion is prepared using bagged black tea leaves dipped for a shorter period of time, whereas in Indian subcontinent and Middle-eastern countries, leaves are boiled for longer (Vinci *et al.*, 2022). Taste, flavor, aroma, color, brightness and astringency of tea infusions are influenced by polyphenols, caffeine, sugars, organic acids, amino acids and volatile flavor compounds (Teshome, 2019). Use of different processes for infusion preparation not only modifies the taste or bioactive contents of the substance, but also is a demand of the modernized fast life of the people (Roy *et al.*, 2017). However, age of tea leaves as well as the methods of preparing tea infusions, i.e. the duration of brewing, temperature, and tea/water ratio, may also play a significant role in the dispersion of bioactives in the infusions (Street *et al.*, 2006). Variations in the method of preparations might lead to difference in the bioactivity of the beverage, especially shorter sipping of bagged tea could produce infusion with less amount of bioactives. Enhancement of tea by addition of milk, sugar, lemon or honey is a common practice, although enhancement of antioxidant potential of the infusions by this process were contradictory (Bartoszek *et al.*, 2018, Saletnik *et al.*, 2018). Earlier studies indicated that antioxidant and pharmacological activities of green tea could be enhanced by adding lemon juice, which might be explained by the effect of ascorbic acid on the increased stability of polyphenols due to lowering of pH (Chakrabarty *et al.*, 2017).

Demand of tea bags in modern day fast life is ever increasing due to different traits including consumer preferences, blended nutraceutical ingredients in small sachets, ease of handling and commercial gain to both consumers and producers. It is the fastest growing segment with 50–60% growth per year in India with tea bags contributing 3-4% of the total tea sale (Bassi *et al.*, 2019). This led the researchers to undertake the present study, where tea bags were prepared using three different types of tea, viz. black tea, oolong tea and green tea, separately combining with different herbal additives, viz. mint (*Mentha spicata*), basil (*Ocimum basilicum*) and ginger (*Zingiber officinale*) that provide flavour and fragrance to adjudicate their antioxidant potential as well as contents of bioactives. The outcome would help in deciphering the commercial aspect of production and marketing of tea bags with herbal additives that would provide health benefits to the consumers.

MATERIALS & METHODS

Chemicals

2,2′-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Himedia, India. Analytical grades of ascorbic acid, gallic acid, Folin-Ciocalteau's solution, sodium hydroxide and sodium carbonate were obtained from SRL, India. Anhydrous aluminium chloride was obtained from Merck, India. Deionized distilled water was used in the entire study.

Preparation of infusions

Disposable tea filter bags (make – Sichumaria, India) were procured from an online marketing concern. Pure leafy black, oolong and green tea were purchased from Navvyad, India. Mint, ginger and basil samples were purchased locally from markets in Barasat, Kolkata. The herbs were checked for damage if any, and then the selected samples without damage were washed thoroughly, sun-dried to get the powdered forms and used in the study. 2 gm each of black, green and oolong tea were put in tea bags separately and coded BT, GT and OT respectively. Powdered herbs were added in an amount of 1 gm each, individually in the tea bags for experimental studies. Each tea bag contains only one type of herb along with the respective tea sample except for the mixed herb sample, where three herbs were put in 1:1:1 weight ratio along with the respective tea sample. Weight of the sample in the tea bags never exceeded 2 gm. The infusions were prepared, individually with the 15 tea bags, by the following procedure – Each tea bag was dipped into 40 ml hot water (~80^{o}C) for 3 minutes (Vuong *et al.*, 2022). After the stipulated time period, the samples were centrifuged at 5000 rpm for 5 min. The clear supernatants were used for *in vitro* assays.

Determination of Tannin Contents

An established method was followed (Atanassova and Christova-Bagdassarian, 2009). 25 ml of the infusion were measured into1 L conical flask, then 25 ml of indigo solution and 750 ml distilled deionised water were added. 0.1 N aqueous solution of $KMnO_4$ was used for titration until the blue coloured solution changed to green colour. Then few drops at time until solution become golden yellow were added. Standard solution of Indigo carmine was prepared as following – 6 g Indigo carmine was dissolved in 500 ml of water by heating, and after cooling, 50 ml of 95-97 % H_2SO_4 was added carefully and the solution was diluted to 1 L and then filtered. The blank tests by titration of a mixture of 25 ml Indigo carmine solution and 750 ml water are carried out. All samples were analysed in triplicates.

Determination of Total Flavonoid and Flavonol Contents

The total flavonoids were estimated according a published method (Nana *et al.*, 2012). $AlCl_3$ methanolic solution (0.5 ml, 2% w/v) was mixed with tea extract solution (0.5 ml). After 10 min, the optical densities were recorded at 415 nm against a blank (mixture of 0.5 ml tea extract solution and 0.5 ml methanol) and compared to the quercetin calibration curve. The amounts of total flavonoids in the tea extracts were expressed as mg of quercetin equivalents (QE)/gm tea of sample. The contents of the total flavonols were determined by the following method – aliquots were prepared by mixing tea extract solutions (0.75 mL, 0.1 mg/ml) and $AlCl_3$ aqueous solution (0.75 ml, 20% w/v). After 10 min of incubation, the optical densities were read at 425 nm against a blank (mixture of 0.75 ml tea extract solutions and 0.75 ml ethanol). The results were expressed as mg of QE/gm tea of sample. The tests were done in triplicates.

Estimation of Total Phenolics Contents

The assay was performed using a previously described procedure (Chakraborty *et al.*, 2015). Briefly, 0.5 ml of tea infusion was mixed with 1.5 ml Folin-Ciocalteau's solution (1:10 v/v diluted with distilled water) and allowed to stand for 28±2°C for 5 min. Then 2 ml of 7% (w/v) aqueous sodium carbonate solution was added and the mixture were allowed stand for another 90 min and at

darkness. The absorbance of the blue color that developed was measured at 725 nm using spectrophotometer (Systronics, Model – 2202). Gallic acid was used to prepare the standard curve (20–100 □g/ml) and the total phenolic concentration in the spice extract was expressed as mg of gallic acid per gram tea sample. All samples were analysed in triplicates.

DPPH radical decolorization assay

The DPPH assay was performed using a previously described procedure (Chakraborty and Bhattacharyya, 2014). 1 ml DPPH solution (prepared by dissolving 3 mg in 25 ml ethanol) was mixed with 0.5 ml sample solution and the decrease in absorbance of the mixture after 20 minutes of incubation in the dark was monitored at 517 nm in a Systronics spectrophotometer (model – 2202). The concentration that causes a decrease in the absorbance of initial oxidants by 50% is defined as IC_{50} of the samples. Gallic acid was used as positive control and comparing with its' IC_{50}, the results were expressed as gallic acid equivalents (µg/gm tea sample). All samples were analysed in triplicates.

Organoleptic assessments of the Tea Infusions

Sensory evaluation of the infusions was carried out using 9-point Hedonic rating scale (Ackbarali and Maharaj, 2014) by 60 semi-trained panel members of the Institute, who declared themselves as regular consumer of tea. The samples were presented nomadically following a completely randomized design. All the samples were appropriately coded before subjecting to sensory evaluation. Taste, colour and overall acceptability were considered for evaluation. The sixty semi trained panellists, were asked to rate the infusions on the basis of a 9-point scale, anchored by – 1 = 'dislike extremely'; 2 = 'dislike very much'; 3 = 'dislike moderately'; 4 = 'dislike slightly'; 5 = 'neither like nor dislike'; 6 = 'like slightly', 7 = 'like moderately', 8= 'like very much' and 9 = 'like extremely'.

Statistical analyses

Experimental results were expressed as mean±SD of three individual experiments. Analyses were done using the software "PSPP ver 1.6.2" (GNU Project).

RESULTS & DISCUSSION

Tea bags are always a better choice in the commercial eateries not only because of the convenience of use, but also due to the fact that substances like green tea may be well restrained in the bags as the leaves are smaller and brittle. A previous study also corroborated this proposition as it showed larger surface area of the tea leaves exposed in the tea bags allow a better extraction of the polyphenols subsequently yielding a higher radical scavenging by the infusions (de Almeida *et al.*, 2019). Considering all these aspects, the commercial tea bags were chosen for the present study. Fig. 1 depicted the samples prepared with the commercial tea bags.

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Figure 1. Samples prepared with commercial empty tea bags filled with different tea samples.

Fig. 2 indicated the contents of tannins in different value-added tea bags. Tannin content in green tea is higher than the other two tea samples, although the higher value compared to the black tea was insignificant. Tannin content was found to be significantly higher in black tea bags when mint leaves were added in the bags. Tea mostly contains condensed tannins, which are formed due to polymerization of flavonoids at lower pH (acid catalysed condensation). Since mint contains higher flavonoid than the other two herbs, viz. ginger and basil, both of which are largely devoid of flavonoids (Bhagwat *et al.*, 2011), we propose that flavonoids leached out during infusion preparation may induce greater condensation at higher temperature, thereby increasing tannin contents in case of black tea. Lower pH of the black tea infusions than the two other tea samples might also facilitated the condensation as earlier reports indicated that black tea infusions possess lower pH than other tea infusions (Bobkova *et al.*, 2021).

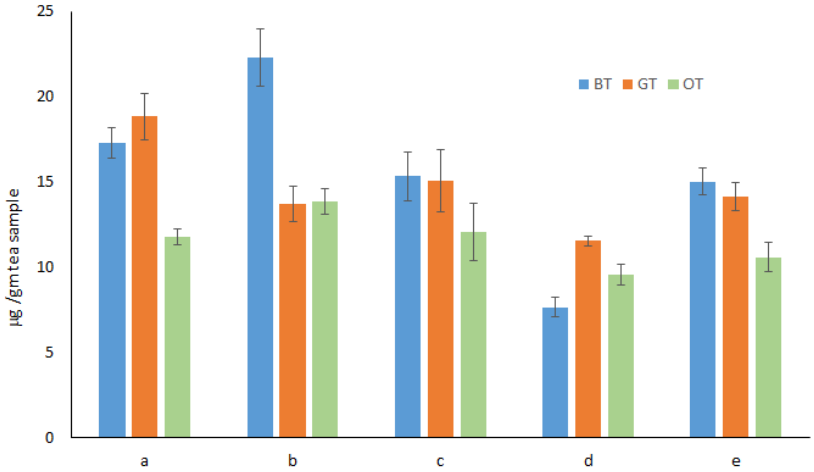

Figure 2. Comparative tannin contents of different tea bags. Results are expressed as mean±SD of three individual experiments. Codes: BT – black tea; GT – green tea; OT – oolong tea; a – tea samples without herbs; b – tea samples with mint; c – tea samples with ginger; d – tea samples with basil; e – tea samples with three herbs.

Fig. 3 indicated the contents of total flavonoids in different value-added tea bags. As mint contains fair amount of flavonoids whereas the other two are largely devoid of that bioactive, there was an

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augmentation of flavonoid content in all the infusions which might produce beneficial effects systemically when taken orally as drinks. The same trend was observed in total flavonol content of the supplemented tea samples (Fig. 4), again substantiating the results observed in Fig. 3. It may also be observed that supplementation with all three herbs improved flavonoid contents in green and oolong tea bags.

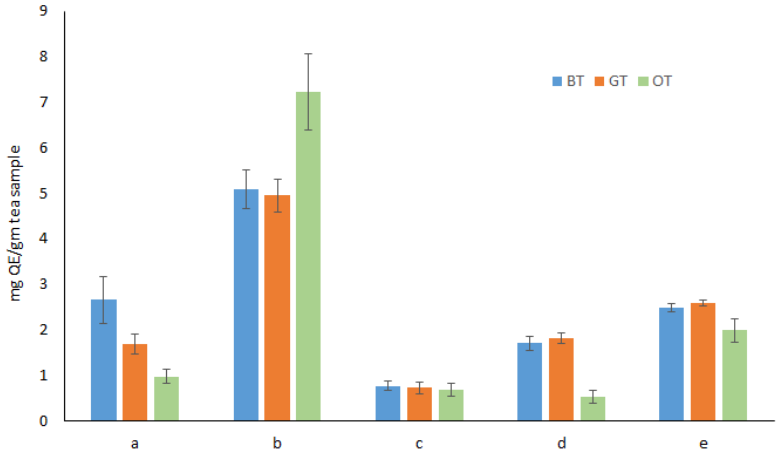

Figure 3. Comparative contents of total flavonoids in different tea bags. Results are expressed as mean±SD of three individual experiments. Codes: BT – black tea; GT – green tea; OT – oolong tea; a – tea samples without herbs; b – tea samples with mint; c – tea samples with ginger; d – tea samples with basil; e – tea samples with three herbs.

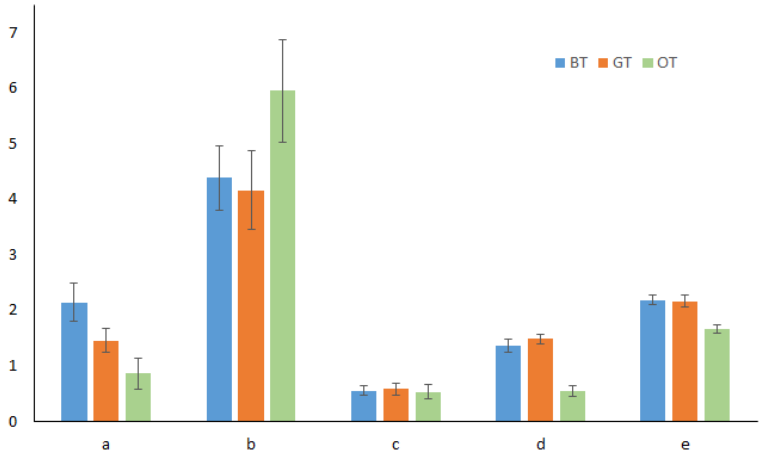

Figure 4. Comparative flavonol contents in different tea bags. Results are expressed as mean±SD of three individual experiments. Codes: BT – black tea; GT – green tea; OT – oolong tea; a – tea samples without herbs; b – tea samples with mint; c – tea samples with ginger; d – tea samples with basil; e – tea samples with three herbs.

Fig.5 indicated that total phenolic content improved in all the supplemented green tea samples after their processing to infusions, although the increment was not always significant. Green tea is generally not undergone the same withering and oxidation processes that have been employed in case of black or oolong tea. This minimizes the degradation of polyphenolic antioxidant bioactives of green tea and hence, the effect has probably been augmented on supplementation of other natural

herbs. It was also observed that supplementation with all three herbs improved overall total phenolic contents of all the three tea samples, which indicated improvement in total antioxidant capacities of the supplemented packaged samples.

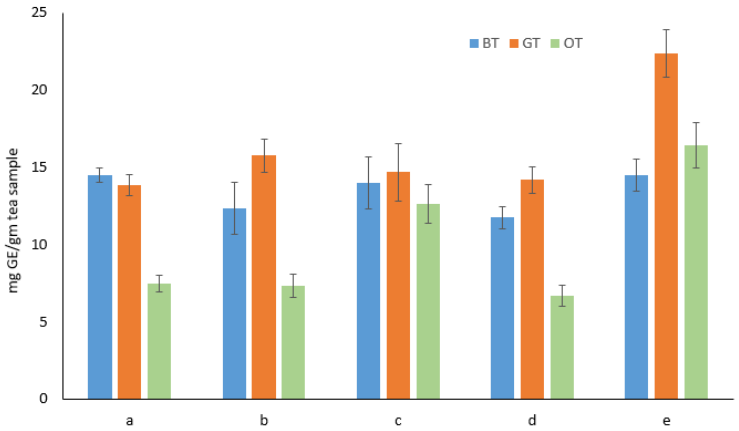

Figure 5. Comparative Total phenolic contents in different tea bags. Results are expressed as mean±SD of three individual experiments. Codes: BT – black tea; GT – green tea; OT – oolong tea; a – tea samples without herbs; b – tea samples with mint; c – tea samples with ginger; d – tea samples with basil; e – tea samples with three herbs.

Fig.6 indicated that DPPH radical scavenging was more for black and green tea than oolong tea without any supplementation. The activity was almost unchanged if black and green tea were supplemented with mint. However with other herbs, radical scavenging activity reduced in all three samples. DPPH assay system involves determination of less polar bioactives of a sample (Chakraborty and Bhattacharyya, 2014). Since all the herbs contain greater proportions of more polar bioactives, it was rightly reflected in the assay results as supplementation augmented more polar bioactives which are beneficial for physiological systems.

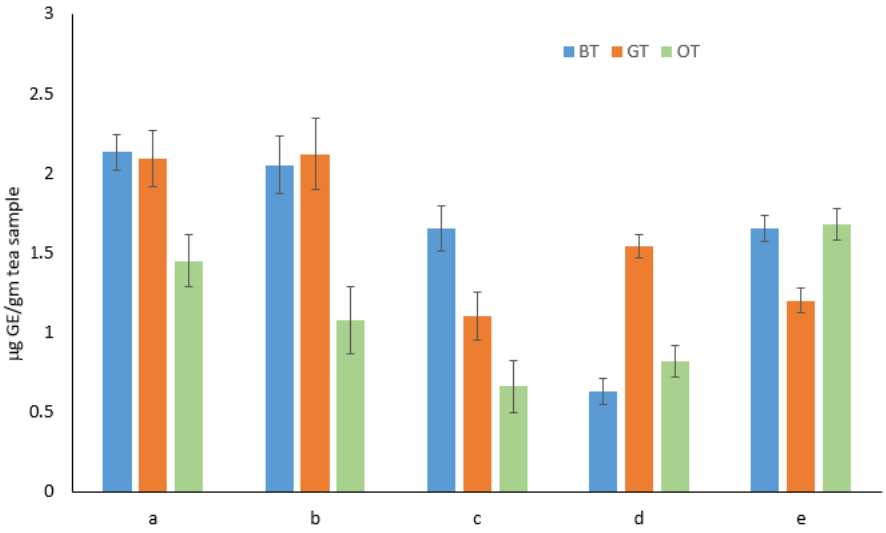

Figure 6. Comparative DPPH radical decolourization activities of different tea bags. Results are expressed as mean±SD of three individual experiments. Codes: BT – black tea; GT – green tea; OT – oolong tea; a – tea samples without herbs; b – tea samples with mint; c – tea samples with ginger; d – tea samples with basil; e – tea samples with three herbs.

Fig. 7 depicted the acceptability of the differently blended tea among the panel members. Results are furnished as per cent people liked the specific recipe. It is clear that there was no difference in the acceptability of the tea before and after addition of the herbs, clearly indicating the commercial potential of the prepared tea bags.

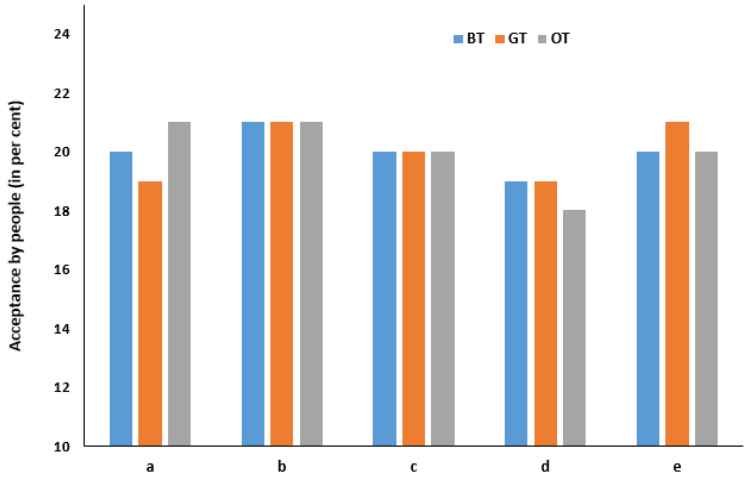

Figure 7. Acceptability of different tea bags among panel members. Results are expressed as per cent member liked the recipe. Codes: BT – black tea; GT – green tea; OT – oolong tea; a – tea samples without herbs; b – tea samples with mint; c – tea samples with ginger; d – tea samples with basil; e – tea samples with three herbs.

CONCLUSION

Although tea bags are a better choice because of the convenience of use in the present day fast life, there were some observations in the present study that should be kept in the mind. Apart from mint supplemented black tea bags, there is a possibility of lowering of tannin content in the infusions which in turn might affect the taste of the infusions for which black tea is treasured worldwide as a popular drink. However, there was an added advantage of mint supplementation in the antioxidant potential of all the three tea samples as the flavonoid contents became higher. Another important observation was that supplementation of other herbs might not augment the total phenolic contents of the samples, but they, on the other hand, did not diminish the quality of the infusions which means that supplementation could not deteriorate the antioxidant potential of the supplemented tea samples. It was also observed that total phenolic contents improved for green and oolong tea bags when the three herbs were supplemented in tandem. Based on the results obtained, it can easily be concluded that tea bags prepared with mint, ginger and basil supplementation might be useful due to their better antioxidant potential, better flavonoid contents and last but not the least, at par acceptability compared to tea bags without any supplementation.

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