



Research Paper

Valorisation of *Camellia sinensis* branches as a raw product with green technology extraction methods



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ABSTRACT

This work deals with the study of tea stalks from pruning debris using environmental friendly extraction technology to offer new healthy properties. In the manufacturing tea industry, tea trees require to be pruned every year and most of their remains are discarded as a waste with no economic value. Microwave aqueous extraction and pressurized hot water extraction process (autohydrolysis) were used to recover bioactive compounds from the tea branches. Operating at a fixed solid: liquid ratio (1:15), the effect of the maximum heating temperatures from 140 to 220 °C was studied. Liquid extracts were analysed for total phenolic, oligosaccharides, protein, mineral and heavy metals content, as well as for antioxidant capacity. The antitumoral possibilities were also determined for selected samples. The obtained results indicated that both processes could be used as an alternative to recover bioactive compounds from tea wastes, although microwave-assisted extraction allowed saving time when compared with autohydrolysis processing. The temperature exhibited a relevant effect on the total phenolic content and antioxidant capacity, decreasing with the microwave treatment and increasing with the autohydrolysis temperature. The obtained extracts could be adequate for incorporation in food and non-food fields.

1. Introduction

Green tea (*Camellia sinensis*) is rich in bioactive compounds and has antioxidant properties which provide favourable effects for health, prevent obesity, reduce the risk of diabetes (Chen et al., 2019) and cardiovascular diseases (Yang and Zhang, 2019), relieve inflammation, and are palliative for some cancer illness (Xing et al., 2019).

To increase the quality and productivity in tea manufacturing is recommended to maintain the shape of the brushes, and to prune them once a year. In this practice, leaves and branches are discarded as a waste material with no economic value. Previous studies (Henriques et al., 2017), have shown that tea plants have polyphenol compounds mostly in their leaves and fruits. Those phenolic compounds with high antioxidant activity can be recovered to give them a profitable value and subsequent application in the field of medicine, pharmacology, nutrition, and beauty while avoiding production of large volumes of organic waste that can affect the environment. Microwave technology or hydrothermic processes could convert these waste products into valuable raw materials with the consequent economic impact (Tsubaki et al., 2010).

Most phenols are found in the vacuoles of plant cells and to release them, it is necessary to break the wall cells. Microwave technologies could accelerate and potentiate the bioavailability of these phenolic

compounds and increase notably the antioxidant activity of the products (Hayat et al., 2019; Feumba Dibanda et al., 2020). Pressurized hot water extraction is another technology that is applied to extract phenolic compounds from terrestrial and marine samples achieving favourable results in terms of recovering bioactive compounds, as previously reported (Rodríguez-Seoane et al., 2019; Flórez-Fernández et al., 2019).

Nowadays, finding greener technologies is a priority in industry, where the extraction times would be as short as possible, in order to work with high efficiency, using non-polluting substances with reasonable cost of maintenance (Chemat et al., 2017). In this case, microwave or autohydrolysis technologies seem to point in this direction.

In this context, the main aim of this study is to acquire new properties from low value stalk and branch wastes of green tea from pruning debris using green extraction technologies.

2. Materials and methods

2.1. Raw materials

Tea branches used as raw materials in this work were kindly supplied by the Orballo Company, located in Donín (A Coruña) in the northwest of Spain. The raw material came from the annual pruning of *Camellia*

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sinensis (green tea) organic fields. In all cases, samples were maintained under cold storage (-20°C) until further processing.

2.2. Chemical characterization of the tea branches

Tea branches were initially crushed, and then their moisture, percentage ash, protein, mineral, heavy metal, and carbohydrate content were analysed to obtain the general chemical characterisation of the raw materials. The moisture and ash content were obtained by a standard gravimetric method using an air convective oven (105°C for 48 h) and a muffle furnace (575°C for 6 h), respectively. The total nitrogen content was assessed by mass spectrometry (FlashEA 1112 Elemental Analyzer, Thermo Fisher Scientific, Germany), and then converted to protein using the general N-protein conversion factor 6.25. The minerals (Na, K, Ca, Mg) and metals (Zn, Cd, Pb, Cu) content were determined by inductively coupled plasma optical emission spectrometry (Optima 4300 DV, PerkinElmer, USA), except for mercury content which was performed using cold vapour atomic absorption spectrometry (Fernández-Fernández et al., 2007). For the carbohydrate determination, milled tea branches were dried ($<10\%$ moisture content), then hydrolysed with H_2SO_4 (72%) in a water bath (30°C for 60 min). The recovered mixture phase was again hydrolysed with H_2SO_4 (4%) in an autoclave (121°C for 60 min). Afterwards, the obtained liquid phase was measured by analysed by high-performance liquid chromatography as explained in section 2.4.3.

2.3. Extraction technologies

Microwave assisted (MW) and autohydrolysis (AH) extraction processing were the two green technologies compared in this study to recover bioactive compounds. Fresh crushed branches were placed in each extraction device using distilled water as a solvent at the following operation conditions: liquid: solid ratio fixed at 15:1 (w/w) and a wide range of extraction temperatures (140, 160, 180, 200, and 220°C) was used. In the case of AH treatment, distilled water and ground sample were processed in a stirred pressure reactor (Parr instruments series 4842, IL, USA) of 0.6 L volume equipped with temperature controller and heater. The same conditions as the autohydrolysis were reproduced in a Microwave reactor (Anton Paar, Monowave 450, Austria) in order to obtain comparative severity factor (log Ro), i.e. the treatment efficiency, according to Overend and Chornet (1987).

The obtained liquid phases were filtrated and separated from the solid phases. The liquors were further analysed to determine their bioactive potential. Solid phases were set aside (-20°C) for future potential agricultural applications as adsorbent or fertilizer.

2.4. Liquor analysis

For each liquid filtrated fraction (liquors) the total phenolic content, antioxidant capacity as well as carbohydrates content and other derived groups were determined. The antitumoral potential of selected samples was also tested.

2.4.1. Total phenolic content

The total phenolic content was determined by the Folin-Ciocalteu test (Singleton & Rossi, 1965), based on the fact that the phenolic compounds react with the Folin-Ciocalteu reagent at basic pH giving rise to a bluish colour that can be determined by spectroscopy. An extract aliquot (0.25 mL) was mixed with distilled water (1.875 mL), the reagent diluted in distilled water (1:1, v/v) (0.125 mL) and sodium carbonate solution (0.25 mL, 10%, w/v) was added. Then, resting for 1 h in darkness at room temperature and the corresponding absorbance was read at 765 nm. The obtained results were reported as mg gallic acid equivalents (GAE)/g extract, since the gallic acid was employed as a standard.

In order to provide the results of this and following methods in g/g units, the dry weight was determined by a standard gravimetric method. Briefly, the liquors obtained by both hydrothermal treatments were

placed in a laboratory oven at 105°C until dried to a constant weight (24–48 h), which allowed defining the grams of solid present in each gram of liquor.

2.4.2. Antioxidant capacity

TEAC assay (Trolox Equivalent Antioxidant Capacity) was used to determine the total antioxidant capacity of the *Camellia sinensis* branches (Re et al., 1999). The assay measures the ability of antioxidants to scavenge the stable radical cation ABTS⁺ (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)), a blue-green chromophore with maximum absorption at 734 nm that decreases its intensity in the presence of antioxidants. Briefly, an aliquot of extract (10 μL) was added to ABTS⁺ solution (1 mL) and incubated at 30°C for 6 min before measuring the absorbance at 734 nm. Note that the obtained results were expressed as g Trolox equivalents antioxidant capacity (TEAC)/g extract.

2.4.3. Carbohydrates and other derived groups content

Carbohydrates and other derived groups were measured by high-performance liquid chromatography (HPLC). Briefly, the oligosaccharides content was obtained after posthydrolysis (H_2SO_4 (4%), 121°C , 20 min). Before HPLC measurements, all tested liquors were filtered through 0.45 μm membranes source. Subsequently, HPLC tests were made on a chromatograph (1100 series Hewlett-Packard) with a refractive index detector operating at 60°C . For these trials, a column (300 \times 7.8 mm, Aminex HPX-87H, BioRad, Hercules, CA) was used with a mobile phase of H_2SO_4 (0.003 M) at 0.6 mL/min.

2.4.4. Antitumoral activity

Cell inhibition for a selected human cell line was evaluated using the extracts with the best phenolic and antioxidant features obtained by microwave assisted (MW) and autohydrolysis (AH) extraction. The selected cell line for a preliminary screening of the antitumoral potential of the extracts obtained from tested wastes was a central nervous system cancer cell line (SF-268). Previous studies found that green tea exhibited potentiality in therapies of central nervous system (Pereira et al., 2016). Extracts were evaluated at different concentrations below 0.1 mg/mL, where no solubility drawbacks were identified.

2.5. Statistical analysis

All experiments were made at least in triplicate. Experimental data were statistically analysed using one-factor analysis of variance, ANOVA. A post-hoc Scheffé test was performed to distinguish means (95% confidence, $p < 0.05$). The PASW Statistics v.22 (IBM SPSS Statistics, New York, USA) software was employed as statistical software.

3. Results and discussion

3.1. Fundamental chemical features of the *Camellia sinensis* branches

Table 1 shows the fundamental chemical properties of the *Camellia sinensis* branches from pruning debris used as raw material in this study. Tea branches contained moisture content around 25% and low ash and protein amounts (about 3.5%). These values are consistent with those previously reported in a comprehensive review for green tea leaves, where protein values varied over the range 2–7% (Chen et al., 2016). Concerning minerals, calcium exhibited the highest values (4.5 g/kg) followed by potassium (3.5 g/kg). Within the metals, the tea branches stood out for their iron (196.5 mg/kg) and zinc (17 mg/kg) content.

Total carbohydrates content accounted about 80% of the tested wastes. Tea branches were mainly composed by glucose (around 43%), xylose (about 16%), mannose (about 10%) or galactose (around 6%). These polysaccharide values were also in close agreement with those previously reported for green tea leaves (Chen et al., 2008, 2016; Wang et al., 2008). The authors are not aware that these chemical data have been previously reported for tea branches.

Table 1
Fundamental chemical characterization of the used raw material.

Properties of tea branches		
Minerals (%)	Moisture (%)	24.74 ± 0.70
	Ash (% , d.b.)	3.57 ± 1.24
	Protein (% , d.b.)	2.30 ± 0.22
	Calcium	0.45 ± 0.01
	Potassium	0.35 ± 0.1
Heavy metals (%)	Magnesium	0.08 ± 0.02
	Sodium	0.03 ± 0.01
	Iron	0.019 ± 0.002
	Zinc	0.02 ± 0.01
	Cadmium	<0.0005 ± 0.0001
Oligomeric carbohydrates and other derived groups (%)	Copper	<0.002 ± 0.001
	Mercury	<0.0001 ± 0.0001
	Lead	<0.001 ± 0.001
	Total	80.89 ± 0.97
	Glucose	42.99 ± 0.54
	Xylose	16.16 ± 0.38
	Galactose	6.01 ± 0.36
	Mannose	9.92 ± 0.11
	Rhamnose	1.64 ± 0.09
	Arabinose	2.86 ± 0.07
	Fucose	1.30 ± 0.04
	Formic acid	2.59 ± 0.19
	Acetic acid	6.25 ± 0.03

3.2. Green extraction processes

The effect of the heating temperature during autohydrolysis and microwave-assisted water extraction of *Camellia sinensis* branches from pruning debris was studied following the scheme displayed in Fig. 1. After extraction, the liquid extracts were analysed for bioactive compounds and the solid phases were cold-stored for future potential applications as fertilizers or adsorbents (Xiao and Jiang, 2015; Hussain et al., 2018).

Fig. 2 shows representative heating and cooling profiles during water extraction of tea branches in the autohydrolysis and microwave-assisted extraction processes. It can be clearly observed that the necessary times in the microwave assisted extraction were notably shorter than those required in the autohydrolysis devices which are conventionally heated. Namely, the rise in operation temperature led to a reduction in extraction times (e.g. 50% for 160 °C and 70% for 220 °C). It should be highlighted that microwave-assisted extraction time of 250–300 s can give severity values comparable to those achieved by autohydrolysis (Table 2).

The extraction yields were slightly higher (about 10%) for samples treated by microwave-assisted extraction when compared with those treated by autohydrolysis. In both cases, the extraction yield exhibited a

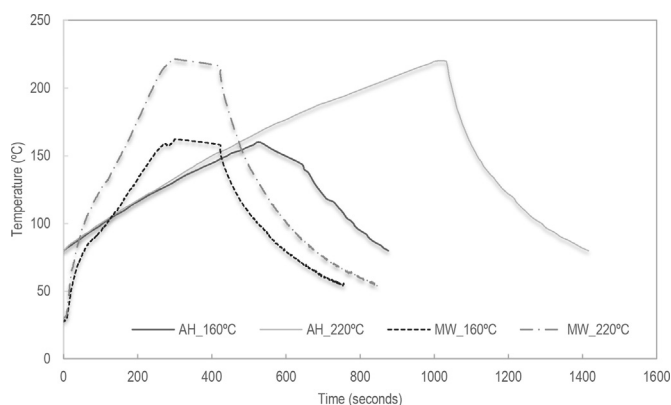


Fig. 2. Severity values for microwave (MW) and autohydrolysis (AH) processes at representative temperatures of 160 °C and 220 °C.

maximum at 220 °C, increasing the extraction yield with rising processing temperatures (between 35% and 45% for microwave and autohydrolysis, respectively). This behaviour was also reported for other natural materials in previous works (Hayat et al., 2019), where microwave heating of fennel seeds yielded better results even at shorter treatment times.

3.3. Liquid extracts features

Fig. 3 shows the effect of temperature on the total phenolic content of the tea branches extracts obtained by microwave and autohydrolysis processing. The total phenolic content statistically (p < 0.05) increased with increasing autohydrolysis temperature, whereas a reverse trend was observed for tea branches treated by the microwave method. It should be noted that the microwave method provided higher phenolic values than autohydrolysis below 180 °C, while the opposite tendency was detected

Table 2
Severity values for microwave and autohydrolysis processes at different tested temperatures.

Temperature (°C)	Range	Mean
140	[3.09–3.16]	3.13
160	[3.60–3.68]	3.65
180	[3.72–4.49]	4.18
200	[4.72–5.13]	4.83
220	[5.20–5.72]	5.42

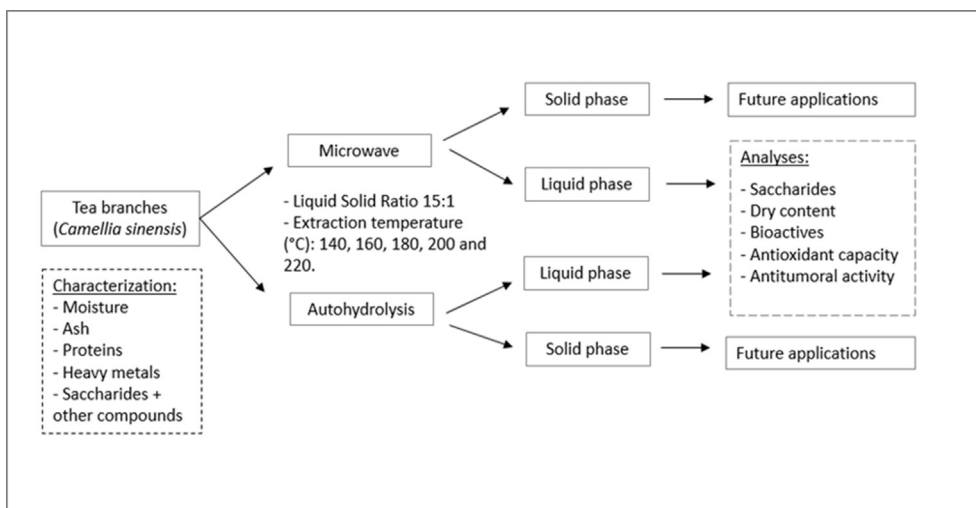


Fig. 1. General view of the performed processing with tea branches.

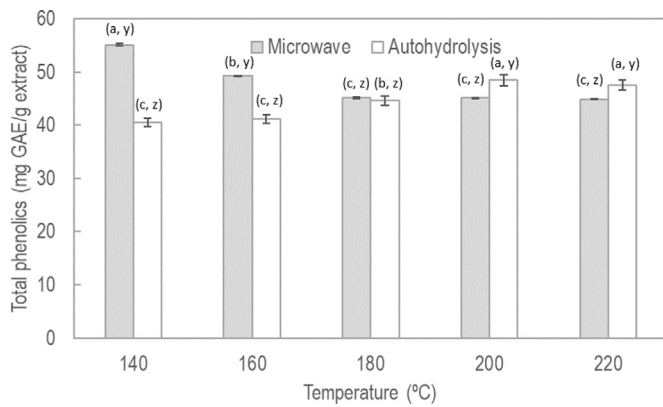


Fig. 3. Total phenolic compounds recovered using microwave (MW) and autohydrolysis (AH) processes. Note here that different letters corresponds with statically different samples ($p \leq 0.05$), in terms of temperature effect for each treatment (a–z) or extraction treatment at fixed temperature (x–z).

above this temperature. No statistical differences were observed between both processes at 180 °C. The highest phenolic content was identified at 140 °C for samples treated by microwave and 220 °C for those processed by autohydrolysis. Other authors (Feumba Dibanda et al., 2020) reported that lower microwave temperatures preserve the phenolic properties of treated samples, as well as some fruit peelings. Even though the obtained phenolic content was in the range of those found for other wastes from natural sources such as the leaves of the pruning branches of *Actinidia deliciosa* in the kiwifruit production (Henriques et al., 2017). Concerning phenolic behaviour with increasing temperature, similar tendencies were reported for other disposal or food materials using the selected environmentally friendly extraction technologies (Flórez-Fernández et al., 2018; Hayat et al., 2019; Rodríguez-Seoane et al., 2019; Feumba Dibanda et al., 2020). Latter works indicated that the differences observed in the thermal effect on the total phenolic content are related to the heating mechanism of both treatments. Microwave is a type of dielectric heating based on ionic and dipolar mechanisms, whereas autohydrolysis involves a conventional heating mechanism. The microwave dynamic movement causes friction and collision of the molecules, increasing the temperature and the structural degradation of the raw material in the solvent. This behaviour suggests a gradual inactivation of polyphenol and the antioxidant compounds deterioration with temperature. In contrast, the temperature increase in autohydrolysis treatment mainly involves a rise in the solubilisation of target components in the solvent.

The ABTS radical scavenging capacity for tea branches extracts exhibited similar tendencies to those observed for the phenolic content, increasing with temperature for extracts treated by autohydrolysis and decreasing for those processing by microwave (Fig. 4). Note here that a more marked drop than in the phenolic content was identified above 180 °C for microwave extracts. Again, the highest values were identified at 140 °C for microwave extracts and 220 °C for autohydrolysis ones. No significant differences were identified between 160 and 180 °C for both processes. The achieved values for the liquors of tea branches were consistent with those reported for other wastes as tea residues (green, oolong, and black) from tea drink manufacturers (Tsubaki et al., 2010) and a number of fruit peelings (Feumba Dibanda et al., 2020).

For both extraction processes, the extraction temperature was influential on the carbohydrates content, increasing with increasing temperature. In the microwave treatment (Fig. 5), the extraction temperature was important under 180 °C, but less influential at higher temperatures. In the autohydrolysis processing (Fig. 6), the carbohydrates recovery was dramatically influenced between 180 and 200 °C (more than the double). The highest carbohydrates content was identified between 180 and 220 °C for microwave processing and between 200 and 220 °C for the autohydrolysis treatment. At the highest temperatures, autohydrolysis led to higher carbohydrates recovery (around 20%). As expected from

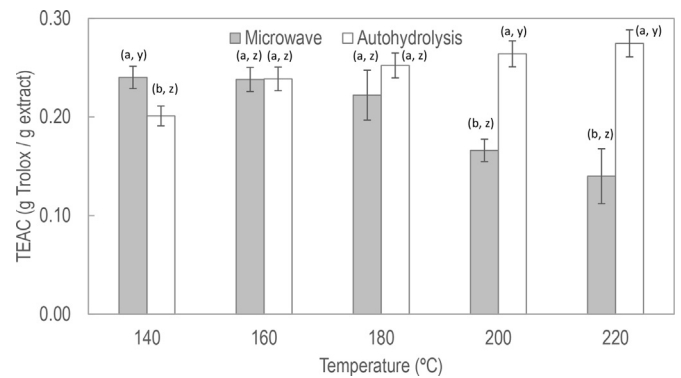


Fig. 4. Antioxidant capacity of liquors recovered from microwave (MW) and autohydrolysis (AH) process. Note here that different letters corresponds with statically different samples ($p \leq 0.05$), in terms of temperature effect for each treatment (a–z) or extraction treatment at fixed temperature (x–z).

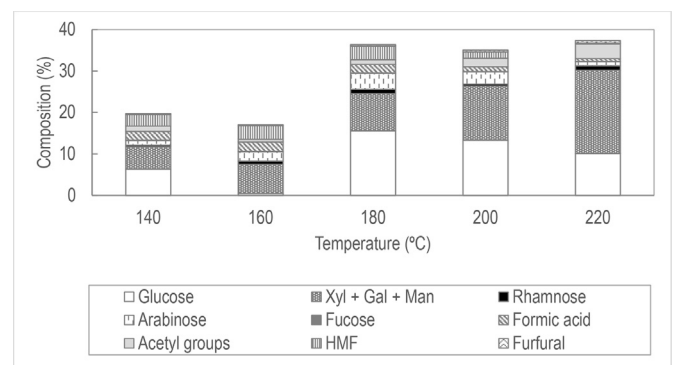


Fig. 5. Oligomeric carbohydrates and other derived groups in the extracts obtained using microwave (MW) treatment.

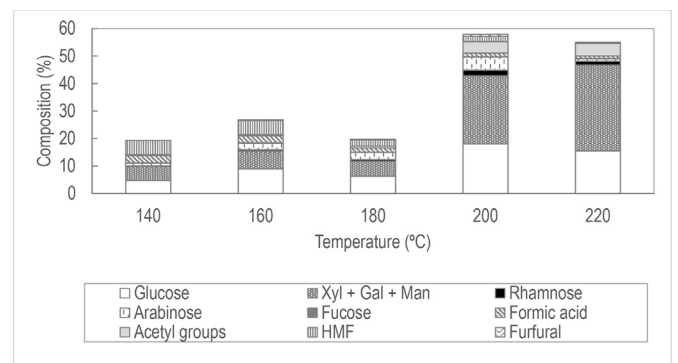


Fig. 6. Oligomeric carbohydrates and other derived groups recovered using autohydrolysis (AH) treatment.

the composition of the raw material, glucose, xylose (Xyl), galactose (Gal) and mannose (Man) accounted for the highest content. The obtained values and tendencies are consistent with those previously identified in tea leaves (Banerjee and Chatterjee, 2015; Chen et al., 2016).

The potentiality of selected extracts (*i.e.* those from microwave at 140 °C and autohydrolysis at 220 °C), with the highest bioactive features, as antitumoral agents for central nervous system cancer (SF-268) was assessed. Preliminary studies were made with this cancer cell line, since some recent works (Pereira et al., 2016; Miyata et al., 2018) suggested that green tea polyphenols from the leaves can be a natural alternative to inhibit carcinogenesis and malignant behaviour in the central nervous system, enhancing the drugs treatment by providing synergistic effects.

Regrettably, these tested extracts showed a low cell growth inhibition (<50%) at the maximum concentration tested (0.1 mg/mL), so it was not possible to perform cell growth inhibition curves. Namely, the cell growth inhibition was 47% for autohydrolysis extracts and 36% for microwave extracts at the maximum tested content. Further studies are required to analyse the possibilities of studied extracts on other cancer cell lines.

Overall, results indicated that both employed technologies could be attractive alternatives to recover bioactive compounds from tea branch wastes. The temperature exhibited a relevant effect on the total phenolic content, antioxidant capacity and carbohydrates content.

4. Conclusions

It can be concluded that microwave-assisted extraction for *Camellia sinensis* branches can save extraction time at analogous severity factors when compared with conventionally heated water extraction equipment. Microwave and autohydrolysis processes allowed value addition to the tea branches from pruning debris through the recovery of bioactive compounds in a broad range of temperature operating conditions. The recovered extracts could be suitable agents for food, pharmaceutical, or cosmetic applications with the main idea to develop new products and processes, which improve the quality of life, through green chemistry technologies minimizing the use of material, reducing energy and maximizing efficiency. Further studies on the investment or operating costs of each technique would be necessary considering a prospect of future industrial application.

Declaration of Competing Interest

Authors declare there is no conflict of interest.

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