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RESEARCH ARTICLE

Effects of Different Cooking Methods on Physicochemical and Antioxidant Properties of Climbing Perch (*Anabas Testudineus)*

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Abstract Climbing perch, *Anabas testudineus* is a freshwater fish recognised for its distinct flavour, high nutritional content, and medicinal properties. This species can be found in almost every Asian country, including Malaysia. However, the cooking methods applied can change the nutritional value of the climbing perch. This study aims to investigate the effect of four different cooking methods for climbing perch, which are frying, grilling, steaming and air frying, on their cooking loss, texture, colour properties, proximate composition, free fatty acid, total phenolic contents and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity. The result showed that the physicochemical properties, proximate composition and antioxidant activity were significantly ($P<0.05$) affected by the cooking method. Fried climbing perch samples were significantly higher ($P<0.05$) for cooking loss and texture. The steamed sample has the lightest colour with 40.39, and the fried sample has the highest value in redness and yellowness with 6.76 and 11.40 respectively. Proximate composition showed the highest value in protein, fat and carbohydrate in fried samples. Furthermore, free fatty acid was highest in the steamed sample (1.98%) and the lowest in the fried sample (1.39%). Fried samples showed the highest total phenolic content (TPC), 1.23 mg GAE/g and DPPH scavenging activity was highest in steamed samples (69.62%). In conclusion, aside from frying, steamed method emerged as the effective cooking method for preserving the nutritional value of the fish, as it exhibited the highest values in most of the analyses conducted.

Keywords: Climbing perch, frying, TPC, antioxidant, DPPH.

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Introduction

Climbing perch, *Anabas testudineus* is a high-priced freshwater fish that belongs to the family of Anabantidae and the order of Perciformes. They are known for their unique taste, great nutritional value, and recuperative and medicinal benefits [1]. This species can be easily found in Bangladesh, India, Pakistan, Burma, Sri Lanka, Thailand, Cochin-China, Tongking, Southern China, Philippines, and Malaysia [1]. It lives in marshlands, lakes, estuaries, canals, paddy fields, pools, swamps, small pits, and ponds [1, 2]..

Freshwater fish typically have a high protein content of 15– 20% [3]. Freshwater fish is also rich in polyunsaturated fatty acids (PUFAs), water-soluble vitamins viz., omega three (ω-3) and omega 6 (ω-6) PUFAs, which are widely recognised for their health benefits [4, 5]. According to [6], the oil extracted from *A. testudineus* exhibited omega-3 content at 2.45%, omega-6 at 5.58%, and PUFA at 55.95%. Omega-3 can inhibit cellular inflammatory processes, where inflammation could play a role in the initiation of some degenerative health disorders, both cardiovascular and endocrine [7]. Fat-soluble vitamins, viz., vitamins A, D, E and K, play crucial roles as essential nutrients in various biological activities within the human body, such as vision and bone maintenance, immune system regulation and reproductive function [4, 8].

Although fish is occasionally eaten raw, it is normally processed through methods such as cooking, grilling, baking, or frying. Heat is applied to food to improve flavour and taste, inactivate pathogenic germs, and extend shelf life [9]. There are three types of cooking methods: dry heat, moist heat, and combination heat. Dry-heat cooking methods use hot air or fat to cook the food (pan-frying, deep-frying, air-frying, grilling, broiling, roasting, and baking). Meanwhile, moist-heat cooking methods use liquid, usually water, stock, or steam, to cook the food (poaching, boiling, steaming), and combination cooking methods combine dry- and moist-heat methods (braising, stewing). Heat produces an edible texture due to protein denaturation, and the texture of fish muscle is altered. Protein denaturation limits muscle's water-holding capacity, resulting in muscle fibre shrinking and a firmer, more compact tissue texture [10]. The degree of heating for the cooking process should be controlled carefully because excessive heating or overcooking damages nutrition, colour, and other qualitative aspects of food [10].

On the other hand, cooking methods can alter the nutritional value of fish, such as proteins, vitamins and fatty acids. The heating produces an edible texture due to protein denaturation, and the texture of fish muscle is altered. Protein denaturation limits muscle's water-holding capacity, resulting in muscle fibre shrinking and a firmer, more compact tissue texture [10]. Grilled samples exhibited the hardest texture and did not contribute any flavour to the food. Loss of water and fat during the grilling process causes increased hardness in grilled samples [11]. Moreover, grilling causes protein denaturation, resulting in the loss of antioxidant enzyme activity or the release of catalytically active iron from metalloproteins [12]. Grilling also causes cell membrane disruption, which brings polyunsaturated fatty acids into contact with pro-oxidants and the thermal decomposition of hydroperoxides to pro-oxidant species like alkoxyl and hydroxyl radicals [12]. These radicals will increase the lipid oxidation chain process, which includes cholesterol.

Despite the widespread consumption in Malaysia, information remains scarce regarding the nutritional content of both raw and cooked climbing perch. Consequently, the health advantages of freshwater fish are often overlooked. Thus, this study aims to determine the effects of frying, grilling, steaming and air frying on the physicochemical properties of the fish following the cooking treatment. The proximate composition, free fatty acid and antioxidant properties of the fish after cooking were investigated.

Materials and Methods

Preparation of Samples

Climbing perch fish, *Anabas testudineus*, were obtained from a freshwater fish pond in Pasir Puteh, Kelantan. On average, the samples' length and weight were 15–20 cm and 30–70 g, respectively. They were transported to the laboratory in a plastic container (size 750ml). The fish were cleaned five times with tap water once they arrived at the lab to remove any remaining blood or excessive mucous. Following that, the fish samples were filleted with a fillet size of about 3 x 4 x1.5 cm, and the fillets were divided into five groups, each with three fillets. The first group was raw, uncooked samples, while the remaining four were processed using four different methods, which were frying, grilling, steaming and air frying.

Cooking Methods

For frying, three fish fillets were fried for 5 minutes in a frying pan with a capacity of 1 L at a temperature of approximately 165°C. About 150 mL of cooking oil was used for pan-frying. For the grilling method, the fish fillets were grilled for 5 minutes on each side of the grilling pan. The steaming method was performed using a domestic steamer at a water temperature of 99–101 °C for 12 minutes. For the air-frying method, the fish fillets were cooked in an air fryer (Khind Air Fryer ARF26) for 15 minutes at a temperature of 160°C. The fresh and cooked fish fillet was grounded using a food mortar and pestle to ensure the homogeneity of the samples for analysis. The remaining fresh samples were stored in polythene bags and kept frozen until further analysis. Each method was analysed in the same way and repeated for three readings each.

Physicochemical Properties Analysis

Cooking Loss

Climbing perch fish samples were weighed on a semi-analytical scale before and after each cooking method for three replicates per treatment, as stated by [13]. The following equation was used to calculate the cooking loss:

$$\text{Cooking Loss (\%)} = \frac{M2-M1}{M2} \times 100\%$$

Where; M_1 = Cooked weight (g)
M_2 = Raw weight (g)

Colour Measurement

The colour of cooked climbing perch fish samples was determined with a Konica Minolta Chromameter (Model CR-400 Chromameter, Konica Minolta, Japan). A white ceramic tile was used to calibrate the chromameter before the first use (Konica Minolta calibration plate). About three different spots on each cooked sample were measured with the chromameter. For each sample, three readings were taken. The colour on the chromameter was described as L*(lightness), a*(redness) and b*(yellowness) [12]. The readings were recorded.

Textural Analysis

Texture profile analysis was carried out following [14] with some modifications made using a texture analyzer (TA-XTPlus,Micro Stable Systems, UK). The samples were compressed by a probe with a blade (HDP/BSK), using pre-test speed of 1.50 mms^{-1}, post-test speed of 10.0 mms^{-1}, test speed of 2.0 mms^{-1} and distance of 20.00 mm. The study looked at shear and firmness in analysing the textural characteristics of the samples.

Determination of Free Fatty Acid (FFA)

Free fatty acid was analysed using an acid-base titration method. Firstly, 2 g of the homogenate samples were added along with 15 mL of solvent (95% ethanol with diethyl ether in a ratio of 1:1). Then, five drops of phenolphthalein indicator were dropped into the homogenates. The homogenates were titrated with 0.1N sodium hydroxide (NaOH) until light pink colour appeared and retained for at least 10 seconds. Free Fatty Acid (FFA) was expressed as oleic acid, and the FFA was calculated as below:

$$\textbf{\%} \text{ Free Fatty Acid} = \frac{mL\ NaOH \times N\ NaOH \times 282}{Sample\ Weight \times 10}$$

Molecular Weight of Oleic Acid = 282

Proximate Composition Analysis

Proximate composition analysis for homogenised samples of raw and cooked fish fillets was carried out in triplicate for protein, moisture, lipid and ash content [15]. The crude protein content was determined by the Kjeldahl method (Distillation Unit, Gerhardt, Germany). Moisture content was determined by oven drying at 105 °C to a constant weight. Total lipid extracted from the muscle tissue was determined by using the Soxhlet method (Soxtherm System, Gerhardt, Germany). For ash content, it was determined gravimetrically in a muffle furnace by heating at 550 °C to a constant weight. The carbohydrate values in fish from frying, baking, grilling and steaming methods were obtained by subtracting the percentage of moisture content, ash content, crude protein and crude fat by 100%.

Sample Preparation for Antioxidant Activity

Fresh and cooked fish were prepared in 90% aqueous methanol solution. Fresh fillets of climbing perch were cleaned with tap water and blotted dry. Approximately 1 g of raw and cooked fish was prepared into a paste using a food mortar and pestle. The paste was made into a final concentration of 100 mg/ml and homogenised separately for each of the samples.

Then, the homogenates were vortexed for 10 minutes and centrifuged at 3,000 g, 25 °C for 10 minutes to get a clear supernatant. Lastly, the clear supernatant was decanted and filtered through Whatman No. 1 filter paper and stored in an amber bottle at -20 °C for subsequent use. Total phenolic content (TPC) and DPPH (2,2-diphenyl-1-picrylhydrazyl) assay were conducted on triplicate samples of the homogenate [16].

Total Phenolic Content (TPC)

The total phenolic content (TPC) in cooked climbing perch extract was determined using the Folin-Ciocalteu method [17]. Gallic acid standard solutions with concentrations of 10-80 µg/ml were prepared in methanol. In 50 µl of methanol, 50 µl of extract (100 mg/ml) or standard solution was added. The mixture was then mixed with 50 µl of 10% F–C phenol reagent and 50 µl of 1 M sodium carbonate solution in a 96-well plate. As a blank, methanol was used. The reactions were incubated in the dark at room temperature for 90 minutes. Using a Microplate Reader, the absorbance was measured at 765 nm (Thermo Scientific, USA).

Determination of Antioxidant Activity by the 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) Method

The antioxidant activity of the samples was evaluated by using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [18] with some modifications. Antioxidant activity was determined by adding 40 µl homogenate of the raw and cooked fish fillet to 160 µl of DPPH solution (prepared as 0.049 mg/ml in methanol) in a 96-well microliter plate. A blank solution that served as a control was prepared using the same volume of methanol and DPPH. The reaction mixture was homogenised using a vortex and placed in the dark for 30 minutes at 37 °C. The absorbance was measured at 517 nm using a Microplate Reader (Thermo Scientific, USA). This absorbance was designated as sample absorbance (A sample) and each sample was analysed in triplicate. Antioxidant activity was expressed as per cent inhibition of DPPH radical using the following equation:

$$\text{Inhibition (\%)} = \left[\frac{Acontrol - Asample}{Acontrol}\right] x100$$

Where *Acontrol* is the absorbance of the 160 µl of DPPH solution and *Asample* is the absorbance of the sample.

Statistical Analysis

The statistical analyses for this research were conducted using IBM Statistical Package for the Social Sciences (SPSS). All the data were analysed using One-Way ANOVA to obtain the mean values from the triplicate samples of climbing perch fish from different cooking methods. Post hoc Duncan's multiple range test was used to determine the significant differences between means among the samples at a significant level of $p< 0.05$.

Results and Discussion

Physicochemical Properties of Cooked Climbing Perch

The physicochemical properties of cooked climbing perch with different cooking methods are presented in Table 1. Pan-fried climbing perch fillets showed the highest cooking loss (33.13%), followed by the air-fried samples (29.89%). Meanwhile, grilled climbing perch samples recorded a 25.58% cooking loss. Cooking loss for steamed samples was the lowest, at 14.73%. The cooking loss happens due to water evaporation and drippings of water and fat. Furthermore, the shrinkage of fish muscles from heat-induced protein denaturation leads to myofiber and collagen fibre shrinking, as well as the gelation of some soluble proteins [19]. This mechanism results in subsequent water expulsion, allowing water to be easily ejected throughout the heating process [20].

The work of shear for fried climbing perch was significantly higher (12.73 N/mm.sec) than other cooking methods. The lowest value of work of shear was recorded for steamed samples (0.49 N/mm.sec). A higher work of shear value indicates a firmer or harder sample, indicating that more force is needed to slice through it. The fried *A. testudineus* reported the highest value of firmness (3.30 N/mm), followed by the air fried (0.86 N/mm) and grilled samples (0.84 N/mm). The hardness can also be explained by observing that when the frying temperature rises, the heat transfer rate increases, leading to the formation of a crust on the surface and greater hardness [21]. Steamed fish exhibited the lowest firmness value of 0.16 N/mm, indicating that it has the softest texture. This can be attributed to the presence of water in the steaming method, which potentially leads to the reduction in muscle structure and the leaching out of soluble protein [22, 23].

Steamed climbing perch showed the lightest colour with the highest value of lightness (L*) (40.39), followed by the air-fried fish sample (38.18) and the grilled sample (31.42). Fried climbing perch was observed to be significantly darkest (30.89). The L* values are affected by the cooking temperature, which causes pigment degradation on the exterior layer of foods [20]. Moreover, the result showed that all cooked climbing perch samples exhibited a more pronounced red colouration as indicated by the values of redness (a*) falling within the range of 4.00 to 6.76. Among the cooking methods employed, the fried climbing perch demonstrated the highest a* of 6.76, followed by grilled samples with a value of 6.28, steamed samples (4.66) and air-fried samples (4.00). Similar findings were observed for yellowness (b*) values, where the fried samples exhibited the highest value of 11.40. Research by [22] deep frying showed a significantly increased ($p<0.05$) redness (a*) and higher yellowness (b*) value (8.23) of keropok lekor compared to boiling, steaming, air-frying, microwaving and oven cooking of keropok lekor. According to [24], mass and heat transfer processes occur during frying, resulting in physicochemical changes that impact the colour of fried Malaysian commercial fish sausage.

Furthermore, when fish are heated, various processes occur, including protein denaturation and browning, which impact the colour and flavour of the food [25].

Table 1. Physicochemical properties of cooked climbing perch using different cooking methods

Sample	Cooking loss (%)	Texture		Colour		
		Work of shear (N/mm.sec)	Firmness (N/mm)	L* (lightness)	a* (redness)	b* (yellowness)
Fried	33.13±0.22^{a}	12.73±3.93^{a}	3.30±1.50 ^{a}	30.89±0.53^{b}	6.76±1.34^{a}	11.40±0.88^{a}
Grilled	25.58±0.86^{b}	3.88±0.79^{b}	0.84±0.06^{b}	31.42±1.08^{b}	6.28±0.76^{a}	10.27±0.58^{ab}
Steamed	14.73±2.76^{c}	0.49±0.05^{b}	0.16±0.02^{b}	40.39±2.06^{a}	4.66±0.82^{ab}	10.67±0.45^{a}
Air fried	29.89±1.87^{a}	4.19±0.36^{b}	0.86±0.04^{b}	38.18±0.78^{a}	4.00±1.27^{b}	9.04±0.78^{b}

Data are expressed as mean ± standard deviation (n=3). Value with different superscript letters within the row a significantly different (P<0.05).

Proximate Composition of Raw and Cooked Climbing Perch

The proximate compositions of raw and cooked climbing perch with different cooking methods are presented in Table 2. The initial moisture content of raw fish was recorded as 70.54%. However, after undergoing heat treatment or cooking, the moisture content of the samples exhibited a significant decrease. Steamed samples showed the highest moisture content (61.31%), followed by grilled (54.49%), air fried (53.43%) and fried samples (44.36%). This result is similar to the previous study by [9], the lowest moisture content was recorded with fried samples of snakehead fish (*Channa striatus*) at 71.6%. As a consequence of a steaming process, the fish fillets absorbed water leading to a higher moisture content compared to other cooking methods. Meanwhile, the fried sample has the lowest moisture content because the high cooking temperature causes a significant loss of tissue fluids (water content) during frying. The process of frying and grilling led to water losses, resulting in higher protein content in fried and grilled fish as compared to the raw fish fillets.

The percentage of ash was significantly higher (P<0.05) in cooked samples when compared to raw fish fillets. The highest ash content (1.50%) was recorded in grilled fish, and the lowest value in raw samples (0.23%). Similar findings by [9] and [11] reported the highest ash content in grilled fish fillets and the lowest in the raw fish fillets of snakehead fish (*Channa striatus*) and anchovy (*Engraulis encrasicholus*), respectively.

The cooked fish resulted in significantly higher (P<0.05) protein content when compared with the raw sample (20.46%). The fried fish sample showed the highest value of protein content (32.48%), followed by grilled fish fillets (30.94%), air- fried fish fillets (29.99%) and steamed fish fillets (29.96%). According to [22], water losses occurring during frying and grilling resulted in a lower moisture content caused higher protein content in fried and grilled fish compared to that of the raw fish. These findings were also supported by [9], which showed that fried fish fillets had significantly higher protein content than raw fillets. As a result, the decrease in moisture content is responsible for the increase in ash, protein, and fat content in fried fish fillets.

The significantly highest (P<0.05) fat content was showed with fried samples (14.75%), followed by grilled (10.86%), air fried (9.13%) and steamed (6.32%), respectively. The rise in fat content of the fried fish fillets is a result of oil absorption during the cooking process. Other than that, oil penetration on the food after some water has evaporated could potentially explain the increase in fat content. A similar result was reported for anchovy (*Engraulis encrasicholus*) fried in sunflower oil [12]. However, other factors, such as the rate of change in food temperature and the temperature of the cooking method (higher in deep frying than in steaming), may impact the results [26].

A significant difference (P<0.05) in carbohydrate content was observed between the fired and air-fried samples compared to the grilled and steamed samples. Almost similar highest amounts of carbohydrate content were reported in large sizes of Thai koi and middle sizes of local koi [27].

Table 2. Proximate composition (%) of raw and cooked samples of climbing perch

Proximate Composition	Raw	Fried	Grilled	Steamed	Air fried
Moisture	70.54±1.66^{a}	44.36±1.23^{d}	54.49±1.06^{c}	61.31±0.21^{b}	53.43±1.41^{c}
Ash	0.23±0.09^{c}	1.24±0.13^{ab}	1.50±0.26^{a}	1.10±0.06 ^{b}	1.32±0.26^{ab}
Protein	20.46±0.77^{d}	32.48±0.03^{a}	30.94±0.28^{b}	29.96±0.59^{c}	29.99±0.26^{c}
Fat	5.70±0.76^{d}	14.75±0.47^{a}	10.86±0.34^{b}	6.32±0.69 ^{d}	9.13±0.22 ^{c}
Carbohydrate	3.06±0.60^{b}	7.17±0.88^{a}	2.20±1.05^{b}	1.29±0.17^{b}	6.13±2.14^{a}

Datata are expressed as mean ± standard deviation (n=3). Value with different superscript letters within a row a significantly different (P<0.05).

The Free Fatty Acid of Raw and Cooked Climbing Perch

Table 3 shows the effects of the cooking method on the free fatty acid (FFA) of climbing perch. Significant reductions (P<0.05) in the FFA content of the samples were observed after undergoing cooking processes. FFA was highest in steamed samples (1.98%) and lowest in fried samples (1.39%). This is probably caused by the loss of volatile FFA during the heating process. FFA content was reduced after cooking because of the inactivation of lipase during the heating process, which may inhibit the release of FFA in cooked samples [28]. Cooking induces various processes, including hydrolysis and oxidation of fatty acids, which impact not only the fatty acid concentration but also the flavour, odour, colour, and texture of the fish [29]. Similar findings were reported in [28], which showed that pan-fried and deep-fried samples had the lowest FFA values. This result is attributed to the frying oil's dilution impact or the greater temperature of the frying process which could lead more FFA to being volatized.

Table 3. Free fatty acid (%) of raw and cooked samples of climbing perch

Samples	Free Fatty acid (FFA) %
Raw	2.70±0.04^{a}
Fried	1.39±0.01^{d}
Grilled	1.88±0.13^{b}
Steamed	1.98±0.05^{b}
Air fried	1.73±0.07^{c}

Data are expressed as mean ± standard deviation (n=3). Value with different superscript letters within a column shows a significantly different (P<0.05).

Antioxidant Activity of Cooked Climbing Perch

Table 4 shows the TPC and DPPH scavenging activity of cooked climbing fish. The phenolic content was reduced by cooking procedures. A significant high value (P<0.05) was observed for the fried climbing fish with a TPC content of 1.23 mg GAE/g. Air fried sample resulted in a TPC of 1.06 mg GAE/g, the grilled sample was 1.02 mg GAE/g, and the steamed sample was 0.95 mg GAE/g. According to [30], the application of heating in cooking techniques can disrupt the structures of phenolic and reduce their content. The higher TPC value observed in the fried sample could be attributed to the difference in temperature and ingredients used between the samples, thus increasing the extractability and bioavailability of antioxidants from fish. A study by [31] stated that temperature can readily impact the phenolic content of foods as well as the polyphenol compounds found in frying oil. In addition, TPC levels in steamed foods are lower due to phenolic compound dissolution in the cooking water. The amount of phenolic compounds lost is also affected by processing time and food size [32].

The value of the percentage inhibition of DPPH for the cooked climbing perch fillets is shown in Table 4. The higher value of percentage inhibition of DPPH interpreted higher antioxidant activity for the sample. The significantly highest value (P<0.05) was shown by the steamed samples (69.62%), and grilled samples showed the lowest value of inhibition (42.80%). The effect of antioxidants on DPPH radical scavenging was assumed to be related to their hydrogen-donating ability [33]. Furthermore, antioxidants are reactive molecules that can easily react with radicals and some oxygen types. Heating could increase the pace of reactivity between antioxidants and oxidants, affecting antioxidant breakdown

and consumption via several mechanisms. As a result, changes in cooking temperature may explain the difference in antioxidant activity reported between samples prepared using different methods [34]. A similar finding was also found by [16] on a small indigenous freshwater fish species, *Puntius sophore*, that was processed by steaming and frying. In addition, the difference in antioxidant activity between the samples is also due to the Maillard reaction. Maillard reaction is a browning reaction that does not involve enzymes. When foods are processed or cooked at high temperatures, a chemical reaction between amino acids and reducing sugars occurs, resulting in the browning effect of the food. It formed melanoidin, known to act as an antioxidant in the food, which contributed to the increase in antioxidant properties [16]. Differences in cooking methods (blanching, boiling, steaming) significantly affected the antioxidant activity in vegetables like turi flower, spinach leaf, papaya leaf, kenikir leaf, yard-long beans, and mung bean sprouts [18].

Table 4. Total phenolic content and inhibition of DPPH of climbing perch cooked in different cooking methods

Samples	Total Phenolic Content (mg GAE/g)	Inhibition of DPPH (%)
Fried	1.23±0.16^{a}	53.48±8.24^{b}
Grilled	1.02±0.05^{b}	42.80±2.70^{c}
Steamed	0.95±0.01^{b}	69.62±3.79^{a}
Air Fried	1.06±0.06^{b}	53.70±5.23^{b}

Data are expressed as mean ± standard deviation (n=3). Value with different superscript letters within a column shows a significantly different (P<0.05).

Conclusions

In conclusion, it was found that cooking methods have a significant effect on the physicochemical properties of climbing perch, which include cooking loss, texture and colour. Fried climbing perch samples showed the highest proximate composition, including protein, fat and carbohydrate likely due to the oil absorption and elevated cooking temperature. The highest TPC also observed in fried climbing perch. In contrast, the steamed samples were the lightest in color and had the highest FFA and DPPH scavenging activity. Further research into the phytochemicals responsible for antioxidant activities in cooked fish is crucial. Consequently, steaming is a healthier alternative to deep-frying for preparing this freshwater fish.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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