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Research Article

Physicochemical characterization of Apis mellifera honey produced in Capanema, Parana: a step towards obtaining the Geographical Indication

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Abstract

The relationship between the physicochemical composition of honey and its phytogeographic origin can be a key factor for its certification and commercialization. This study analyzed the physicochemical parameters of 37 honey samples from *Apis mellifera* produced in Capanema, Paraná. The moisture, hydrogen potential (pH), ash, reducing sugars, non-reducing sugars, hydroxymethylfurfural according AOAC Internacional. The color of honey was evaluated by spectroscopy at 650 nm. Moisture (16.00% - 19.22 %) and hydroxymethylfurfural (0.11 - 55.61 mg Kg^{-1}) were the only properties that did not present significant differences at 5% significance among the honeys studied. Significant differences were observed in the pH (3.46 - 4.75), acidity (16.36 - 54.50 meq Kg-1), ash (0.12% - 0.41%), reducing sugars (44.78% - 73.75%), non-reducing sugars (0.61% - 33.30%) and color (0,049 - 0,304 nm). In addition, 18.9% of the samples presented lower inverted sugar content, and 59.4% presented higher sucrose content than that permitted by the Technical Regulation for the Identity and Quality of Brazilian Honey. The most prominent honey colors were White Water (40% of samples) and Extra White (20% of samples), obtained from apiaries bordering the Iguassu National Park and up to the border with Argentina. This information points to a strong link between the composition of honey and regional phytogeographic characteristics, which could support the application of a Geographical Indication. Furthermore, it's necessary to further validate through palynological studies.

Keywords: *Apis Mellifera*; Physical-Chemical Parameters; Capanema Honey; Phytogeography; Beekeeping

Introduction

Honey is a food product made from flower nectar, plant secretions, and secretions of sap-sucking insects are transported by honeybees and stored for maturation [1]. Honey is a concentrated sugar solution with a predominance of monosaccharides (glucose and fructose - 65%) and water (approximately 18%). Minor components include proteins, organic acids, minerals, aromatic substances, vitamins, phenolic compounds, and flavonoids. Honey is a complex food produced by practically all countries and widely used for human consumption due to its therapeutic potential, such as immunological, antibacterial, anti inflammatory, analgesic, sedative, and expectorant effects [2]. Honey cannot be considered a complete food by human nutritional standards; however, it is a healthy alternative because it can provide nutrients not found in commercial sugar, such as vitamin B. The original composition of this food depends on many factors, such as botanical origin, hive management, collection, processing, storage, and others. After harvesting, honey undergoes

chemical, physical, and sensory changes (color, flavor, odor), creating the need to produce it at high-quality levels, controlling all stages of its processing. Normative Instruction No. 11 of the Ministry of Agriculture and Food Supply [1] establishes general and specific compositional characteristics of the main varieties of honey commercialized in Brazil. According to this directive, the main labeling requirements indicate monofloral, multifloral, or honeydew origin, origin, sensory characteristics, and physical chemical properties. In Brazil, honey production is an essential economic activity. According to IBGE, in 2022, the country produced 60,966 tons of honey, generating a gross production value of R$ 957.811 million, with Paraná contributing significantly. The state of Paraná stands out in the Brazilian context for the production of honey and honey products, occupying the second position among the producing states in 2023, moving around 8.6 thousand tons of honey and being responsible for 15.7%, on average, of the production in the entire country [3]. Located in the southwest of Paraná, Capanema borders Argentina and the Iguassu National Park, and due to its native vegetation, it is possible to produce honey with unique characteristics, mainly in color. In this sense, the characterization of the honey produced in Capanema from native flowers is essential as part of a strategy to add value to the product and protect biodiversity and regional identity, known as Geographical Indication.

Geographical Indication (GI) refers to a quality attributed to a product originating from a territory whose characteristics are inherent to its geographical origin. It represents a quality related to the natural environment or human factors, which gives it notoriety and territorial specificity. The registration of a GI enables refractory gains in a multifaceted range of potentialities in development. GI products are higher quality than individual brand products [4]. On the other hand, by offering higher quality products, producers can access markets more easily, which is one of the reasons for adopting GI [5,6].

Furthermore, higher prices are consistently seen as one of the primary benefits producers can access by participating in a GI [7,8]. This is because consumers tend to be willing to pay more for a higher quality product [9]. Another benefit is increased production [10] and promoting collaboration between local actors [8]. GI can be used as a management strategy to promote regional development, which is related to economic growth and quality of life. It is also essential to highlight the conservation of the environment, strengthening the sense of belonging, and the protagonism of the inhabitants of that region.

Because of the above, this study analyzed samples of honey originating from Capanema. Its goal was to identify differences in physical and chemical parameters concerning the location of the different communities within the municipality and verify whether the samples met the maximum and minimum limits determined by Brazilian legislation.

Material and Methods

Sample collection

Thirty-seven (total n = 37) honey samples from Africanized bees (*Apis mellifera*) were collected and provided by Capanema and Region Beekeepers Association beekeepers from the 2022/2023 harvest. These samples were distributed across seven communities: Santa Clara (n = 3), Santa Ana (n = 8), Cristo Rei (n = 5), São Pedro (n = 2), São Luiz (n = 3), São Francisco (n = 11), and Lajeada (n = 5), as shown in figure 1.

The municipality of Capanema is located in the southwest of the state of Paraná and borders Argentina, on the banks of the Iguassu River, at an altitude of 368 meters, latitude 25º 40’ 19”S, longitude 53º 48’ 32” W and a total area of 418.7 km2. The climate is characterized as a humid mesothermal subtropical climate, with hot summers (average temperature above 22 °C) and a tendency for concentrated rainfall and winter (average temperature below 18 °C) with infrequent frosts, with no defined dry season.

The samples were stored in transparent plastic containers with screw caps, with a capacity of 250 g, as they are commercially available, and sent to the laboratory, where they were stored in the dark at room temperature until the time of analysis.

Analytical procedures

Analyses of moisture, hydrogen potential (pH), acidity, ash, and hydroxymethylfurfural to characterize the honey were performed according to the methodology established by AOAC methods [11]. The samples were diluted to 1 g L^{-1} for the total sugar analyses, according to the 3-5-dinitrosalicylic acid colorimetric

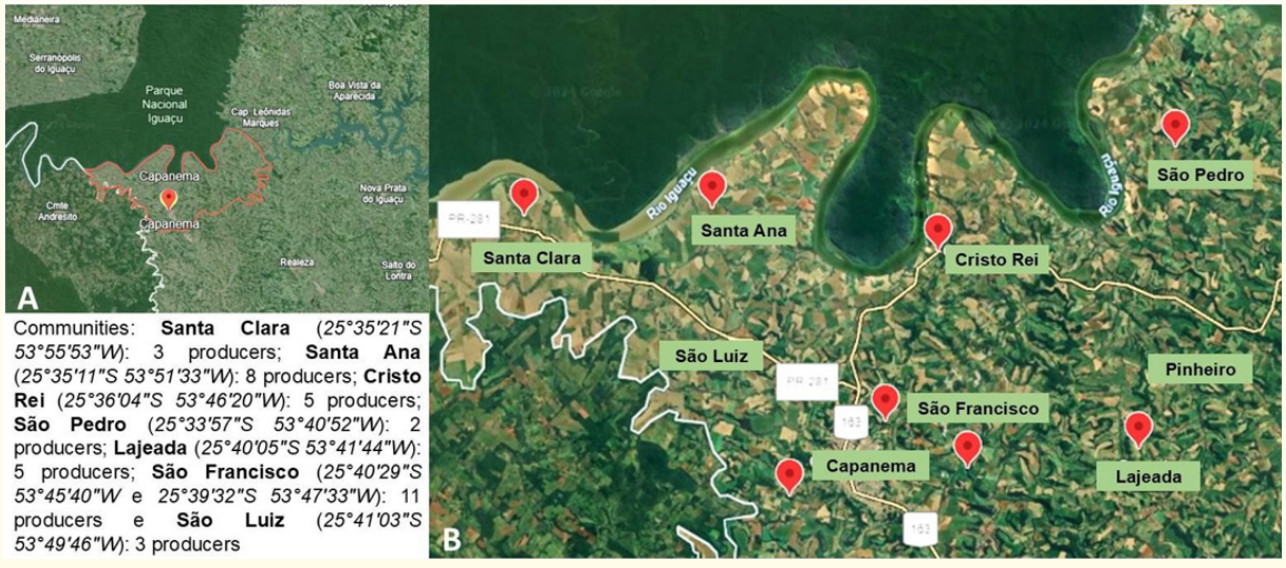

Figure 1: (A): Geographic location of Capanema – PR; (B): Location of honey-producing communities in Capanema – PR.

method [12]. The sugars were quantified by spectrophotometry (Thermo Scientific™ Evolution 60S) at a wavelength of 540 nm, using a glucose standard curve whose concentration ranged from 0.0275 g L^1 to 0.3725 g L^{-1} (R^2 = 0.987). To determine the color of honey, the Bianchi method was used [13], 5 g of honey was dissolved in water. Subsequently, the volume was completed in a 10 ml volumetric flask, homogenized, poured into a cuvette, and left to stand for 15 minutes. The reading was performed at 635 nm, using distilled water as a blank. The color was calculated using the equation: Color = – 38.70 + 371.39 * Abs and expressed in mm PFund (Table 1).

Color							
	Water white	**Extra white**	**White**	**Extra light amber**	**Light amber**	**Amber**	**Dark amber**
mm Pfund	0 - 8	8 - 17	17 - 37	34 - 48	48 - 83	83 - 114	> 114
Abs*	< 0.125	0.125 - 0.148	0.148 - 0.195	0.195 - 0.238	0.238 - 0.333	0.333 - 0.411	> 0.411

Table 1: Honey color classification and respective ranges according to the PFund scale.
*Absorbance at 650 nm. Source: Adapted from Montenegro., *et al*. (2005).

Statistical analyses

All analyses were performed in triplicate, providing excellent reliability to the results obtained at the Multiuser Laboratory of Food and Environmental Biotechnology from the Universidade Tecnológica Federal do Paraná (UTFPR), Câmpus Dois Vizinhos, PR. The results were analyzed using analysis of variance (ANOVA) and F test at a 5% significance level.

Results and Discussion

Honey commonly presents variations in its physical and chemical composition, given that several factors interfere with its quality, such as climatic conditions, stage of maturation, bee species, processing and storage, and the type of flowering [14]. The results obtained from the physical-chemical parameters of the 37 samples of

honey from *Apis mellifera* bees analyzed in this study are presented in table 2. At a 5% significance level, there was a difference between the honey samples produced in the studied communities, except for the humidity and hydroxymethylfurfural variables.

Physicochemical parameters (n = 37)	Minimum	Maximum	Average ± s*	*p-valor*
Moisture (g $100g^{-1}$)	16.00	19.22	17.96 ± 0.53	0.56
pH	3.46	4.75	4.27 ± 0.28**	0.00
Acidity (meq Kg^{-1})	16.36	54.50	31.04 ± 8.8**	0.00
Ash (g $100g^{-1}$)	0.12	0.41	0.18 ± 0.03**	0.00
Reducing sugars (%)	44.78	73.75	64.01 ± 6.42**	0.00
Non-reducing sugars (%)	0.61	33.30	10.24 ± 7.43**	0.00
Hydroxymethylfurfural (mg Kg^{-1})	0.11	55.61	13.43 ± 13.3	0.06
Color (650 nm)	0.049	0.304	0.143 ± 0,.5**	0.00

Table 2: Physicochemical parameters (minimum, maximum, average, and standard deviation values) of Capanema's 2022/2023 Apis mellifera honey harvest.
*s: Standard Deviation, **means differ statistically from each other, in the same line, by the F test ($p < 0.05$).

The moisture content in the honey samples produced in seven communities in the municipality of Capanema did not differ statistically ($p > 0.05$). It was lower than the moisture content of the honey produced in the municipalities of Santa Helena and Terra Roxa [15], within the parameters established by current Brazilian legislation, which establishes a maximum of 20 g of moisture 100 g^{-1} of honey [1]. Water content above 21% favors the development of yeasts naturally present in honey. The percentage of water in honey is important for its quality since it influences the viscosity, specific weight, maturity, crystallization, flavor, and conservation of this food [16]. Honey moisture depends on the composition of the nectar used by the bees, the time of year and the degree of maturity reached during honey collection, environmental humidity [17, 18], honey processing techniques and product storage conditions [19]. [20] evaluated the organic honey harvest 2011/2012 from *Apis mellifera* produced by beekeepers in the center-south and southeast regions of Parana, obtaining 19% moisture.

The average pH value (4.27±0.28) and free acidity (31.04±8.8 meq Kg^{-1}) of honey samples from the municipality of Capanema differed statistically ($p < 0.05$) among the communities evaluated (**Table 2**). The pH may change based on collection and storage conditions, and the acidity of honey can influence determining flavor and stability against microbial growth. [21] state that the pH of the nectar, the composition of the soil, or the association of botanical varieties in the composition of the honey are factors that affect this parameter. Honey with a pH lower than 4.0 is acidic, which can help preserve the product by inhibiting microbial growth. Although pH is not a mandatory criterion for quality control, it is an important parameter because it influences the speed of formation of hydroxymethylfurfural, an indicator of honey quality [14,15]. working with honey samples from Santa Helena and Terra Roxa in Paraná, reported values of 3.98 and 3.94, respectively. [22], in honey samples from different regions of Paraná, showed a higher range, from 3.30 to 5.50, reaching higher values, as did [14], who reported pH values between 3.54 and 5.30 for honey from different flowering periods produced in the State of Piauí, and [23] who studied samples of *A. mellifera* honey from the State of Ceará and obtained values between 3.4 and 5.3.

Other studies carried out in Brazil have reported pH values between 3.63 and 4.68 in the South [24], 2.30 and 4.60 in the Southeast [25], 3.30 to 3.97 in the North [26] and between 3.65 and 4.25 in the Northeast [27]. According to national and international regulations - Adolfo Lutz, Codex, and European Union standards [28] - pH determination should be performed at a fixed concentration of 10% (w/v) honey. However, pH analysis is influenced by sample dilution, the presence of ions, and their interactions with other components such as sugars, amino acids, and salts. This, pH value is related to soil properties and the geographic origin of the honey.

The acidity of honey has its origin in the variation of organic acids present in different sources of nectar (floral origin), by the action of the enzyme glucose-oxidase, which produces gluconic acid through the action of bacteria during honey maturation and by the amounts of minerals present in it. In addition, these organic acids in honey remain in balance with internal esters, lactones, and some inorganic ions such as phosphates, sulfates, and chlorides [29]. The acidity of honey contributes to its stability against the development of microorganisms, but on the other hand, it may indicate irregular storage conditions and the occurrence of fermentation. Although acidity is not directly related to soil characteristics, it can be attributed to its different floral origins. Among the producing communities, four of them - Santa Clara, Santa Ana, Cristo Rei, and São Pedro - border the Iguassu River and the Iguassu National Park, which is known for housing the largest remnant of Atlantic Forest in the Southern Region of Brazil and for having a diverse flora, with different botanical species. This variability of botanical origin influences the composition of organic acids and amino acids and may be directly related to the location of each of the properties.

The ash content is determined mainly by the soil and climate characteristics and may have a complex function of floral and geographic origin. Low ash content may indicate that the honey is monofloral, just as honey with high ash content may indicate poor management and/or collection by beekeepers [30]. Brazilian legislation allows an ash content (quantity of minerals in honey) of no more than 0.6% for *Apis mellifera* honey (1). All samples analyzed presented values consistent with the legislation (0.18±0.03 g $100g^{-1}$), and the value significantly differed among honey from the communities. On the other hand, the ash content directly relates to the honey's color. [31] showed that darker honey may have four to six times more mineral salts than lighter honey, emphasizing the presence of manganese, potassium, sodium, and iron.

Sugars are the main constituents of honey, with reducing sugars, that is, the monosaccharides glucose and fructose, found in more significant quantities, making up an average of 64% of the total amount of sugars in the honey evaluated. Disaccharides, non-reducing sugars, such as sucrose and maltose, represent approximately 10%. The Technical Regulation for the Identity and Quality of Honey (RTIQ-MEL) [1] specifies that the content of reducing sugars must be at least 65g $100g^{-1}$. The content of reducing sugars showed an average of 64.01% (Table 2), and 18.9% of the samples presented an inverted sugar content lower than that established by legislation. The values of this study are lower than those reported by [32], who reported a variation of 73.29 to 79.05%. According to RTIQ-MEL (1), the sucrose concentration must be less than 6g $100g^{1}$ for floral honey. Of the honey evaluated, 59.4% had sucrose content above that permitted by law. [33] when analyzing honey samples from the state of Ceará, found that 10% of the honey samples were above the permitted by law, with an average of 2.71%. [34] reported that sucrose concentrations above those permitted by law may indicate premature harvesting of the honey, in which the sucrose has not yet been completely converted into fructose and glucose by the action of the enzyme invertase. On the other hand, the higher sucrose content could be because of their low moisture content levels [35]. [23] found that 5.8% of the samples analyzed were above the regulatory limits [1]. They suggested the bees may have collected other material besides nectar to form honey. Sucrose concentration is a criterion for differentiating monofloral from polyfloral honey. In the western region of Paraná, beekeepers have been adopting winter feeding with crystal sugar supplementation in surface feeders, claiming that bees do not incorporate this solid sugar into the honey. [32] even obtaining sucrose values within the maximum limit of current legislation, highlighted that the sucrose concentration in the western region was higher ($p > 0.05$) than in other areas of Paraná. These excess values in some samples may indicate the addition of sucrose to the honey or adulteration through contamination by leftovers from winter feeding intentionally added or not removed by the beekeeper. According to [1], honey cannot have added sugars or other substances that alter its original composition. Therefore, in the honey nutritional table, sugars must be counted as total sugars. In these cases, the amount of added sugars will be zero.

Honey sugars, especially hexoses such as fructose and glucose, can be acid-catalyzed and dehydrated, generating the compound hydroxymethylfurfural (HMF). The production of HMF causes the formation of a dark liquid layer on the surface, rendering

the honey unusable for consumption. Low HMF content is recognized as a parameter of honey freshness, while high levels of HMF may indicate overheating, inadequate processing, or adulteration with syrups. The levels of HMF accepted by the European Community and Brazilian legislation are a maximum of 40 mg Kg^{-1} and 60 mg Kg^{-1}, respectively [1,28]. Of the samples evaluated, there was no significant difference ($p > 0.05$) of HMF in honey (Table 2), and all are classified as fresh honey (0.10 - 55.61 mg Kg^{-1}) by Brazilian legislation; however, only one sample (55.61 mg Kg^{-1}) falls above European legislation. Studying the freshness index of honey samples from the Bordj Bou Arreridj region, [36] reported HMF indices ranging from 2.24 to 27.88 mg Kg^{-1}, and one sample with a higher value (45.25 mg Kg^{-1}) than that established by European legislation.

The color of honey is the main factor for its commercialization due to its visual aspect, which defines consumers' quality, preference, and acceptance. It is characterized as one of the parameters of sensory characteristics and can vary from almost colorless to dark brown. Through color analysis, it was observed (Figure 2) that most samples from Capanema were classified as being Water White (40% of samples) to Extra White (20% of samples), which represents a maximum absorbance value of 0.148, at 635 nm, and in practice indicates honey with a very light color. It is suggested that light honey contains lower concentrations of reducing sugars. The honey color is an important quality parameter that needs to be evaluated. It can be influenced by floral origin, mineral content, nitrogen, free amino acids and sugars, and also by processing and storage conditions, climatic factors, the temperature at which honey is produced inside the hive, moisture content, carotenoids and flavonoids. Darker honey has a higher nutritional value. However, honey with lighter coloration tends to be more accepted by consumers and has a higher sales value.

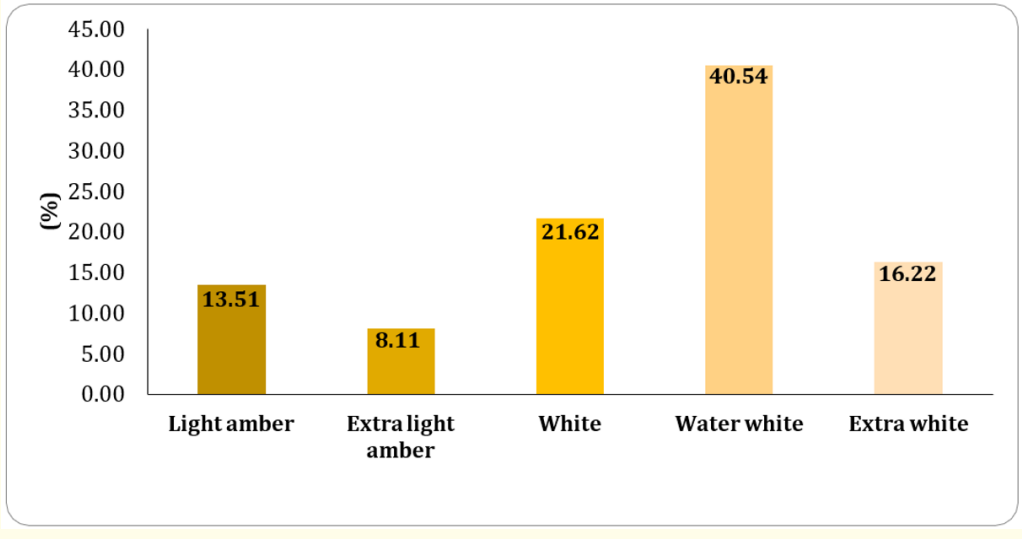

Figure 2: Comparison, in percentage, of the color of Capanema's 2022/2023 *Apis mellifera* honey harvest. The column names refer to the color honey established by [13].

Furthermore, the color may also vary depending on the management adopted by the beekeeper, with honeycombs from the first harvest having lighter colors than those from combs with dark wax. In the Capanema region, beekeepers use Langstroth hives, which are composed of modules, including the bottom, the brood chamber, one or more supers, and the lid. Due to their construction details, their structure make it easier for beekeepers to manage the hives. Continuous management of the nest and supers influences the final color of the product since it prevents the bees from depositing honey in combs darkened by the deposition of propolis. Another factor related to management indicates that harvesting honey immediately after the combs percolate also prevents the bees from eating part of this honey due to climatic influences.

Then, they fill these combs with honey from flowering, naturally producing darker honey. Furthermore, the precise honey color is guaranteed when the combs are centrifuged without bee eggs, larvae, or pupae.

Regarding coloration, some authors reported that lighter-colored samples were predominant in lakeside municipalities, while in municipalities far from lakes, samples with darker colors predominated [15,37], corroborating the results obtained for the evaluated honey. In Capanema, the native or reforested bee pasture is predominantly made up of flowering species native to the Atlantic Forest, and the species known as Louro-Branco (*Bastardiopsis densiflora*) and Cambará (*Aloysia virgata*) can be found with remarkable recurrence. [37] found that in honey classified as white or extra light amber from Santa Helena, pollen grains of *Hovenia dulcis* predominated, followed by *Eucalyptus* sp., while in amber honey from Terra Roxa, pollen grains of *Glycine max* predominated, followed by *Mimosa scabrella*, an exotic bee plant, widely planted by beekeepers in the region. Another important factor related to the variation in color between harvests and within the same harvest is that the harvest is commonly considered from September to April, and some beekeepers harvest a single harvest while others harvest several, separating more specific honey (from certain flowering periods).

Conclusion

According to the physical and chemical parameters analyzed, the honey samples generally meet the parameters recommended by Brazilian legislation, demonstrating a quality standard that ensures food safety for the product. The composition of the honey from the different apiaries was influenced by the different types of flowers make up the flora of each location. Regarding the physical and chemical characterization of the honey from Capanema-PR, the laboratory and statistical analyses showed similarities between the samples and their physical and chemical profile for some parameters for the harvest evaluated. The predominant color of the lighter honey is located in the region bordering the Iguassu National Park, mainly in the communities of Santa Clara, Santa Ana, Cristo Rei, and São Pedro and on the border with Argentina, near the community of São Luiz. On the other hand, some particularities were pointed out by the same statistical analyses about some parameters, such as the content of reducing sugars and sucrose. This information, supplemented by research conducted on pollen composition, indicates a strong link between honey composition and the phytogeographic characteristics of the region, which could supports the request for a Designation of Origin for honey produced by the Capanema and Region Beekeepers Association. For beekeepers, GI represents a concrete option to add value and generate wealth linked to the development of agribusiness, being able to serve the domestic and foreign markets.

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Conflicts of interest/Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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