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Characterization of *Chlorella vulgaris* and *Spirulina platensis* extracts: Feasibility of functional drinks formulation

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Abstract

Background and Objective: Microalgae, such as *Chlorella (C.) vulgaris* and *Spirulina (S.) platensis*, are rich sources of bioactive compounds, including proteins, polyphenols, carotenoids, and essential fatty acids, which can enhance the nutritional and functional profiles of beverages. This study aimed to extract ethanolic and hydro-ethanolic extracts from *C. vulgaris* and *S. platensis*, evaluate their functional properties, and develop a consumer-acceptable functional beverage.

Materials and Methods: The nutritional composition (protein, lipid, and mineral content) of the microalgae was determined. Both ethanolic and hydro-ethanolic extracts were prepared, and their antioxidant activities were analyzed using DPPH, ABTS, and FRAP assays, alongside the determination of total phenolic content (TPC). For beverage formulation, varying concentrations (0.5%, 1%, and 1.5%) of the extracts were incorporated, followed by sensory evaluation. Physical and chemical parameters, including Brix, pH, titratable acidity, reducing sugars, sucrose, ethyl alcohol, and formol number, were measured for the optimized formulations.

Results: The hydro-ethanolic extracts exhibited significantly higher antioxidant activity and phenolic content compared to the ethanolic extracts. *S. platensis* showed higher protein and lipid contents, while *C. vulgaris* was superior in mineral composition. Sensory analysis revealed that the beverage containing 0.5% of the microalgal extract mixture achieved the highest consumer acceptance. The selected formulations maintained acceptable physicochemical properties and high sensory scores.

Conclusion: The hydro-ethanolic extracts of *Chlorella* and *Spirulina* serve as potent natural antioxidant sources for functional beverage production. This study demonstrates that incorporating these microalgal extracts can result in a beverage with enhanced nutritional value and high bioactivity without compromising consumer acceptability.

What is "already known":	• Inherent antibacterial and antioxidant properties of these microalgae can potentially reduce the necessity for synthetic additives in food formulations

What this article adds:	• The aqueous-ethanolic extracts had higher antioxidant activity and polyphenol content than the ethanolic extracts. • Spirulina algae showed higher protein and fat content, while Chlorella was rich in minerals. • The selected beverage formulas had acceptable physicochemical properties and high sensory acceptance.

1. Introduction

In recent years, the global demand for health-promoting food products, particularly functional beverages, has witnessed a significant surge. Beyond fulfilling basic nutritional requirements, these products contain bioactive compounds with potential health benefits, playing a crucial role in preventing chronic diseases and enhancing quality of life [1]. Among the diverse sources of functional ingredients, microalgae have garnered substantial attention. Specifically, *S. platensis* and C. *vulgaris* have been extensively applied in the food [2,3] and pharmaceutical productions due to their high protein content, essential fatty acids, vitamins, minerals, and bioactive pigments such as phycocyanin and chlorophyll [4-6].

Spirulina, characterized by a protein content exceeding 60% and a complete profile of essential amino acids, B-group vitamins, and antioxidant compounds like beta-carotene, has been reported to improve immune function, mitigate oxidative stress, and support cardiovascular health [7]. Conversely, *Chlorella*, in addition to its significant protein levels, is renowned for its dietary fiber, chlorophyll, and natural detoxifying agents, which contribute to improved digestive function, modulation of gut microbiota, and the elimination of heavy metals from the body [8]. Furthermore, the inherent antibacterial and antioxidant properties of these microalgae can potentially reduce the necessity for synthetic additives in food formulations [9-13]. The incorporation of microalgae into beverage formulations not only enhances nutritional value but also improves the functional characteristics of the final product. However, challenges such as color stability, palatability, microbial load control, and overall sensory acceptance necessitate rigorous investigation [14, 15]. Despite the growing number of studies on the extraction of bioactive compounds from *C. vulgaris* and *S. platensis* and their incurporation into a few functional foods and beverages, several important knowledge gaps remain. Most previous investigations have focused either on optimizing extraction efficiency or on evaluating the nutritional and antioxidant properties of individual microalgal species, while comprehensive studies integrating extraction strategy, bioactive characterization, nutritional composition, beverage formulation, physicochemical quality, and sensory acceptability are still limited. In particular, a direct comparison between ethanolic and hydro-ethanolic extraction systems and their influence on the functionality and consumer acceptance of a beverage containing a combination of both microalgae has not been sufficiently explored. Therefore, this study was designed to comparatively evaluate these extraction systems and to investigate the feasibility of developing a consumer-acceptable functional beverage enriched with *C. vulgaris* and *S. platensis* extracts.

2. Materials and Methods

Commercial dried powders of *C. vulgaris* and *S. platensis* were obtained from [Islamic Azad University, Tehran, Iran]. The materials were supplied as food-grade dried microalgal powders and stored in airtight containers at 4°C until analysis. According to the supplier's specifications, the biomass was produced under controlled cultivation conditions suitable for food applications. Detailed information regarding the harvesting procedure, drying method, and batch number was not available from the manufacturer.

2.1. Extraction of microalgal extracts

Microalgal extraction was performed using two solvents: ethanol and a hydro-ethanolic mixture.

2.1.1. Hydro-ethanolic Extraction

Bioactive compounds were extracted according the method presented by Tavakoli et al.[15] with minor adjustments. Briefly, 10 g of microalgal powder was

mixed with 70% ethanol to a final volume of 100 mL. The mixture was exposed to ultrasonic treatment in an ultrasonic bath for 30 min. The resulting solution was centrifuged (2795 g) and subsequently filtered. The filtrate was concentrated in a temperature-controlled oven and stored in amber containers under refrigeration until further analysis.

2.1.2. Ethanolic Extraction

The ethanolic extraction followed the procedure of Georgiopoulou et al. [16] with slight adjustments. In this method, 96% ethanol was used instead of 70%, and the sonication time was increased to 60 min. After filtration, the extract was concentrated and stored in dark vessels to prevent photo-oxidation [16].

2.2. Evaluation of bioactive properties

2.2.1. DPPH Radical Scavenging Assay

The DPPH assay was conducted based on the method of Brand-Williams et al. [17] with minor modifications. A 0.1 mM DPPH solution in methanol was prepared, and 1 mL of this solution was mixed with 1 mL of the extract. After incubation (30 min) in the dark at ambient temperature, the absorbance was measured at 517 nm. The DPPH radical scavenging activity was calculated as the percentage of radical inhibition and expressed as micromoles of Trolox equivalents per gram of extract (µmol TE/g).

2.2.2. ABTS Radical Scavenging Assay

The $ABTS^{+}$ cation radical scavenging capacity was determined based on the method by Re et al.[18]. The ABTS radical was generated by reacting ABTS with potassium persulfate for 12–16 h in the dark. The solution was diluted to reach an absorbance at 734 nm. After adding the sample, the absorbance decrease was measured via spectrophotometry after 5 min. Results were presented as Trolox equivalents (µmol TE/g).

2.2.3. Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing power of the extracts was measured using the FRAP assay, based on the reduction of Fe^{3+} to Fe^{2+} in the presence of TPTZ [19]. A fresh FRAP reagent was prepared by mixing 25 mL of acetate buffer (pH 3.6), 2.5 mL of TPTZ solution, and 2.5 mL of $FeCl_3$. Then, 50 µL of the sample was mixed with 1.5 mL of the FRAP reagent, and the absorbance was immediately read at 593 nm.

2.2.4. Total Phenolic Content (TPC)

The TPC was measured using the Folin-Ciocalteu method as described by Carloni et al. [20]. Briefly, 100 µL of the extract was combined with 200 µL of 50% Folin–Ciocalteu reagent. Following a 3 min reaction period, 600 µL of 20% sodium carbonate solution was added. The reaction mixture was incubated at room temperature for 2 h, after which the absorbance was recorded at 765 nm. The total phenolic content (TPC) was determined using a gallic acid calibration curve (25–500 mg/L) and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract).

2.2.5. Mineral Content Analysis

The mineral composition was determined using Atomic Absorption Spectroscopy according to the method of White & Gadd [21]. One gram of microalgal powder was digested with concentrated nitric acid in a thermal digestion block. After cooling, the samples were diluted with 6 M hydrochloric acid and distilled water. Concentrations were calculated based on specific standard curves and expressed as mg/kg on a dry weight basis.

2.2.6. Lipid Determination

Total lipid content was extracted using the Soxhlet method with hexane as the solvent, according to AOAC guidelines [22]. Three grams of dried sample were placed in a filter paper thimble and subjected to continuous extraction for 4 h. After solvent evaporation, the lipid content was determined gravimetrically.

2.2.7. Total Protein Determination

Total protein content was determined using the Kjeldahl method. Following acid digestion, distillation, and titration, the nitrogen content was quantified and converted to protein content using a conversion factor of 6.25. The results were expressed as percentage protein on a dry weight basis [22].

2.3. Formulation of the fortified beverage

A functional base beverage was developed using potable water, sucrose, pectin, microalgal extracts, and food-grade preservatives. The base formulation consisted of 88% water, 9% sucrose, 1% pectin, 1%

microalgal extract, and 1% preservative. Lemon essence was added to enhance the flavor profile. The process involved dissolving sugar in water, followed by the gradual addition of pectin to prevent clumping, and finally incorporating the microalgal extracts and preservatives under controlled stirring and temperature to preserve bioactive integrity. The samples were analyzed for Brix, pH, acidity, reducing sugars, sucrose, formol number, and ethanol content to ensure compliance with non-carbonated beverage standards.

2.4. Physicochemical Characterization of the Beverage

Physicochemical attributes, including Brix, titratable acidity, pH, reducing sugars, sucrose, formol index, and ethyl alcohol, were measured according to the Iranian National Standard No. 2685 [23].

2.5. Sensory evaluation

Sensory evaluation was conducted by 30 panelists (15 males and 15 females) aged between 22 and 45 years. The panel consisted of semi-trained individuals familiar with sensory evaluation of food products. Samples were presented in a randomized order and evaluated using a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely) for color, aroma, taste, texture, and overall acceptability. Drinking water was provided between samples to minimize carry-over effects.

2.6. Statistical Analysis

All analytical measurements were performed in triplicate as independent technical replicates using the same batch of each microalgal extract. Data were analyzed using SPSS version 21. A completely randomized design (CRD) was employed, and significance was determined via Analysis of Variance (ANOVA). Mean comparisons were conducted using Duncan's Multiple Range Test at a significance level of $p < 0.05$.

3. Results and Discussion

3.1. DPPH Radical Scavenging Activity

As shown in Table 1, the IC_{50} values for the DPPH radical scavenging activity of the hydro-ethanolic extracts of *C. vulgaris* and *S. platensis* were 267.77 mg/mL and 272.56 mg/mL, respectively. Lower IC_{50} values indicate greater radical scavenging activity. The IC_{50} values obtained in the present study indicate that both microalgal extracts possess measurable antioxidant activity; however, their activity is lower than that reported for several plant extracts with stronger radical scavenging capacity. Differences among studies may be attributed to variations in microalgal species, cultivation conditions, extraction solvent composition, extraction procedures, and the profile of bioactive compounds. Nevertheless, the substantially lower IC_{50} values of the hydro-ethanolic extracts compared with the ethanolic extracts indicate that the hydro-ethanolic solvent system improved the recovery of antioxidant constituents under the experimental conditions employed in this study. These findings align with previous literature; for instance, DPPH inhibition of up to 78% has been reported for hydrolyzed peptides from *Spirulina* and *Chlorella* [24]. Similarly, studies on *Spirulina*-fortified beverages reported antioxidant activities in the range of 45% [25]. This consistency suggests that the hydro-ethanolic solvent effectively facilitates the extraction of antioxidant compounds, including phycocyanin, polyphenols, and carotenoids. In terms of bioactive composition, *S. platensis* plays a significant role in radical scavenging due to the presence of phycocyanin, a pigment-protein complex with robust antioxidant properties [26]. In contrast, *C. vulgaris* benefits from higher concentrations of chlorophyll, beta-carotene, and Vitamin C. Furthermore, phenolic compounds—specifically p-coumaric and ferulic acids—alongside carotenoids like lutein, are key contributors to the antioxidant capacity of both microalgae [27]. The superior performance of hydro-ethanolic extracts compared to pure ethanolic extracts may be attributed to the synergistic effect of hydrophilic and lipophilic compounds in the binary solvent system, which enhances extraction efficiency.

3.2. ABTS Radical Scavenging Activity

The IC_{50} results for ABTS radical inhibition were 264.52 mg/L for *C. vulgaris* and 246.15 mg/L for

S. platensis, indicating potent antioxidant activity (Table 1). These *values* are slightly higher than those reported for hydrolyzed peptides [24], which can be related to differences in extraction methods and solvent polarity. The bioactive agents responsible for this effect include phycocyanin, polyphenols, carotenoids, and vitamin C. Phycocyanin, particularly abundant in *Spirulina*, serves as the primary radical scavenger, while *Chlorella* is notably rich in Vitamin C. Polyphenols and carotenoids present in both species inhibit the ABTS radical through hydrogen and electron transfer mechanisms. The hydro-ethanolic extracts, due to their optimized balance of polar and non-polar properties, exhibited higher activity than purely ethanolic extracts, confirming their potential for use in functional food formulations.

3.3. Ferric Reducing Antioxidant Power:FRAP

The FRAP assay revealed that both extracts possess a significant capacity to reduce ferric ions (Fe^{3+}). However, the hydro-ethanolic extract of *S. platensis* (72.4 µmol TE/g DW) outperformed *C. vulgaris* (58.9 µmol TE/g DW) (Table 1). The higher FRAP values observed for *S. platensis* may be attributed to its overall antioxidant composition rather than to a single bioactive compound. Previous studies have reported that pigments such as phycocyanin, together with phenolic compounds and other antioxidants, *contribute* to the reducing capacity of *Spirulina*. However, since phycocyanin was not quantified in the present study, its specific contribution cannot be confirmed. Although *C. vulgaris* is known to contain considerable amounts of vitamin C and carotenoids, its reducing power was lower than that of *S. platensis* under the extraction conditions employed. Additionally, small peptides derived from protein hydrolysis may also contribute to the ferric reducing capacity through their electron-donating functional groups. These findings suggest that the hydro-ethanolic solvent system was more effective in extracting a broad spectrum of antioxidant constituents, resulting in enhanced ferric reducing antioxidant power.

3.4. Total Phenolic Content (TPC)

As detailed in Table 1, *C. vulgaris* extracts were richer in phenolic compounds compared to *S. platensis*. The hydro-ethanolic extracts of *C. vulgaris* and *S. platensis* contained 41.47 mg GAE/L and 27.59 mg GAE/L, respectively. These concentrations were significantly higher than those found in ethanolic extracts, underscoring the efficiency of the hydro-ethanolic solvent in recovering phenols and flavonoids. Active compounds include flavonoids such as catechin, quercetin, myricetin, and luteolin, as well as algae-specific phenolics like phanicul and chlorogenin. Interspecies differences are linked to distinct biosynthetic pathways; for example, *Spirulina* possesses specialized pathways for phycocyanin and specific flavonoid production. These findings are consistent with prior studies [16, 25], confirming that hydro-ethanolic extracts are excellent sources of active polyphenols for functional beverages.

3.5. Lipid Content

According to Table 2, the lipid content of *S. platensis* (8.96%) was significantly higher than that of *C. vulgaris* (2.42%). This difference is attributed to their fatty acid profiles; *Spirulina* is rich in polyunsaturated fatty acids (PUFA), including gamma-linolenic acid (GLA) and omega-3 acids like linoleic acid, whereas *Chlorella* primarily consists of saturated and monounsaturated fatty acids with lower PUFA levels. Environmental factors such as light, temperature, and nutrient concentration also influence lipid synthesis and storage. These findings are in agreement with previously reported data [28].

Table 1. Antioxidant activity (DPPH, ABTS, FRAP) and total phenolic content of Spirulina and Chlorella extracts compared to Trolox[†]

Treatments	DPPH IC50 (mg/mL)	ABTS (IC50)	FRAP µmol TE/g DW	Phenolic Content (Gallic Acid Eq.(mg/g))
Chlorella, Ethanolic Extract	576.57±1.09^{c}	539.42 ±6.87^{c}	46.6±0.97^{a}	30.45±0.56^{c}
Chlorella, Hydroethanolic Extract	267.77±0.96^{a}	264.52 ±0.78^{b}	58.9±1.01^{c}	41.47±1.04^{d}
Spirulina, Ethanolic Extract	613.41±1.99^{d}	617.59 ±0.85^{d}	52.5±0.38^{b}	18.41±0.10^{a}
Spirulina, Hydroethanolic Extract	272.56±0.95^{b}	246.15 ±1.23^{a}	72.4±1.55^{d}	27.59±0.42^{b}

[†] Mean ± SD. Different letters indicate significant differences at $p<0.05$ within each column.

3.6. Protein Content

The total protein content of *S. platensis* (55.09%) was higher than that of *C. vulgaris* (49.51%) (Table 2). This variation is largely dependent on the species, the composition of water-soluble proteins (especially phycocyanin), cell wall thickness, and cultivation conditions. While *Chlorella* has high protein potential (40–55% DW), its thick cell wall can limit extractable protein compared to *Spirulina* (50–60% DW). Factors such as nitrogen and phosphorus availability significantly impact protein synthesis. These results align with earlier studies [29].

3.7. Mineral Content

C. vulgaris exhibited a higher mineral density compared to *S. platensis*. Iron, manganese, and potassium levels in *Chlorella* were 14,780 ppm, 48 ppm, and 322 ppm, respectively, compared to 1,650 ppm, 9.7 ppm, and 36 ppm in *Spirulina* (Table 2). This discrepancy arises from the biological characteristics and growth media of the microalgae; the cell wall is capable of biosorbing ions from the environment, which then play roles in enzymatic activities and metabolic pathways such as photosynthesis and osmotic regulation. These findings confirm *Chlorella*'s superior capacity for mineral accumulation [30].

Table 2. Contents of fat, protein, and mineral elements (iron, manganese, potassium) in Spirulina and Chlorella extracts[†]

Microalgae	Fat (%w/w)	Protein (%w/w)	Fe (ppm)	Mn (ppm)	K(ppm)
Chlorella	2.42±0.90*	49.51±2.40	14780	48	322
Spirulina	8.96±0.98	55.09±4.30	1650	9.7	36

[†] Mean ±SD

3.8. Beverage Formulation

In the present study, functional beverages were initially developed by incorporating a 1:1 mixture of *C. vulgaris* and *S. platensis* extracts at concentrations of 0.5%, 1%, and 1.5% (w/v). These formulations were then subjected to sensory evaluation to determine the proper concentration. In all treatments, the base ingredients remained constant, including sucrose (9%), flavoring essence (1%), and citric acid (1%). Pectin was utilized as a stabilizing agent to maintain the structural integrity of the beverage and prevent the sedimentation of microalgal solids. The sucrose level was selected to emulate the mouthfeel of commercial beverages, while the combination of lemon essence and citric acid was effective in masking the characteristic marine-like odor and aftertaste of the microalgae, thereby enhancing palatability. The natural green hue provided by the microalgal pigments, complemented by the added flavorings, contributed to a high degree of sensory appeal. The results of the sensory analysis are summarized in Table 3.

Table 3. Sensory evaluation of formulated beverages by algae extract[†]

Test Parameter	formulated beverage 1 (0.5% algae extract)	formulated beverage 2 (1% algae extract)	formulated beverage 3 (1.5% algae extract)
Taste	4.143±0.377[a]	2.667±0.516[b]	2.000±0.577[c]
Odor	4.286±0.488[a]	2.333±0.816[b]	2.286±0.755[b]
Off-Flavour	4.000±0.816[a]	2.500±0.836[b]	1.714±0.952[b]
Color	4.714±0.488[a]	2.333±0.816[b]	2.000±0.817[b]
Appearance	4.714±0.487[a]	1.833±0.408[b]	2.428±0.976[b]
Total acceptance	4.000±0.000[a]	2.000±0.000[b]	2.286±0.488[b]

[†]Mean ± SD. Different letters indicate significant differences at $p<0.05$ within each row

3.9. Physicochemical Characterization of the Selected Beverage

Table 4 presents the physicochemical attributes of the selected beverage (containing 0.5% microalgal extract mixture) in comparison with national standard requirements. The measured Brix value was 18.85, which is higher than the standard limit (maximum 11). This elevated Brix is attributed to the relatively high pectin concentration required for stabilizing microalgal extracts, a phenomenon consistent with prior research where values around 14% were reported [31].

The total sugar content in both formulations remained within the permissible range of the Iranian National Standards. The sucrose concentration was meticulously adjusted to maintain a balanced sweetness profile without becoming cloying. Due to the presence of citric acid (at a level that does not impart excessive sourness), lemon essence, and pectin, the pH of the beverage was slightly higher than the standard threshold. Furthermore, the alkaline nature of certain microalgal components may contribute to a reduction in titratable acidity. Similar pH values (approximately 3.2) have been documented in related studies [31].

A significant advantage of the developed beverage is the use of natural microalgal extracts as the sole coloring agent, eliminating the need for synthetic dyes. The characteristic yellowish-green color is derived directly from chlorophyll and phycocyanin. Previous literature suggests that synthetic colors can sometimes interfere with the stability of algal-based beverages; thus, this formulation remains additive-free in that regard. Additionally, no chemical preservatives, such as sorbates or benzoates, were incorporated. Evidence indicates that *Spirulina* compounds possess inherent antimicrobial properties, providing natural preservation [32]. Finally, sucrose was employed as the exclusive sweetener, avoiding artificial alternatives like sucralose or aspartame, which have been reported to potentially interfere with the metabolic benefits of microalgal bioactives [33].

Table 4. Physicochemical properties of formulated beverages compared with Iranian national standards[†]

Test Parameter	Formulated Beverage (0.5% extract)	Acceptable Range / Limit (Iran National Standard 2837-23107)
Ethyl alcohol	0.099±0.001	Max. 0.15% (ISIRI 2837)
Formalin index	0.725±0.021	-
Sucrose	9.175±0.106	Max. 10.5% (ISIRI 23107)
Reducing sugars	0.550±0.035	-
pH	4.190±0.014	2.5 – 4.1 (ISIRI 23107)
Titratable acidity	0.535±0.021	-
°Brix	18.849±0.071	Max. 11 (ISIRI 23107)

[†]Mean ± SD

4. Conclusion

The findings of this research demonstrate that the incorporation of *C. vulgaris* and *S. platensis* extracts into functional beverages significantly enhances their nutritional and functional profiles. Hydro-ethanolic extracts exhibited superior antioxidant activity compared to pure ethanolic extracts, primarily due to their higher recovery of polyphenols, proteins, and essential fatty acids. Mineral analysis further confirmed the superiority of *C. vulgaris* in terms of iron, manganese, and potassium content. These microalgae serve as potent sources of bioactive compounds including polyphenols, proteins, and essential minerals which contribute to robust radical scavenging and reductive capacities. Most of the physicochemical evaluation (pH, acidity, reducing sugars, sucrose, ethyl alcohol, and formol number) indicated that the selected formulations comply with Iranian National Standards, ensuring product quality and safety. The use of pectin as a stabilizer successfully immobilized the microalgal extracts and improved the visual and sensory characteristics of the beverage. Sensory analysis revealed that the fortified beverages achieved high levels of acceptability in terms of color, flavor, aroma, and mouthfeel. Specifically, the inclusion of natural flavorings effectively balanced the characteristic marine notes of the algae, which is crucial for the development of innovative and market-acceptable functional products. In conclusion, the production of functional beverages enriched with *C. vulgaris* and *S. platensis* extracts represents an effective strategy for utilizing sustainable natural resources to promote public health. The developed beverage demonstrates promising potential as a functional beverage formulation. However, further studies evaluating storage stability, microbiological safety, antioxidant retention, color stability, sedimentation behavior, and shelf-life are required before industrial-scale production and commercialization can be recommended.

5. Declarations

5.1. Acknowledgements

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5. 2. Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

5.3. Authors Contributions

Mehdi Jarrahzadeh: Methodology, Validation, Investigation, Resources, **Maryam Moslehishad:** Conceptualization, Project administration, Writing Review & Editing, **Fataneh Hashempour-baltork:** Writing Original Draft, Visualization, Supervision

5.4. Using Artificial Intelligent chatbots

AI chatbots used for translating and Language edition were used in this research.

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