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QUALITY CHARACTERISTICS OF OLIVE OIL AND BY-PRODUCTS FROM OIL MECHANICAL EXTRACTION PROCESS

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Abstract

The paper presents the characterization of four olive oil samples, originating from olives from the Mediterranean area, to which a set of analyses were applied. The identification of the merceological parameters and their reporting to the specialized literature, leads to the classification of the quality of the olive oil samples. Determination of parameters regarding the quality and/or phenolic composition of the samples leads to the subsequent analysis of by-products from the mechanical extraction process such as olive pomace and the production of phenolic powder obtained from mill waste water. Multiphase decantation for industrial olive oil extraction generates large quantities of a new by-product called olive paste, which consists of wet pomace pulp, which can be utilized.

Key words*: antioxidant activity, olive oil, olive powder, polyphenols, sustainability.*

INTRODUCTION

The food industry and agriculture are the most important branches of the agri-food industry in Europe (Palvic et al., 2023). Due to the high polyphenol content of extra virgin olive oil (EVOO), lipids are protected against oxidation. Regular consumption of 20 grams of EVOO (containing five grams of hydroxytyrosol and derivatives)/day contributes to a beneficial effect on health, according to Commission Regulation (EU) No. 432 (2012). The anticancer, chemopreventive, antioxidant, anti-inflammatory activities of extra virgin olive oil are due to the presence of hydrophilic substances (Beauchamp, 2005; Hashim et al., 2007; Covas, 2008; Servili et al., 2009). Virgin olive oil (VOO) contains numerous phenols (phenolic acids, alcohols, flavonoids, lignans, secoiridoids) (Obied et al., 2008; Servili et al., 2004). Secoiridoids are the main bioactive compounds in VOO, both quantitatively and qualitatively (Servili et al., 2004). EVOO from Mediterranean areas is obtained mainly by centrifugation technique (Roig et al., 2006), and the content of phenolic compounds in VOO and by-products resulting during the technological process is influenced by a series of agronomic, genetic etc. factors (Servili et al., 2009). In Italy, the three-phase centrifugation system is widely used because it ensures a dilution of the olive paste, producing between 50 and 90 litres of olive pomace water per second/100 kg of olive pomace. The resulting waste is an emulsion formed by pectin, oil and mucilage (Servili et al., 2011). Due to the high value of biochemical oxygen consumption, the polluting potential of olive pomace water is high (Niaounakis & Halvadakis, 2004), and its recovery is important due to the high biological value in secoiridoids (Roig et al., 2006) according to olives containing between 1-3% phenolic compounds in the weight of the fresh pulp (Garrido Fernandez, 1997). Improper management of waste resulting from the primary processing of plant-based raw materials, as well as waste resulting from product processing, leads to the quantitative formation of waste and, implicitly, to environmental pollution (Awasthi et al., 2021). The global waste problem (Lamolinara et al., 2022) could be solved by implementing zero-waste production schemes, using different

PREPARATION OF OLIVE OIL SAMPLES

The tested samples consisted of olive oils from different lots the olives coming from the Mediterranean area. The oil samples subjected to analysis were numbered as follows:
P1 - olive oil from olives in the Mediterranean area (Spain, Italy, Greece);
P2 - olive oil from 100% Italian olives from the Umbria region;
P3 - olive oil from olives from 100% Italian olives, Tuscany region;
P4 - olive oil from olives from 100% Italian olives, Terni, region of Umbria;
Laboratory analyses were performed within the Erasmus+ internship mobility, at the University of Perugia, Italy.

MATERIALS AND METHODS

The olive oil & olive paste samples were subjected to laboratory tests in order to: determine the moisture content, determination of free fatty acids, of the peroxide index, of the quality indices (K232, K270, ΔK) and extraction of polyphenols from olive oil for high-performance chromatographic analysis (HPLC), determination of lipid content, of the ash content, until obtaining the phenolic concentrate.

Reagents & Materials

The reagents and materials used were those available in the laboratory of the University of Perugia at the time of the experimental research, namely: acetic acid-chloroform mixture, ratio 3:1; saturated solution of KI; sodium thiosulfate ($Na_2S_2O_3$), starch solution 1%, methanol (N_2) methanol 80%, alcohol-ether mixture 1:1, phenolphthalein; alcoholic solution of NaOH (1%), NaOH (0.1N); olive oil from olives from different regions of Europe.

Methods for olive oil analysis

Determination of moisture

The moisture content of the olive oil was determined using a drying oven, according to the method described in ISO 662:1998, adapted to the working conditions.

Determination of free fatty acids

The determination of the acidity of olive oils was carried out in accordance with Regulation (EEC) No. 2568/91. About 5 g of olive oil sample was weighed into an Erlenmeyer flask (250 ml) and 50 ml of alcohol-ether solution was added. The titration was carried out with NaOH (0.1N) under continuous stirring in the presence of phenolphthalein as an indicator, until the colour changes.

Determination of the peroxide index

The peroxide value represents the amount of substances in the sample (expressed in millie-quivalents of active oxygen per kilogram) that oxidize potassium iodide under working conditions.
The sample in the form of a solution in a mixture of acetic acid and chloroform is treated with a solution of potassium iodide. Characteristics of olive oils and oils from olive residues, as well as methods of their analysis for the determination of peroxide value, was carried out in accordance with Commission Regulation (EEC) No. 2568/91 of 11 July 1991.In a glass Erlenmeyer flask (300 ml) with a capacity of 300 ml, weigh accurately 1-1.5 g of olive oil sample and add 25 ml of acetic acid-chloroform mixture and 0.5 ml of saturated KI solution. Leave for 2 minutes in the dark, then dilute with 25 ml of water and titrate with $Na_2S_2O_3$ using starch solution as indicator, until the color changes.

Determination of quality indices (K232, K270, ΔK) of olive pomace

Quality (K232, K270, ΔK) indices were determined using ultraviolet spectrophotometric analysis, according to the official analysis methods described in Regulation (EEC) no 2568/91. The olive oil sample must be perfectly homogeneous and free of suspended impurities. The olive oil sample was filtered using filter paper at a temperature of 30°C. Weigh about 0.25g (with a precision of 1 mg) of the sample thus prepared in a 25 ml graduated flask and fill it up to graduation with the isooctane solvent and homogenize. The solution+n obtained must be perfectly clear. The obtained samples are inserted into the spectrophotometer to read the absorbance at different wavelengths absorbance at different wavelengths (λ=232; λ=266; λ=270; λ=274), and subsequently the calculation formula:

$$K\lambda = A\lambda/(b*c)$$

where:
Aλ = absorbance read at the wavelength;

b = length of the quartz cuvette in cm;
c = concentration of the solution in g/100 ml.
Subsequently the calculation formula was applied to determine the variation of the specific extinction $\Delta K=K_{270}-(K_{266}+K_{274})/2$. The results obtained are expressed to two decimal place.

Extraction of polyphenols from olive oil for high-performance chromatographic analysis (HPLC)

Extraction of polyphenols from olive oil samples for high-performance chromatographic analyses was performed following the method described by (Taticchi et al., 2021), being adapted to the working conditions, as follows: the extract was taken with 2 ml of pure methanol, evaporated to dryness under a stream of nitrogen and stored at -25°C until analysis. HPLC of the phenolic extract was performed using Agilent Technologies 1100.
The analysis was performed after solubilization of the sample with 1 ml of methanol/water solution (50:50 v/v), and subsequent filtration.
The mobile phase was: 0.2% acetic acid (pH=3.1) in solvent A (water) with solvent B (methanol).

Methods for olive paste analysis

Olive paste production

Olive pomace samples were obtained from the Frantoio variety, and by using Multi-Phase Decanter technology (Leopard, Pieralisi Maip S.p.A., Jesi, AN, Italy) the olive pâté resulted, a pomace with high humidity and without pits.

Determination of moisture content

The moisture content of olive paste was evaluated using a drying oven, according to method B described in ISO 662:1998.
The method was adapted to specific working conditions. The olive paste stain sample was distributed on the surface of the aluminium capsule (±3.5 g) and placed in an oven (Binder GmbH, Tuttlingen, Germany) at 103±2°C, 24 hours until constant values were obtained.

Determination of lipid content

The lipid content of the olive paste was analysed using a Soxhlet extractor, the process lasting six hours.
Hexane was used as the extraction solvent, the solvent being removed using a Rotavapor R-210 rotary evaporator (Buchi Italia s.r.l Cornaredo, Italy).
The residual oil content was determined according to A.O.A.C., (1995).

Determination of ash content

For the determination of olive pomace ash the specific method was applied to soybeans, cakes, cottonseed meal, etc., as described in the A.O.C.S. (1983).

Characterization of pomace olive samples

Polyphenols were extracted as reported by (Selvaggini et al., 2014) with some modifications. Twenty grams of sample were ground with a blender (Osterizer Blender Cycle Blend Pulse 4153-50, Sunbeam Products, Inc., Boca Raton, FL, USA), then 5 g were collected, extracted with 50 ml methanol/water (80:20, v/v), which contained 20 mg/L butylated hydroxytoluene.
The methanol was evaporated using a rotary evaporator and subsequently the aqueous extract was used for solid phase extraction of phenols.
The extract was evaporated at 30°C, under vacuum, and the residue obtained was dissolved in 1 mL of methyl alcohol and analysed using a 1100 series high-performance liquid chromatography system (Agilent Technologies, Santa Clara, CA, USA), consisting of a vacuum degasser, quaternary pump, auto sampler, thermostatic column compartment and detectors and equipped with a column (Spherisob ODS-1 (250 nm*4.6 nm) and 5 nm particle size provided by Phase Separation Ltd. For quantitative analysis, an external calibration curve was used for each compound, except for isobascoside, quantified using the textuscoside response factor.
High-performance liquid chromatography (HPLC) analysis was performed after solubilizing the sample with 1 ml methanol/water (50:50 v/v) and filtering through a filter with a pore size of 0.2 μm.

Phenolic powder obtaining

The phenolic powder was obtained using spray-dry technology, maltodextrin was added as a stabilizer in the technological process (Extracta S.n.c., Muggio (MB), Italy) (Figure 1).

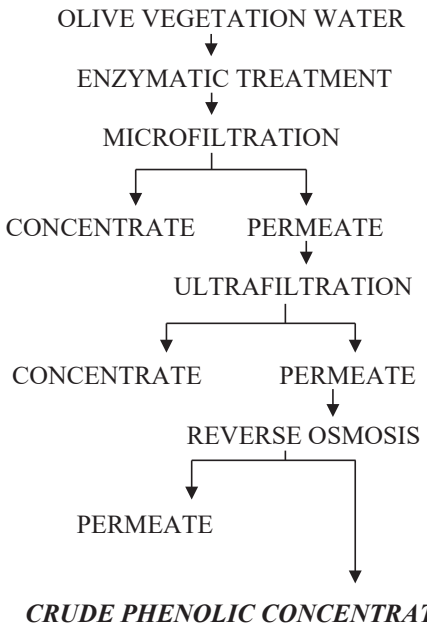

Figure 1. Obtaining the crude phenolic concentrate from the vegetation waters of olive mills (Servili et al., 2014)

RESULTS AND DISCUSSIONS

Current trends towards improving the sustainability of food systems indicate a transition towards increased reliance on plant-based foods, which include olives (Graça et al., 2019).

Olive oil

The olive oil samples tested achieved the parameters that determine their inclusion in the EVOO category, according to Annex 1 of the Official Journal of the European, Regulation (EC) No. 2568/91. The qualitative parameters, namely: total acidity, peroxide index, extinsion coefficient K_{232}, extinsion coefficient K_{270}, variation extinction coefficient ΔK, are presented in Figures 2, 3, 4, 5 and 6 According to Annex 1, the olive oil samples had an acidity value of ≤ 0.8, a peroxide number (mcq/O2/kg) of ≤ 20, a K_{232} value of ≤ 2.50, a K_{270} value of ≤ 0.22, and a ΔK value of ≤ 0.01, which allows the samples to be classified as extra virgin olive oils.

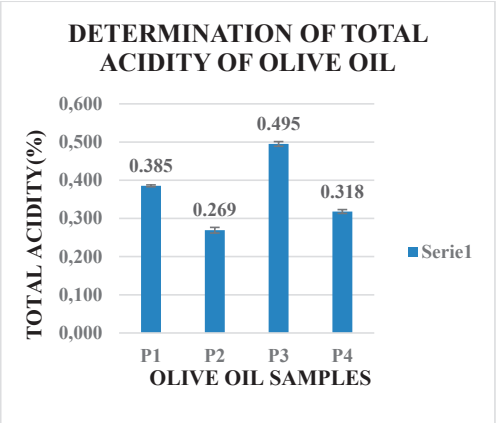

Figure 2. Determination of the acidity

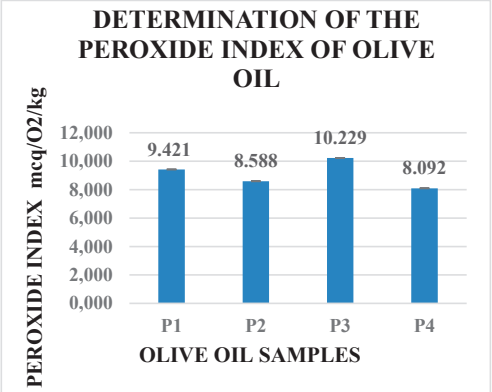

Figure 3. Determination of the peroxide index of olive oil

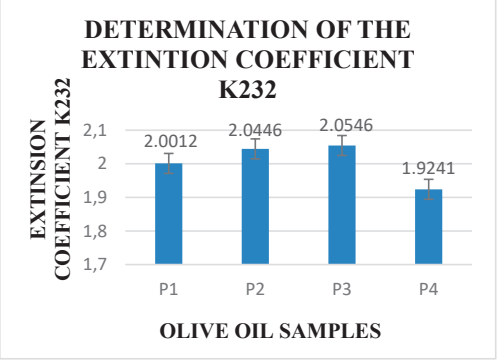

Figure 4. Determination of the extinction coefficient K_{232}

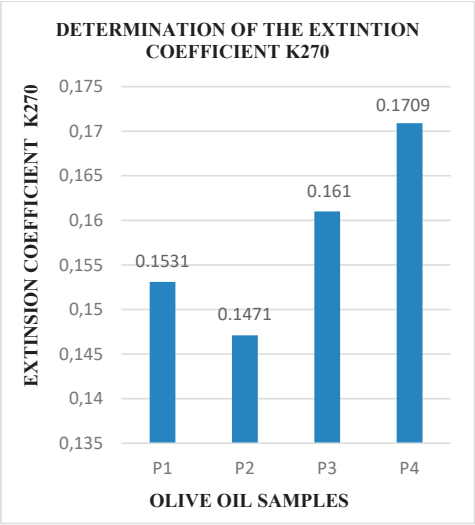

Figure 5. Determination of the extinction coefficient K_{270}

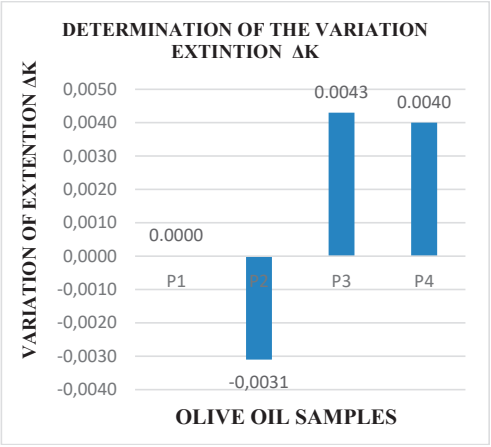

Figure 6. Determination of the variation extinction coefficient ΔK

The moisture content of the olive oil samples was: P1=0.00001; P2=0.00001; P3=0.00001 and P4=0.00001.

The polyphenol content of the analyzed olive oil samples is presented according to Tables 1, 2, 3, and 4.

The antioxidant activity determines the validity of the analyzed oils, as well as their quality.

In this case, the highest content in polyphenols is owned by sample P2, P4, P1, with sample P3 in last place

Table 1. Phenolic composition of olive oil (P1)

Phenolic composition of olive oil (mg/kg)*	P1		
Hydroxytyrosol (3,4-DHPEA)	**15.9**	±	0.2
Tyrosol (*p*-HPEA)	10.9	±	0.2
Vanillic acids	0.4	±	0.0
Oleacein(3,4-DHPEA-EDA)	271.9	±	1.5
Oleocanthal (*p*-HPEA-EDA)	81.9	±	0.5
(+)-1-Acetoxypinoresinol	21.7	±	0.001
(+)-Pinoresinol	16.6	±	0.004
Oleuropein aglycone isomer (3,4-DHPEA-EA)	188.6	±	0.2
Ligstrozide aglycone	14.2	±	13
Total phenols	**622.2**	**±**	**2.1**
Sum of oleuropein derivatives	476.4	±	1.5
Sum og ligstroside derivatives	107.0	±	1.4
The amount of lignans	38.3	±	0.004

*Results are the mean of two determinations ± standard deviation.

Table 2. Phenolic composition of olive oil (P2)

Phenolic composition of olive oil (mg/kg)*	P2		
Hydroxytyrosol (3,4-DHPEA)	**9.0**	±	0.2
Tyrosol (*p*-HPEA)	9.9	±	0.7
Vanillic acids	0.6	±	0.3
Oleacein (3,4-DHPEA-EDA)	382.9	±	4.8
Oleocanthal (*p*-HPEA-EDA)	148.7	±	2.4
(+)-1-Acetoxypinoresinol	31.4	±	0.02
(+)-Pinoresinol	21.0	±	0.03
Oleuropein aglycone isomer (3,4-DHPEA-EA)	279.6	±	3.9
Ligstrozide aglycone	30.2	±	0.9
Total phenols	**913.2**	**±**	**7.9**
Sum of oleuropein derivatives	671.4	±	6.2
Sum og ligstroside derivatives	188.7	±	2.7
The amount of lignans	5.4	±	0.04

*Results are the mean of two determinations ± standard deviation.

Table 3. Phenolic composition of olive oil (P3)

Phenolic composition of olive oil (mg/kg)*	P3		
Hydroxytyrosol(3,4-DHPEA)	**19.9**	±	0.6
Tyrosol (*p*-HPEA)	14.4	±	1.1
Vanillic acids	0.7	±	0.0
Oleacein (3,4-DHPEA-EDA)	244.2	±	7.9
Oleocanthal (*p*-HPEA-EDA)	72.7	±	0.1
(+)-1-Acetoxypinoresinol	34.6	±	0.005
(+)-Pinoresinol	15.9	±	0.01
Oleuropein aglycone isomer (3,4-DHPEA-EA)	201.3	±	4.7
Ligstrozide aglycone	14.9	±	1.1
Total phenols	**618.5**	**±**	**1.5**
Sum of oleuropein derivatives	465.4	±	9.3
Sum og ligstroside derivatives	102.0	±	1.5
The amount of lignans	50.4	±	0.01

*Results are the mean of two determinations ± standard deviation.

Table 4. Phenolic composition of olive oil (P4)

Phenolic composition of olive oil (mg/kg)*	P4		
Hydroxytyrosol (3,4-DHPEA)	**12.1**	±	0.1
Tyrosol (*p*-HPEA)	8.6	±	0.1
Oleacein (3,4-DHPEA-EDA)	345.2	±	3.0
Oleocanthal (*p*-HPEA-EDA)	109.3	±	0.7
(+)-1-Acetoxypinoresinol	28.2	±	0.004
(+)-Pinoresinol	17.1	±	0.01
Oleuropein aglycone isomer (3,4-DHPEA-EA)	261.3	±	4.9
Ligstrozide aglycone	26.0	±	0.03
Total phenols	**807.9**	**±**	**7.5**
Sum of oleuropein derivatives	618.7	±	5.7
Sum og ligstroside derivatives	143.9	±	0.7
The amount of lignans	45.3	±	0.01

*Results are the mean of two determinations ± standard deviation.

All the EVOOs showed high quality characteristics mainly related to phenolic content responsible of the health and sensory properties of the final product. Samples P1 and P3 showed a medium/high phenolic composition whereas the other two EVOOs (P2 and P4) showed and high phenols content with the P2 oil that reach the maximum level over 900 mg/g. All the EVOOs were abundantly included in the health CLAIM for the olive oil polyphenols (No.432, 2012).

Olive paste

The recently introduced multiphase decantation technology for industrial olive oil extraction generates large quantities of a new by-product (olives paste) consisting of partially defatted moist pomace pulp containing very few traces of pith (Durante M., 2019). Two samples of olive pomace from different lots, from the Frantoio olive cultivar, were tested.

Olive pomace samples were coded: PM1; PM2, where: PM1-Olive paste from the Frantola olive variety, lot 1 and PM2-Olive paste from the Frantola olive variety, lot 2.

The moisture content of olive paste samples is presented in Figure 7.

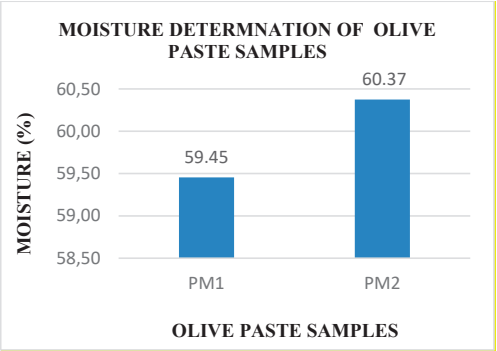

Figure 7. Moisture content of samples

The lipid content of olive paste samples is presented in Figure 8.

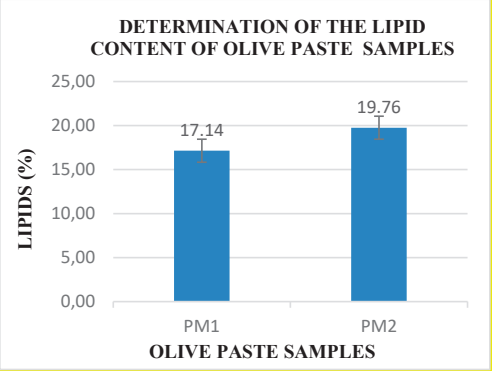

Figure 8. Lipid content of olive paste samples

The ash content of the two olive paste samples is graphically represented in Figure 9.

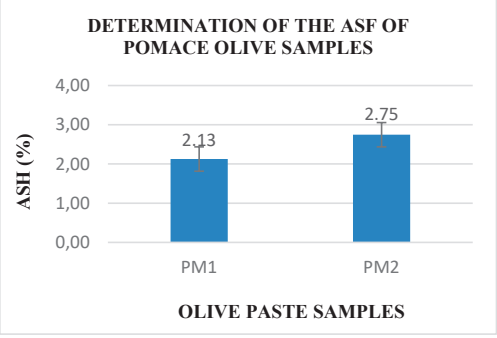

Figure 9. Determination of the ash of olive paste samples

The phenolic content of olive paste (mg/kg) is presented in Table 5.

Table 5. Characterization of phenols in olive paste (mg/kg)

*Phenolic content of olive paste (mg/kg)	PM1	PM2
Hydroxytyrosol (3,4-DHPE)	339.8±1.9	401.8±1.8
Tyrosol (*p*-HPEA)	74.5±0.9	163.6±0.3
Vanillic acids	5.4±0.001	5.1±0.002
Caffeic acid	7.3±0.01	14.5±0.04
Verbascoside	67.4±0.2	47.1±0.01
Oleacein (3,4-DHPEA-EDA)	371.7±2.4	80.9±0.5
Oleochanthal (*p*-HPEA-EDA)	152.9±0.5	89.7±1.2
(+)-1-Acetoxypinoresinol	16.4±0.07	15.9±0.01
Total phenoli	**1035.3±3.1**	**818.6±1.8**

*Results are the mean of two determinations ± standard deviation.

CONCLUSIONS

The results of the analyzed olive oil samples were compared with the parameters in Annex 1 of Annex 1 of the Official Journal of the European Union, Regulation (EC) No. 2568/91, resulting in the inclusion of the samples in the EVOO category. Due to the rich content of polyphenols, the analysed olive oils have a reduced antioxidant activity, which determines their life span. The quality of the oils, as well as their durability over time, depends on the physicochemical parameters that olive oils possess. In this case, the highest content in polyphenols is held by the P2 olive oil sample from 100% Italian olives from the Umbria region, followed by the P4 olive oil sample from 100% Italian olives, Terni, from the Umbria region, then P1- olive oil from olives from the Mediterranean area (Spain, Italy, Greece), in last place being sample P3- olive oil from olives from 100% Italian olives, the Tuscany region. Recycling the powder obtained from the vegetation waters of olive mills into new matrices could contribute to achieving a circular bioeconomy with zero waste, as well as environmental sustainability.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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