

Bioactive Colored Extracts from Agro-Industrial Food Wastes: Functional Ingredients to Improve Food Quality and Health

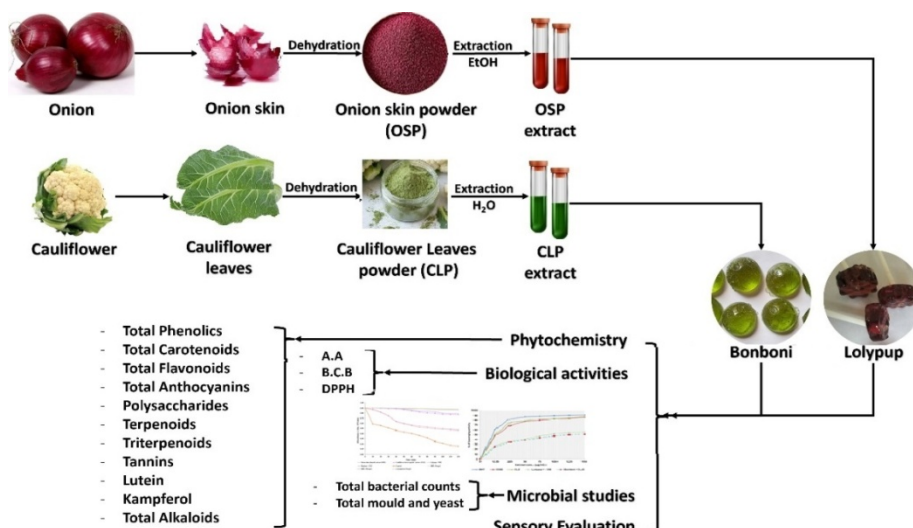
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Abstract Agro-industrial food wastes, including onion peels and cauliflower leaves, are rich in bioactive compounds like polyphenols, carotenoids, flavonoids, and polysaccharides. This study prepared colored extracts from these wastes and evaluated their bioactive contents and application in sugar confectionery products, Bonboni and Lolypup. Onion skin ethanolic extract (OSE) exhibited higher total phenolics (397.67 ± 9.88 mg GAE/g) and flavonoids (39.11 ± 3.14 mg resveratrol equivalent/g), while cauliflower leaves aquatic extract (CLA) contained higher carotenoids (131.17 ± 8.12 mg rutin equivalent/g) and polysaccharides (116.12 ± 7.15 mg starch/g). Anthocyanin contents were similar in both extracts. CLA also showed higher lutein (81.43 ± 6.09 mg/g) and alkaloids (15.73 ± 1.04 μ g atropine/g), whereas OSE had more terpenoids (34.21 ± 3.04 μ g/g). Incorporating these extracts into Bonboni and Lolypup significantly increased bioactive compound levels compared to controls. Antioxidant activity tests revealed high antioxidant percentages for OSE (89.21%) and CLA (90.65%), though synthetic standards BHT and α -tocopherol exhibited superior activity. Fortified sweets showed enhanced antioxidant activity which recorded 47.69% and 51.83% for Bonboni + CLE and Lolypup + OSE than the controlled samples. β -carotene bleaching and DPPH assays confirmed moderate antioxidant effects of the extracts and products, with IC₅₀ values indicating reduced activity after incorporation. Microbial analysis demonstrated that adding extracts suppressed bacterial and fungal growth during 60 days of room temperature storage, with total plate counts increasing to 1232 ± 38 cfu/g for Lolypup + OSE versus 2780 ± 45 cfu/g in controls. Sensory evaluation showed improved appearance, color, taste, odor, texture, and overall acceptability in enriched products, with Bonboni + CLA achieving top scores (appearance 9.12 ± 0.07 , texture 9.17 ± 0.20 , acceptability 9.13 ± 0.06). These results support the valorization of agro-industrial wastes as natural functional ingredients to enhance food quality and shelf life.



Keywords: Onion skin, cauliflower leaves, bioactive compounds, biological activities, sugar confectionery, microbial analysis, Sensory evaluation

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1. Introduction

Food quality refers to the set of attributes and characteristics that determine the acceptability of food to consumers, including sensory, nutritional, microbiological, and safety-related factors. According to Nwadi and Okonkwo, [1] food quality encompasses both external features, such as color, texture, appearance, and aroma, and internal attributes like nutrient content and safety. From a consumer perspective, the sensory characteristics are often the most immediate determinants of quality, while producers and regulatory agencies emphasize microbiological safety and nutritional adequacy [2]. Food safety is a critical component of food quality, as contamination by biological, chemical, or physical hazards can pose serious health risks [3]. Nutritional quality, another key dimension, relates to the presence of essential nutrients such as proteins, vitamins, and minerals that contribute to human health [4].

In recent years, the importance of food quality has expanded beyond immediate consumption to include broader societal concerns such as sustainability, ethical sourcing, and environmental impact. Sustainable food systems aim to maintain food quality while minimizing resource use and ecological footprint [5]. Ensuring high-quality food is not only crucial for public health but also for economic development, as consumer trust and product marketability are closely tied to consistent quality standards [6]. Moreover, with the increasing globalization of food markets, harmonized quality regulations and certifications such as ISO 22000 have become essential for international [7].

Food quality encompasses a range of attributes that determine the acceptability of food to consumers, including sensory characteristics, nutritional content, and safety. However, the inclusion of artificial additives and colorants in food products has raised significant health concerns. These synthetic substances, commonly used to enhance the appearance, taste, and shelf-life of processed foods, have been linked to various adverse health effects. Artificial food colorings, such as tartrazine, erythrosine, and sunset yellow, are among the most scrutinized additives. Studies have shown that these dyes can cause allergic reactions, including urticaria, rhinitis, and angioedema, particularly in sensitive individuals [8]. Additionally, there is evidence suggesting that artificial colors may exacerbate symptoms of attention-deficit hyperactivity disorder (ADHD) in children [9,10]. The mechanisms underlying these effects are thought to involve neurotoxicity, oxidative stress, and alterations in neurotransmitter function [11,12].

Beyond behavioral issues, some artificial colorants have been implicated in more severe health problems. For instance, erythrosine has been associated with thyroid tumors in animal studies, raising concerns about its safety in food products [13]. Similarly, the use of brominated vegetable oil (BVO) in beverages has been linked to neurological damage and endocrine disruption, leading to its ban in several countries (Brominated vegetable oil, 2024). The consumption of these additives is particularly concerning among children, who are more susceptible to

their harmful effects due to their developing bodies and higher intake relative to body weight [8]. Despite regulatory measures, the widespread use of artificial additives in processed foods continues to pose a significant public health challenge.

Sugar confectionery, which includes sweets such as candies, chocolates, jellies, and toffees, plays a significant role in global food markets and cultural practices. As of 2023, the global sugar confectionery market was valued at over USD 85 billion, driven by increased urbanization, rising disposable income, and a growing demand for convenient snack options [14]. These products are particularly appealing to children due to their sweet taste, colorful appearance, and aggressive marketing strategies [15]. While occasional consumption of sugar confectionery may be harmless, excessive intake is associated with several health concerns, especially in children. Frequent consumption contributes to a higher risk of obesity, insulin resistance, and dental caries [16]. Moreover, sugar-rich diets are often energy-dense and nutrient-poor, leading to poor dietary quality among young consumers [17].

Another major concern in sugar confectionery production is the use of artificial additives and synthetic colorants. Colors such as Tartrazine (E102), Sunset Yellow (E110), and Allura Red (E129) are commonly added to enhance visual appeal. However, several studies have linked these additives to behavioral problems in children, including increased hyperactivity and attention deficits [18,19]. Regulatory agencies have taken notice, with the European Union mandating warning labels for products containing certain dyes, and the U.S. FDA currently reviewing the long-term safety of these substances [20,21]. In light of these risks, public health authorities recommend limiting children's access to sugar confectionery and encouraging consumption of natural and minimally processed alternatives [17]. Educating parents and caregivers about these issues remains essential in reducing childhood exposure to harmful food components.

Agro-industrial food wastes refer to the by-products generated during the industrial processing of agricultural raw materials, including fruit peels, seeds, pulps, pomace, bran, and husks. These wastes, though often discarded, are rich in bioactive compounds such as polyphenols, dietary fibers, natural pigments, and essential oils [22]. Globally, food systems produce nearly 1.3 billion tonnes of food waste each year, with a substantial portion originating from industrial processing stages [23]. Economically, these residues represent untapped resources; when effectively utilized, they offer cost-efficient and sustainable alternatives to synthetic food additives and colorants. The valorization of agro-industrial by-products has become a vital component of the circular economy, creating added value while minimizing environmental pollution [24].

A growing body of research supports the use of agro-industrial residues as sources of natural food colorants and functional ingredients. For instance, grape pomace is a valuable source of anthocyanins, tomato skins are rich in lycopene, beetroot waste yields betalains, and citrus peels contain flavonoids and carotenoids [25]. These natural pigments not only impart vibrant colors but also possess

health-promoting properties. Functional ingredients derived from these wastes exhibit antioxidant, anti-obesity, anti-inflammatory, antimicrobial, and anticancer activities, making them suitable for application in food preservation and nutritional enhancement [26]. Additionally, dietary fibers extracted from cereal brans and fruit pulps have prebiotic properties, contributing to improved gut health and immune function [27]. Integrating agro-industrial waste valorization into modern food systems plays a crucial role in advancing sustainability by aligning with multiple Sustainable Development Goals (SDGs). This approach not only reduces the volume of food waste generated during industrial processing but also transforms these residues into value-added products with functional and nutritional benefits. As noted by Benavides et al., [27] the extraction of natural additives and bioactive compounds from such waste supports environmentally responsible practices, including reduced reliance on synthetic additives, lower production costs, and the promotion of zero-waste models. Ultimately, this strategy enhances economic efficiency, supports public health through the incorporation of natural and functional ingredients, and contributes significantly to reducing the environmental footprint of food production.

In line with all the aforementioned objectives, the present study was conducted with the aim of preparing certain colored extracts from selected agro-industrial food wastes that are commonly available both locally and globally (onion peels and cauliflower leaves), and to investigate the biologically active and bioactive compounds present in these extracts. Additionally, the study explored the potential application of these functional ingredients to improve the nutritional and health quality of an important food product, sugar confectionery (bonbons and lollipops).

2. Materials and Methods

2.1. Materials

2.1.1. Plant Parts

Fresh samples of cauliflower (*Brassica oleracea*) leaves were collected in December 2023 through coordination with local farmers in various villages around Shebin El-Kom City, Menoufia Governorate, Egypt. Onion (*Allium cepa*) skins were sourced from the New Bani Suef Company for Preservation, Dehydration, and Industrialization of Vegetables, located in Bani Suef El-Goudida City, east of the Nile, Bani Suef, Egypt.

2.1.2. Chemicals

Standards of bioactive compounds such as gallic acid (GA), catechin (CA), α -tocopherol, linalool, ursolic acid, and butylated hydroxytoluene (BHT), along with DPPH (2,2-diphenyl-1-picrylhydrazyl), copper sulfate (CuSO_4), dimethyl sulfoxide (DMSO), and various vitamins, were obtained from Sigma Chemical Co., St. Louis, MO, USA. Unless otherwise noted, all other chemicals, solvents, and reagents used were of analytical grade and procured from El-Ghomhorya Company for Trading Drugs, Chemicals, and Medical Instruments, Cairo, Egypt.

2.1.3. Equipment

A UV-160A spectrophotometer (Shimadzu Corporation, Kyoto, Japan) was utilized to measure absorbance for various analytical assays. For determining mineral content, an atomic absorption spectrophotometer (Perkin-Elmer, Model 2380, Waltham, MA, USA) was employed. Total nitrogen was measured using a semi-automatic Micro-Kjeldahl apparatus from Velp, Italy, and crude fat was quantified with a Soxhlet semi-automatic extractor, also from Velp, Italy.

2.2. Methods

2.2.1. Preparation of Plant Powder

The preparation of cauliflower leaf and onion skin powders followed the procedure described by Elhassaneen et al., [28]. Initially, the plant materials were washed thoroughly to eliminate surface contaminants. Cauliflower leaves were dried in a horizontal forced air oven (Velp Inc., Italy) at 70°C for 10 hours, while onion skins were dried at 60°C for 4 hours until reaching a final moisture content of approximately 7%. The dried materials were ground using a Moulinex miller (Moulinex Egypt, ElAraby Co., Benha, Egypt). The resulting fine powder was passed through an 80-mesh sieve, packed in polyethylene bags, and stored at 4°C for subsequent analyses.

2.2.2. Preparation of Plant Extracts

Dried plant materials (20 grams) were extracted using 180 ml of solvent. For onion skin, 80% aqueous ethanol (distilled water: ethanol) was used, while cauliflower leaf was extracted using distilled water only. The extraction process was conducted in an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany) for 3 hours at 60°C (ethanolic extraction) and 80°C (aqueous extraction). The mixtures were filtered through Whatman No. 5 filter paper with a Buchner funnel. Solvent removal was performed using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany) under reduced pressure—organic solvents at 40°C and water at 60°C. Extracts were then stored at 4°C until further use.

2.2.3. Bonboni and Lolypup Manufacturing Method

The table (1) presents the formulation of two candy products—Bonboni and Lolypup—showing both the weight (in grams) and percentage composition of each ingredient. This table showcases a comparative formulation strategy using both traditional confectionery components (ES: 464-1/2005 and ES: 464-2/2006) and novel functional ingredients derived from vegetable waste. The inclusion of onion skin ethanolic extract (OSE) in Lolypup and cauliflower leaves aquatic extract (CLA) in Bonboni introduces potential health benefits and aligns with sustainable food processing practices. Additionally, both recipes maintain consistency in sweetness and texture through similar levels of sucrose and glucose syrup.

2.2.3.1. Bonboni Manufacturing Method

Bonboni is manufactured in accordance with the Egyptian Standard Specifications (ES: 464-1/2005 and ES:

464-2/2006). Sugar, glucose syrup, and water are placed in a stainless steel pot and heated over medium heat with continuous stirring until the sugar is completely dissolved. The mixture is then boiled until it reaches a temperature of 145–150°C (the hard crack stage), using a candy thermometer to accurately control the temperature. Once the desired temperature is reached, the mixture is removed from the heat, and cauliflower leaf extract and spearmint oil are added and mixed thoroughly. The mixture is then poured into non-stick molds or onto a lightly oiled surface and left to cool and solidify.

2.2.3.2. Lolypup Manufacturing Method

Lolypup is manufactured in accordance with the Egyptian Standard Specifications (ES: 464-1/2005 and ES: 464-2/2006). As with Bonboni, sugar, water, and glucose syrup are mixed and heated until dissolved. The mixture is cooked to the hard crack stage by boiling it until it reaches 145–150°C. After removing the mixture from the heat, onion peel extract and spearmint oil are added. The mixture is then poured into lollipop molds (containing sticks) and left to cool and take the shape of a “Lolypup.”

Table 1. Recipes of the selected products (Bonboni and Lolypup)

Component	Bonboni		Lolypup	
	Gram	Percent (%)	Gram	Percent (%)
Water	50	14.64	60	17.32
Sugar (Sucrose)	200	58.57	200	57.72
Glucose syrup	80	23.43	80	23.09
Onion skin ethanolic extract (OSE)	-----	-----	5.0	1.44
Cauliflower leaves aquatic extract (CLA)	10	2.93	-----	-----
Strawberry essence	-----	-----	-----	-----
Spearmint essence	1.50	0.44	1.50	0.43
Total	341.5	100	346.5	100

2.2.4. Chemical Analysis of Plant Powder

The chemical composition of the onion skin and cauliflower leaf powders was assessed to determine their nutritional components. This analysis included measurements of moisture content, crude protein (determined by calculating total nitrogen $\times$ 6.25 using the micro-Kjeldahl method with a semi-automatic analyzer from Velp, Italy), crude fat (extracted using a Soxhlet system from Velp, Italy, with petroleum ether as the solvent), ash, crude fiber, and total dietary fiber. These analyses were conducted according to the standard procedures described by AOAC, [29]. The carbohydrate percentage was derived indirectly by subtracting the combined percentages of moisture, protein, fat, ash, and fiber from 100%, using the formula:

Carbohydrates (%) = 100 - (% moisture + % protein + % fat + % ash + % fiber).

2.2.5. Determination of Bioactive Compounds in Plant Extracts

The total phenolic content in extracts derived from onion skins and cauliflower leaves was analyzed using the Folin-Ciocalteu method, following the protocols of Singleton and Rossi, [30] and Wolfe et al., [31]. The results were presented as gallic acid equivalents (GAE).

Carotenoids were quantified in 80% acetone extracts using the procedure described by Litchenthaler, [32] and reported as micrograms of carotenoids per gram of dried extract. Total flavonoid content was determined via a colorimetric assay based on the method of Zhisen et al., [33], with catechin used as the standard. The values were calculated using the standard curve equation: $y = 0.0003x - 0.0117$, $r^2 = 0.9827$, and expressed as milligrams of catechin equivalents (CAE) per gram of dry extract. Polysaccharide concentration was estimated using the method reported by Vazirian et al., [34] where starch served as the standard. Results were expressed in milligrams of starch equivalents per gram of dry weight (dw). The total terpenoid content was extracted and measured in accordance with Ghorai et al., [35] using linalool as the reference compound, with outcomes given in milligrams of linalool equivalents per gram of dw. Total triterpenoids were evaluated following the procedure by Schneider et al., [36] using ursolic acid as a reference. The content was expressed in milligrams of ursolic acid per 100 grams. Anthocyanin levels were measured according to Sharif et al., [37] and expressed as milligrams of cyanidin-3,5-diglucoside per 100 grams. Tannin content was determined based on the method of Van-Burden and Robinson, [38] using gallic acid (GA) to establish the calibration curve. Lastly, total alkaloids were measured following the procedure described by Zhao and Wang, [39] with atropine used as the reference for creating the calibration curve, allowing for estimation of alkaloid concentration in the samples.

2.2.6. Biological Activities

2.2.6.1. Antioxidant Activities (AA) and β -carotene Bleaching (BCB) Assay

The antioxidant potential (AA) of extracts from onion skin, cauliflower leaves, and derived products, along with standard antioxidants such as α -tocopherol and BHT, was assessed using the β -carotene bleaching (BCB) method, adapted from the procedure established by Marco [40]. In this method, 1 mL of a β -carotene solution (0.2 mg/mL in chloroform) was transferred into a 50 mL round-bottom flask, followed by the addition of 0.02 mL linoleic acid and 0.2 mL of Tween 20. To this mixture, 0.2 mL of either 80% methanol (as the control), a plant extract, or a reference standard was added. After the organic solvent was removed under vacuum at ambient temperature, 50 mL of oxygen-saturated distilled water was introduced, and the mixture was vortexed to form a stable emulsion. The resulting solution was incubated at 50°C for two hours to induce thermal auto-oxidation. Absorbance at 470 nm was measured at 10-minute intervals using a Beckman DU-50 spectrophotometer. The degradation rate of β -carotene was evaluated via linear regression analysis. Each test was conducted in triplicate. Four methods were employed to determine antioxidant activity:

A plot of absorbance over time was used to generate a slope; the absolute value of this slope represented the antioxidant value (AOX), following the method by Al-Saikhan et al [41].

Antioxidant activity (AA) was also calculated as a percentage of inhibition relative to the control using the formula: $AA (\%) = [(R \text{ control} - R \text{ sample}) / R \text{ control}] \times$

100, where $R_{control}$ and R_{sample} represent the β -carotene degradation rates in the control and sample mixtures, respectively.

The oxidation rate ratio (ORR) was calculated based on the approach by Marinova et al. [42] using the formula: $ORR = R_{sample} / R_{control}$.

The antioxidant activity coefficient (AAC) was determined using the method of Mallet et al. [43], with the formula:

$$AAC = [(AbsS_{120} - AbsC_{120}) / (AbsC_0 - AbsC_{120})] \times 100,$$

where $AbsS_{120}$ is the absorbance of the sample at 120 minutes, $AbsC_{120}$ is the absorbance of the control at 120 minutes, and $AbsC_0$ is the absorbance of the control at the initial time point.

2.2.6.2. DPPH Radical Scavenging Assay

The capacity of onion skin, cauliflower leaves, and related products to scavenge free radicals was assessed using the DPPH assay, as described by Desmarchelier et al., [44]. A reaction mixture was prepared by combining 2.4 mL of a 0.1 mM methanolic DPPH solution with 1.6 mL of extract at varying concentrations (ranging from 12.5 to 150 μ g/mL). This mixture was vigorously vortexed and allowed to stand in darkness at room temperature for 30 minutes. Post-incubation, the absorbance was read at 517 nm using a UV-160A spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Butylated hydroxytoluene (BHT) served as the positive control. The DPPH radical scavenging efficiency was calculated using the equation:

$$\text{Scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100,$$

Where: A_0 is the absorbance of the control (without extract), and A_1 is the absorbance after the addition of the extract or BHT. The resulting inhibition percentages were plotted against the concentrations to determine the IC_{50} value from the dose-response curve.

2.2.7. Microbiological Examination

2.2.7.1. Total Bacterial Counts

Total bacterial counts of frozen vegetables samples were determined according to the American Public Association, [45] by plating suitable dilution in duplicates using nutrient agar medium [46]. This medium consists of: beef extract (3 g/L), bacto peptone (5g/L), agar (15 g/L), sodium chloride (5 g/L) and distilled water to 1000 ml PH 7. Plates were incubated at 32°C for 3 days before counting and recording the results.

2.2.7.2. Total Mould and Yeast

Potato dextrose agar recommended by the Oxoid Manual, [47] was used for the enumeration of molds and yeast's. This medium consists of: potatoes extract (4 g/L), dextrose (20 g/L), agar (15 g/L) and PH (5.6). Plates were incubated at 20 – 25 ° before counting.

2.2.8. Sensory Evaluation

A sensory assessment was conducted with a panel of 10 postgraduate students from Menoufia University, Shebin El-Kom, Egypt. Each participant received six sweet product samples, randomly placed on a rectangular plastic tray. The samples were individually sealed in pouches and

labeled with unique three-digit codes to ensure anonymity. The sample set included four different composite sweet variants and one control sample without plant extract additions. To maintain palate neutrality, water was provided for rinsing between tastings. Panelists evaluated six sensory attributes: color, appearance, odor, taste, texture, and overall acceptability, using the 9-point hedonic scale. The scale ranged from 1 = dislike extremely to 9 = like extremely.

2.3. Statistical Analysis

All experimental measurements were carried out in triplicate to ensure accuracy, repeatability, and reliability of the results. The data obtained from these repeated trials were statistically analyzed and expressed as the mean $\pm$ standard deviation (SD), which provides a measure of central tendency and the degree of variability within the dataset. Statistical analysis was performed using Student's *t*-test, and data were processed using MINITAB version 12 (Minitab Inc., State College, PA, USA).

3. Results and Discussion

3.1. Chemical Composition of Onion Skin and Cauliflower Leaves Powder

The data presented in Table 2 underscore the chemical composition and nutritional potential of two underutilized vegetable by-products, onion skin powder (OSP) and cauliflower leaf powder (CLP), highlighting their applicability in functional food development due to their richness in dietary fiber, minerals, and plant-based proteins. Notably, CLP exhibited significantly higher moisture content ($8.09 \pm 0.65\%$) than OSP ($6.22 \pm 0.41\%$), suggesting that OSP may offer better shelf-stability, a claim supported by previous studies such as Buvaneswari et al., [48] and Florina et al., [49]. In terms of protein, CLP surpassed OSP ($7.21 \pm 0.59\%$ vs. $4.01 \pm 0.12\%$), aligning with the nutritional profiles reported by Wani et al., [50] and Tukassar, [51], thus positioning CLP as a promising ingredient for protein enrichment, especially in plant-based diets. Conversely, OSP demonstrated a significantly higher crude fat content ($8.98 \pm 0.84\%$) compared to CLP ($2.08 \pm 0.12\%$), likely due to the presence of lipid-soluble compounds such as quercetin and sulfur compounds, as noted by Florina et al., [49], enhancing OSP's functional value. The most pronounced disparity between the two powders was in crude fiber content, where OSP ($21.89 \pm 2.11\%$) far exceeded CLP ($13.98 \pm 0.94\%$), corroborating findings by Michalak-Majewska et al., [52] and emphasizing OSP's potential for digestive health applications. Mineral content, as indicated by ash values, was also significantly higher in OSP ($7.01 \pm 0.63\%$) than in CLP ($3.02 \pm 0.10\%$), with studies like Chadorshabi et al., [53] confirming the abundance of calcium, potassium, and phosphorus in onion skins. On the other hand, CLP presented higher carbohydrate levels ($65.62 \pm 1.98\%$) compared to OSP ($51.89 \pm 2.37\%$), a trait attributed to its starch and sugar content, as documented by Buvaneswari et al., [48] making CLP an energy-dense ingredient. Additionally, such data are in accordance with

that observed by several authors [28,54,55]. Collectively, these findings affirm the potential of vegetable by-products as valuable functional ingredients: OSP's high fiber, fat, and mineral content suits applications aimed at gut health and micronutrient enhancement, while CLP's elevated protein and carbohydrate levels make it suitable for protein- and energy-fortified food products. This aligns with current sustainability trends promoting the valorization of agricultural waste to enhance nutritional profiles, support food security, and minimize environmental impact [49,53].

Table 2. Chemical composition of onion skin and cauliflower leaves powder

Component	Onion skin powder	Cauliflower leaves powder
Water	6.22 ± 0.41 ^b	8.09 ± 0.65 ^a
Total Protein	4.01 ± 0.12 ^b	7.21 ± 0.59 ^a
Crude fat	8.98 ± 0.84 ^a	2.08 ± 0.12 ^b
Crude fiber	21.89 ± 2.11 ^a	13.98 ± 0.94 ^b
Ash	7.01 ± 0.63 ^a	3.02 ± 0.10 ^b
Carbohydrates	51.89 ± 2.37 ^b	65.62 ± 1.98 ^a

Values represent the mean ± SD (n=3). Means in the same row with the different superscript letters were significantly (p≤0.05).

3.2. Documented Analysis of Bioactive Compounds in Onion Skin Ethanolic (OSE) and Cauliflower Leaves Aquatic (CLA) Extracts

Table 3. Some bioactive compounds content of onion skin ethanolic (OSE) and cauliflower leaves aquatic (CLA) extracts

Compounds	Onion skin ethanolic (OSE) extract	Cauliflower leaves aquatic (CLA) extract
Total phenolics (mg gallic acid equivalent, GAE. g ⁻¹)	397.67 ± 9.88 ^a	181.12 ± 11.45 ^b
Total carotenoids (mg rutin equivalent, . g ⁻¹)	16.98 ± 2.01 ^b	131.17 ± 8.12 ^a
Total flavonoids (mg resveratrol equivalent. g ⁻¹)	39.11 ± 3.14 ^a	16.14 ± 1.22 ^b
Total anthocyanin's (mg cyanidin-3,5-diglucoside. g ⁻¹)	14.67 ± 0.94 ^a	11.60 ± 1.16 ^a
Polysaccharides (mg starch. g ⁻¹)	89.45 ± 4.67 ^b	116.12 ± 7.15 ^a
Terpenoids (µg linalol. g ⁻¹)	34.21 ± 3.04 ^a	21.71 ± 3.07 ^b
Triterpenoids (µg ursolic acid.g ⁻¹)	14.06 ± 1.03 ^b	30.53 ± 4.1 ^a
Tannins (mg tannic acid equivalent. g ⁻¹)	2.12 ± 0.06 ^a	4.30 ± 0.62 ^a
Lutein (mg. g ⁻¹)	6.04 ± 0.44 ^b	81.43 ± 6.09 ^a
Kaempferol (mg.g ⁻¹)	0.605 ± 0.013 ^a	0.483 ± 0.06 ^a
Total alkaloids (µg.atropine.g ⁻¹)	8.23 ± 0.77 ^b	15.73 ± 1.04 ^a

*Each value represents the mean of three replicates ±SD. Means with the superscript letters in the same row were significant different at p≤0.05.

Table 3 presents a comparative profile of key bioactive compounds in onion skin ethanolic (OSE) and cauliflower leaves aquatic (CLA) extracts, revealing distinct phytochemical compositions attributable to both plant species and extraction methods. OSE demonstrated a

significantly higher total phenolic content (397.67±9.88 mg GAE/g) compared to CLA (181.12 ± 11.45 mg GAE/g), corroborating prior studies which identify onion skins as a rich source of phenolics with potent antioxidant activities [56,57]. Phenolic compounds are widely recognized for their free radical scavenging abilities, contributing to the prevention of oxidative stress-related chronic diseases [58]. Conversely, CLA showed a markedly higher concentration of total carotenoids (131.17 ± 8.12 mg rutin equivalent/g) relative to OSE (16.98 ± 2.01 mg/g). This aligns with findings that cauliflower leaves are rich in carotenoids such as lutein and beta-carotene, essential for eye health and possessing anti-inflammatory properties [59,60]. The aquatic extraction used for CLA likely preserved carotenoids more effectively than the ethanolic extraction employed for onion skin, as carotenoids are sensitive to solvent polarity and extraction conditions [61]. Total flavonoids were significantly higher in OSE (39.11 ± 3.14 mg resveratrol equivalent/g) than in CLA (16.14 ± 1.22 mg/g), consistent with the flavonoid-rich nature of onion skin, particularly quercetin and kaempferol derivatives, which exhibit anti-inflammatory and antioxidant effects [62,63]. Ethanolic solvents are known to efficiently extract flavonoids due to their solubility characteristics, explaining the higher flavonoid content in OSE [64]. Total anthocyanins content was comparable between OSE and CLA, with values of 14.67 ± 0.94 mg/g and 11.60 ± 1.16 mg/g, respectively. Anthocyanins are potent antioxidants linked to cardiovascular and neuroprotective effects [65] and the similar levels suggest both extracts could contribute similarly to these health benefits. Polysaccharide content was higher in CLA (116.12 ± 7.15 mg starch/g) compared to OSE (89.45 ± 4.67 mg/g). Plant polysaccharides function as prebiotics and immunomodulators, which may support gut health and overall immunity [59]. The aquatic extraction method is favorable for retaining high molecular weight carbohydrates like polysaccharides, accounting for this observation. Terpenoids, with recognized antimicrobial and anti-inflammatory properties [66] were more abundant in OSE (34.21 ± 3.04 µg/g) than CLA (21.71 ± 3.07 µg/g). Conversely, CLA had a significantly higher triterpenoid content (30.53 ± 4.1 µg/g) compared to OSE (14.06 ± 1.03 µg/g), compounds known for anticancer and anti-inflammatory effects [67]. These variations reflect the inherent metabolic profiles of the plants as well as the selective extraction efficiency of solvents. Tannin levels were similar between the two extracts (2.12 ± 0.06 mg/g in OSE and 4.30 ± 0.62 mg/g in CLA), both contributing antioxidant and antimicrobial activities [68]. Lutein, a carotenoid vital for eye health, was substantially higher in CLA (81.43 ± 6.09 mg/g) compared to OSE (6.04 ± 0.44 mg/g), highlighting the potential use of CLA as a natural source for lutein-enriched functional foods [60]. Kaempferol concentrations were slightly higher in OSE (0.605 ± 0.013 mg/g) compared to CLA (0.483 ± 0.06 mg/g), consistent with the known flavonoid profile of onion skin and its associated antioxidant properties [63]. Lastly, total alkaloid content was notably higher in CLA (15.73 ± 1.04 µg atropine/g) than in OSE (8.23 ± 0.77 µg/g). Alkaloids exhibit diverse pharmacological activities including anti-inflammatory and analgesic effects, further

supporting the therapeutic potential of CLA [69]. The differences observed can be explained by the plant species' intrinsic biochemical makeup and the polarity and efficiency of extraction solvents—aqueous extraction favoring water-soluble compounds such as polysaccharides and carotenoids, while ethanolic extraction enhances recovery of phenolics and flavonoids [64]. The significance of these compounds lies in their combined antioxidant, anti-inflammatory, antimicrobial, and anticancer effects, making these extracts promising candidates for functional food ingredients and nutraceuticals development [70].

3.3. Comprehensive Analysis of Bioactive Compounds in Bonboni and Lolypup Products Supplemented with Onion Skin Ethanolic (OSE) and Cauliflower Leaves Aquatic (CLA) Extracts

Table 4. Some bioactive compounds in Bonboni and Lolypup products control and supplemented with onion skin ethanolic (OSE) and cauliflower leaves aquatic (CLA) extracts

Compounds	Bonboni		Lolypup	
	Control	Control + CLA	Control	Control + OSE
Total phenolics (mg gallic acid equivalent, GAE. 100g ⁻¹)	0.00	573.11 ± 12.56 ^a	0.00	589.45 ± 11.45 ^a
Total carotenoids (mg rutin equivalent. 100g ⁻¹)	0.00	385.24 ± 9.67 ^a	0.00	37.23 ± 3.76 ^b
Total flavonoids (mg resveratrol equivalent. 100g ⁻¹)	0.00	44.10 ± 4.31 ^b	0.00	59.41 ± 3.45 ^a
Total anthocyanin's (mg cyanidin-3,5-diglucoside. 100g ⁻¹)	0.00	31.65 ± 3.55 ^a	0.00	23.06 ± 1.59 ^b
Polysaccharides (mg starch. 100g ⁻¹)	2.37	346.59 ± 8.73 ^a	1.48	134.45 ± 6.23 ^b
Terpenoids (µg linalol. 100g ⁻¹)	0.00	60.43 ± 4.22 ^a	0.00	39.98 ± 4.20 ^b
Triterpenoids (µg ursolic acid. 100g ⁻¹)	0.00	90.21 ± 6.09 ^a	0.00	26.17 ± 2.04 ^b
Tannins (mg tannic acid equivalent. 100g ⁻¹)	0.00	14.17 ± 1.45 ^a	0.00	4.17 ± 0.23 ^b
Lutein (mg. 100g ⁻¹)	0.00	211.90 ± 7.23 ^a	0.00	9.89 ± 0.31 ^b
Kampferol (mg. 100g ⁻¹)	0.00	1.51 ± 0.11 ^a	0.00	0.91 ± 0.08 ^a
Total alkaloids (µg atropine. 100g ⁻¹)	0.00	43.03 ± 3.34 ^a	0.00	10.45 ± 0.19 ^b

*Each value represents the mean of three replicates ±SD. Means with the superscript letters in the same row were significant different at $p \leq 0.05$.

Table 4 clearly demonstrates a remarkable enhancement of various bioactive compounds in Bonboni and Lolypup products upon supplementation with cauliflower leaves aquatic (CLA) and onion skin ethanolic (OSE) extracts, respectively, whereas the controls exhibited no detectable levels of these compounds. Both Bonboni + CLA and Lolypup + OSE showed substantial increases in total phenolics, aligning with previous studies that identify onion skins and cauliflower leaves as rich sources of potent antioxidant phenolics mitigating oxidative stress linked to chronic diseases [56,57,58]. Carotenoid content was notably higher in Bonboni + CLA than in Lolypup + OSE, reflecting the higher intrinsic carotenoid levels in cauliflower leaves and the efficacy of aquatic extraction in

preserving these compounds compared to ethanolic extraction for onion skin [59,60]. Conversely, flavonoids were more abundant in Lolypup + OSE, consistent with the flavonoid-rich nature of onion skins and their enhanced solubility in ethanol [62]. Anthocyanins were also elevated, with Bonboni + CLA surpassing Lolypup + OSE, reinforcing the antioxidant and cardiovascular benefits of these pigments [65]. The significantly higher polysaccharide content in Bonboni + CLA highlights the advantage of aquatic extraction in preserving prebiotic carbohydrates important for gut health [59]. Terpenoids and triterpenoids were markedly greater in Bonboni + CLA, underscoring their antimicrobial and anticancer potential [66,67]. Similarly, tannins and lutein, both with antioxidant and eye health benefits—were substantially higher in Bonboni + CLA, affirming CLA's value for functional food development [60,68]. Kaempferol and alkaloids, known for anti-inflammatory and pharmacological properties, were also more abundant in Bonboni + CLA, suggesting enhanced therapeutic potential [63,69]. These differences arise primarily from the distinct phytochemical profiles of the source plants and the extraction methods, with aqueous extraction favoring water-soluble compounds like polysaccharides and carotenoids, while ethanolic extraction favors flavonoids and phenolics [64]. The presence and increase of these bioactive compounds are significant due to their well-documented antioxidant, anti-inflammatory, antimicrobial, and anticancer effects, supporting the feasibility of enriching food products with plant extracts to meet growing consumer demand for nutraceutical-rich foods [57,70]. Phenolics, a broad class of bioactive compounds, primarily function as antioxidants by scavenging free radicals and chelating metals, thereby modulating oxidative stress and inflammation and offering protection against chronic diseases including cardiovascular disorders and cancer [58,70]. Carotenoids such as lutein act as potent antioxidants and photoprotective agents critical for eye health by preventing age-related macular degeneration and enhancing immune responses [60,71]. Flavonoids, a diverse subgroup of phenolics, exhibit strong anti-inflammatory, antiviral, and anticancer activities by modulating cell signaling pathways and inhibiting enzymes [57,72]. Polysaccharides from plants and algae show immunomodulatory, antiviral, and antitumor effects through enhancement of macrophage function and cytokine stimulation [73,74]. Anthocyanins, pigments responsible for red to blue coloration in plants, provide antioxidant, anti-inflammatory, and cardiovascular protective benefits by improving endothelial function and reducing oxidative damage [75]. Terpenoids and triterpenoids, structurally diverse compounds, contribute anti-inflammatory, antimicrobial, and anticancer effects by interfering with signaling cascades and inducing apoptosis in malignant cells [76,77]. Tannins are polyphenolic compounds exhibiting strong antioxidant and antimicrobial properties, including protein precipitation which underlies their astringent taste and roles in gut health and pathogen inhibition [78,79]. Lutein, a carotenoid concentrated in the retina, protects against oxidative stress and light-induced damage, supporting visual performance and reducing risks of cataracts and

macular degeneration [60,80]. Kaempferol, a flavonoid widely found in fruits and vegetables, exhibits antioxidant, anti-inflammatory, and anticancer activities through modulation of apoptotic pathways and inhibition of oxidative stress [63].

3.4. Antioxidant Activity of Different Extracts Compared to Standards

Data in Table 5 indicated that the antioxidant activity of onion skin ethanolic extract (OSE) and cauliflower leaves aquatic extract (CLE) was assessed and compared with synthetic standards BHT (butylated hydroxytoluene) at two concentrations and α -tocopherol, as well as a control, using a set of comprehensive parameters—antioxidant value (AOX), antioxidant activity percentage (AA%), oxidation rate ratio (ORR), and antioxidant activity coefficient (AAC)—to provide an integrated evaluation of antioxidative performance. Both OSE and CLE exhibited strong antioxidant activities, recording AOX values of 0.061 ± 0.011 A/h and 0.053 ± 0.016 A/h, and high AA% values of 89.21% and 90.65%, respectively, consistent with earlier findings that underscore the potent free radical scavenging abilities of flavonoid- and phenolic-rich onion skins [56,57] and phenolic- and carotenoid-rich cauliflower leaves [59]. Notably, CLE showed a marginal advantage over OSE in antioxidant activity percentage and coefficient (AAC: 748.22 ± 30.24 vs. 723.19 ± 24.56), possibly due to the efficient retention of water-soluble antioxidants such as polysaccharides and carotenoids during aqueous extraction, which may act synergistically to quench singlet oxygen or enhance stability [61]. Compared to these natural extracts, synthetic antioxidants—BHT and α -tocopherol—demonstrated markedly superior antioxidant activities, with AOX values of 0.017 ± 0.004 and 0.013 ± 0.002 A/h, and AA% of 96.98% and 97.64%, respectively, reflecting their well-established efficacy in lipid oxidation inhibition and radical scavenging [68,70], and further confirmed by higher AAC values (858.27 ± 22.57 for BHT and 869.74 ± 18.44 for α -tocopherol). The control sample, lacking any antioxidant addition, showed the highest oxidation rate (AOX: 0.565 ± 0.031 A/h), validating the sensitivity of the method. The slight variations between OSE and CLE in AOX and AAC values can be explained by the nature of their bioactive constituents and extraction solvents—ethanol being more efficient in recovering flavonoids and phenolic acids, known for metal chelation and hydrogen donation [62,63] while water extraction tends to preserve hydrophilic antioxidants and compounds like polysaccharides and carotenoids, which contribute through different antioxidative mechanisms [59,60]. These findings align with a wide range of previous studies examining antioxidant profiles in plant materials [54,55,81] [82,83,84] [85,86,87] [88,89], which consistently report a strong correlation between antioxidant capacity and the presence of diverse phytochemicals including flavonoids, phenolic acids, carotenoids, terpenoids, tannins, anthocyanins, alkaloids, and polysaccharides. The functional roles of these compounds go beyond radical scavenging, extending to modulation of oxidative stress-related pathways implicated in chronic diseases such as obesity,

cardiovascular diseases, neurodegenerative disorders, and cancer [28,58] [67,90] [91,92] [93,94]. Importantly, these findings support the application of natural antioxidants like those in OSE and CLE as safer and multifunctional alternatives to synthetic antioxidants, which—despite their potency—may pose long-term toxicity or health concerns [69].

3.5. Antioxidant Activities of Different Products Compared to Standards

3.5.1. Antioxidants Activity

Data in Table 6 indicated that the antioxidant activity of commercial sweet products Lolypup and Bonboni, tested both alone and in combination with natural plant extracts, namely onion skin ethanolic extract (OSE) and cauliflower leaves aquatic extract (CLE), as assessed and compared against standard antioxidants (BHT and α -tocopherol) and a control, using comprehensive parameters such as antioxidant value (AOX, A/h), antioxidant activity percentage (AA%), oxidation rate ratio (ORR), and antioxidant activity coefficient (AAC). The results revealed that Lolypup and Bonboni, in their unmodified forms, exhibited no antioxidant activity (AOX = 0, AA% = 0), highlighting the absence of intrinsic antioxidant capacity, which is typical for many food items lacking functional bioactives [70]. However, upon fortification with OSE and CLE respectively, both products demonstrated significant improvements: Lolypup + OSE recorded an AOX of 0.27 ± 0.021 A/h and AA% of $51.83 \pm 4.41\%$, while Bonboni + CLE showed a slightly higher AOX of 0.296 ± 0.022 A/h but a marginally lower AA% of $47.69 \pm 4.61\%$, indicating that the inclusion of natural extracts enhanced their antioxidative performance. This enhancement is attributable to the presence of bioactive phenolics, flavonoids, and carotenoids in OSE and CLE, compounds known for their radical scavenging, metal chelation, and lipid peroxidation inhibition properties, as supported by earlier studies [56,59]. Despite these improvements, the antioxidant activity of the fortified sweets was still significantly lower than that of the standards; BHT at 50 mg/L and 100 mg/L exhibited superior antioxidant efficacy (AA% of $88.06 \pm 2.47\%$ and $96.98 \pm 1.16\%$, respectively), and α -tocopherol at 50 mg/L demonstrated the highest AA% ($97.64 \pm 2.13\%$), reaffirming its efficiency as a lipid-soluble antioxidant that disrupts lipid oxidation chain reactions [60,63]. The reduced activity of OSE and CLE in food systems compared to isolated antioxidant standards is likely due to the dilution effect within complex food matrices, possible antagonistic interactions between antioxidants and food ingredients, and matrix-related constraints on compound bioavailability or stability [95]. Furthermore, food systems often harbor pro-oxidants such as metal ions or unsaturated lipids that can accelerate oxidative reactions and diminish antioxidant efficacy. Nonetheless, the moderate yet significant antioxidant effects observed in the enriched products confirm the relevance of phenolics and carotenoids, which exert antioxidant action through hydrogen atom donation, radical quenching, and metal ion chelation [58,61]. These findings not only support the potential health-promoting and preservative benefits of

OSE and CLE but also highlight their viability as natural antioxidant alternatives in food applications. In conclusion, although synthetic antioxidants like BHT and α -tocopherol remain more potent under controlled conditions, the integration of natural plant extracts into food matrices such as Lolypup and Bonboni significantly enhances their antioxidant properties, representing a valuable step toward clean-label, plant-based antioxidant strategies for improved food quality and health outcomes.

3.5.2. β -Carotene Bleaching, (BCB)

Table 7 and Figure 1 presents the antioxidant activity of various extracts and sweet product formulations evaluated using the β -carotene bleaching (BCB) assay over 120 minutes, with α -tocopherol (50 mg/L) serving as the standard reference. This assay assesses the ability of antioxidants to inhibit lipid peroxidation by tracking the discoloration of β -carotene in a linoleic acid emulsion under oxidative stress, where a slower decline in absorbance reflects stronger antioxidant efficacy. As expected, the control sample lacking antioxidants showed the most rapid β -carotene degradation, dropping from 0.903 to 0.151, affirming the necessity of antioxidant protection [96]. Among tested agents, BHT at 100 mg/L and α -tocopherol exhibited the highest protective effects, maintaining absorbance above 0.86, which aligns with their established efficacy as chain-breaking antioxidants that scavenge lipid peroxyl radicals and stabilize free radicals [60,97]. Moderate protection was observed with BHT at 50 mg/L, onion skin ethanolic extract (OSE), and cauliflower leaves aquatic extract (CLE), all stabilizing absorbance around 0.76–0.79, suggesting that phenolic compounds like quercetin in OSE and chlorogenic acid

and ascorbic acid in CLE provide appreciable antioxidant activity [56,59]. However, the antioxidant performance of the food formulations—Lolypup + OSE and Bonboni + CLE, was lower, with absorbance declining to ~0.47, indicating only moderate antioxidant potential. This decline is likely due to dilution of active compounds in the food matrix and possible interactions with other ingredients, such as lipids, proteins, and carbohydrates, which may hinder antioxidant functionality [95]. Food matrices may also introduce pro-oxidants or transition metal ions that catalyze oxidative reactions, further reducing efficacy [70]. The application of the BCB method has been widely validated for assessing antioxidant activity in various plant extracts [83,90,91,98,99], reinforcing its utility here. The superior results from α -tocopherol and high-dose BHT are attributed to their stable chemical structure and efficient free radical scavenging mechanisms, whereas natural extracts like OSE and CLE, though rich in bioactive compounds, contain complex phytochemical mixtures that may exhibit reduced potency per unit compared to pure synthetic antioxidants [12,80]. Nonetheless, these natural compounds are increasingly valued not only for their antioxidant capacity but also for additional health benefits such as anti-inflammatory, antidiabetic, and anticancer properties [65,92]. The moderate activity observed in food-extract combinations underscores the challenge of translating in vitro antioxidant performance to real food systems, where formulation, storage, and matrix complexity influence bioactivity and stability, highlighting the need for optimized concentrations and formulation strategies to achieve desired protective effects [59].

Table 5. Antioxidant activity of different extracts compared to standards

Extract*	Antioxidant value ^a AOX (A/h)		Antioxidant activity ^b AA (%)		Oxidation rate ratio ^c (ORR)		Antioxidant activity coefficient ^d (AAC)	
Onion skin ethanolic extract (OSE)	0.061±	0.011 ^c	89.21±	3.99 ^b	0.107±	0.031 ^c	723.19±	24.56 ^b
Cauliflower leaves aquatic extract (CLE)	0.053±	0.016 ^c	90.65±	6.36 ^b	0.093±	0.022 ^d	748.22±	30.24 ^b
Control	0.565±	0.031 ^a	0.00±	0.00	0.998±	0.067 ^a	0.00±	0.00
BHT, 50 mg/L	0.067±	0.008 ^c	88.06±	2.47 ^b	0.119±	0.042 ^c	703.20±	19.41 ^b
BHT, 100 mg/L	0.017±	0.004 ^d	96.98±	1.16 ^a	0.030±	0.021 ^d	858.27±	22.57 ^a
α -tocopherol, 50 mg/L	0.013±	0.002 ^d	97.64±	2.13 ^a	0.023±	0.009 ^d	869.74±	18.44 ^a

^a Antioxidant value (AOX, A/h) = The absolute value of slope (Abs was plotted against time).

^b Antioxidant activity (AA, %) = (R control - R sample) / R control x 100 where: R control and R sample were the bleaching rates of beta-carotene in reactant mixture without antioxidant and with plant extract, respectively

^c Oxidation rate ratio (ORR) = R sample / R control

^d Antioxidant activity coefficient (AAC) = (Abs S 120 - Abs C 120) / Abs C 0 - Abs C 120 x 1000 where: Abs S 120 was the absorbance of the antioxidant mixture at time 120 min, Abs C 120 was the absorbance of the control at time 120 min, Abs C 0 was the absorbance of the control at zero time.

^e Each value represents mean $\pm$ SD. Mean values with the different superscript letters in the same raw mean significantly different at level $p \leq 0.05$

* BHT, Butylated hydroxytoluene. In each column different letters mean significant differences ($p \leq 0.05$).

Table 6. Antioxidant activity of different products compared to standards

Extract*	Antioxidant value ^a AOX (A/h)		Antioxidant activity ^b AA (%)		Oxidation rate ratio ^c (ORR)		Antioxidant activity coefficient ^d (AAC)	
Lolypup	0.00±	0.00	0.00±	0.00	0.00±	0.00	0.00±	0.00
Bonboni	0.00±	0.00	0.00±	0.00	0.00±	0.00	0.00±	0.00
Lolypup + OSE	0.27±	0.021 ^d	51.83±	4.41 ^c	0.481±	0.031 ^b	73.35±	42.78 ^c
Bonboni + CLE	0.296±	0.022 ^b	47.69±	4.61 ^c	0.522±	0.053 ^b	1.38±	35.54 ^d
Control	0.565±	0.031 ^a	0.00±	0.00	0.998±	0.067 ^a	0.00±	0.00
BHT, 50 mg/L	0.067±	0.008 ^c	88.06±	2.47 ^b	0.119±	0.042 ^c	703.20±	19.41 ^b

Extract ^a	Antioxidant value ^a AOX (A/h)		Antioxidant activity ^b AA (%)		Oxidation rate ratio ^c (ORR)		Antioxidant activity coefficient ^d (AAC)	
BHT, 100 mg/L	0.017±	0.004 ^d	96.98±	1.16 ^a	0.030±	0.021 ^d	858.27±	22.57 ^a
α -tocopherol, 50 mg/L	0.013±	0.002 ^d	97.64±	2.13 ^a	0.023±	0.009 ^d	869.74±	18.44 ^a

^a Antioxidant value (AOX, A/h) = The absolute value of slope (Abs was plotted against time).

^b Antioxidant activity (AA, %) = (R control - R sample) / R control x 100 where: R control and R sample were the bleaching rates of beta-carotene in reactant mixture without antioxidant and with plant extract, respectively

^c Oxidation rate ratio (ORR) = R sample / R control

^d Antioxidant activity coefficient (AAC) = (Abs S 120 - Abs C 120) / Abs C 0 - Abs C 120 x 1000 where: Abs S 120 was the absorbance of the antioxidant mixture at time 120 min, Abs C 120 was the absorbance of the control at time 120 min, Abs C 0 was the absorbance of the control at zero time.

^e Each value represents mean $\pm$ SD. Mean values with the different superscript letters in the same raw mean significantly different at level $p \leq 0.05$

^{*} BHT, Butylated hydroxytoluene. OSE, onion skin ethanolic extract; CLE, cauliflower leaves aquatic extract

Table 7. Antioxidant activity of different extracts and products assayed by the β -carotene bleaching method (Alpha-tocopherol at 50 mg/L concentration was used as a standard)^{*}

Samples	Time (min)												
	0	10	20	30	40	50	60	70	80	90	100	110	120
OSE	0.903	0.903	0.902	0.899	0.899	0.883	0.857	0.838	0.835	0.827	0.798	0.790	0.790 ^b
CLE	0.903	0.902	0.900	0.893	0.891	0.873	0.848	0.825	0.810	0.801	0.780	0.776	0.771 ^b
Lolypup + OSE	0.903	0.875	0.821	0.753	0.642	0.599	0.564	0.546	0.529	0.511	0.497	0.487	0.476 ^c
Bonboni + CLE	0.903	0.869	0.817	0.737	0.633	0.589	0.554	0.521	0.510	0.491	0.481	0.471	0.461 ^c
Control	0.903	0.585	0.556	0.507	0.461	0.430	0.403	0.364	0.312	0.246	0.213	0.173	0.151 ^d
BHT, 50 mg/L	0.903	0.902	0.900	0.889	0.883	0.869	0.839	0.817	0.791	0.784	0.769	0.765	0.763 ^b
BHT, 100 mg/L	0.903	0.903	0.903	0.899	0.896	0.890	0.888	0.889	0.885	0.878	0.871	0.870	0.864 ^a
α -toc., 50 mg/L	0.903	0.903	0.903	0.899	0.899	0.894	0.893	0.891	0.889	0.883	0.880	0.878	0.877 ^a

^{*} Mean values with the different superscript letters in the same raw mean significantly different at level $p \leq 0.05$. OSE, onion skin ethanolic extract; CLE, cauliflower leaves aquatic extract; α -toc, α -tocopherol; BHT, butylated hydroxytoluene.

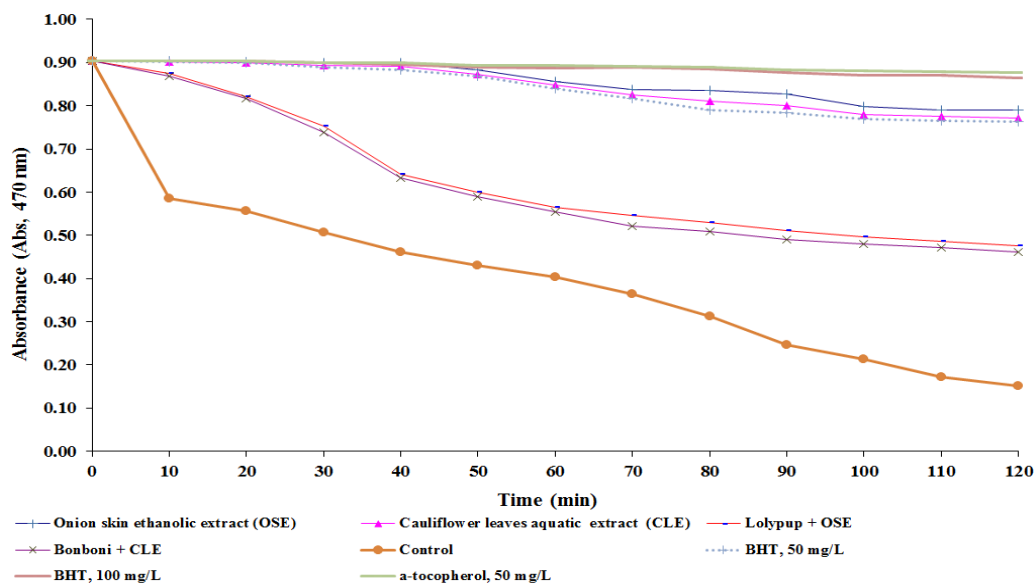


Figure 1. Antioxidant activity of different extracts and products assayed by the β -carotene bleaching method (BHT, butylhydroxy toluene and α -tocopherol were used as a reference)

3.5.3. DPPH Radical Scavenging Activity

Table 8 and Table 9 and Figure 2 presents the half maximal inhibitory concentration (IC₅₀) values from the DPPH free radical scavenging assay for BHT (a synthetic antioxidant), onion skin ethanolic extract (OSE), cauliflower leaves aqueous extract (CLE), and their respective food product formulations Lolypup + OSE and Bonboni + CLE, where lower IC₅₀ values denote stronger antioxidant activity. BHT recorded the lowest IC₅₀ (11.25

$\pm 0.17 \mu\text{g/mL}$), confirming its potent antioxidant capacity in line with its widespread use in food preservation [95,97]. The natural extracts OSE ($15.18 \pm 0.81 \mu\text{g/mL}$) and CLE ($14.14 \pm 0.69 \mu\text{g/mL}$) also demonstrated substantial antioxidant potential, supported by studies showing that onion skins are rich in quercetin and other flavonoids [56] while cauliflower leaves provide phenolics and vitamin C with similar activity (Zhang et al., 2022). However, when incorporated into food matrices, Lolypup + OSE ($92.04 \pm 0.93 \mu\text{g/mL}$) and Bonboni + CLE (142.05

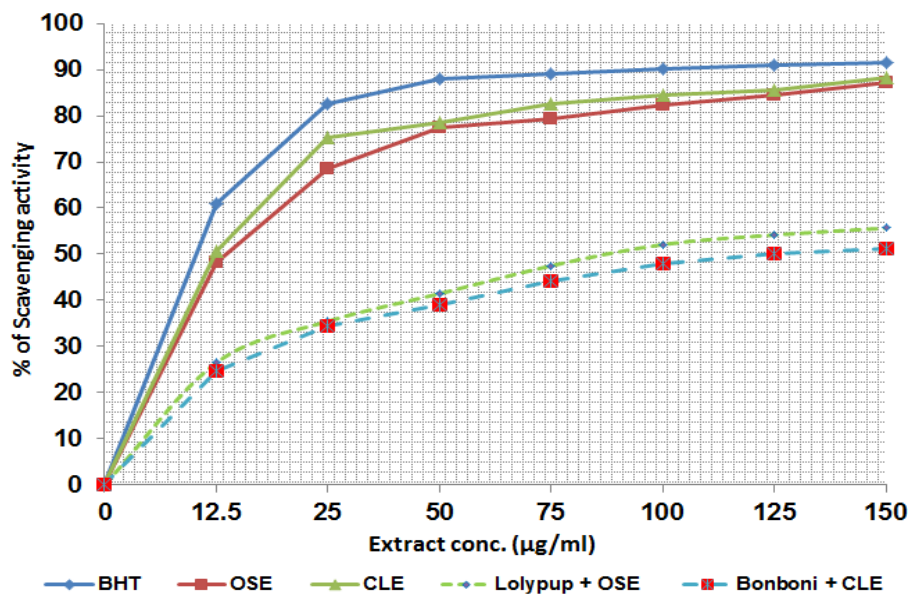
$\pm 0.78 \mu\text{g/mL}$) showed significantly higher IC_{50} values, reflecting reduced antioxidant efficacy. This reduction is likely due to the dilution of active compounds in the food matrix and interactions with ingredients like fats, proteins, and carbohydrates, which can limit bioavailability or inactivate antioxidant compounds [70,95]. Additionally, food processing and storage may degrade or alter antioxidant molecules, further diminishing their potency [58]. These differences highlight the complexity of translating antioxidant effects from isolated extracts to real food systems, where matrix components may hinder the stability or effectiveness of bioactive compounds or even promote oxidation by introducing pro-oxidant factors like metal ions [70]. Interestingly, CLE outperformed OSE slightly, which may result from its broader spectrum of water-soluble antioxidants like ascorbic acid and synergistic phenolic acid [59], though Bonboni + CLE still

had a higher IC_{50} than Lolypup + OSE, likely due to variations in food formulation and processing. The use of DPPH assay in this context is consistent with numerous earlier investigations on plant-derived antioxidant extracts containing compounds found in OSE and CLE [54,85,87,89,100,101,102] which confirmed the ability of these compounds to donate electrons or hydrogen atoms, thereby neutralizing free radicals. Phenolic compounds such as quercetin and chlorogenic acid are widely recognized for this function, offering protection against oxidative stress-related conditions including obesity, cancer, diabetes, cardiovascular and neurodegenerative diseases, and organ dysfunctions [58,63,88,89,103]. Therefore, although the antioxidant activity is reduced in the final food products compared to the pure extracts, incorporating OSE and CLE into sweets not only aids preservation but may also confer functional health benefits.

Table 8. DPPH radical scavenging activity (%) of Lolypup and Bonboni mixing with OSE and CLE as well as standard (BHT)*

Tested materials Conc. ($\mu\text{g/mL}$)	DPPH radical scavenging activity (%)				
	BHT	OSE	CLE	Lolypup + OSE	Bonboni + CLE
0	0.00	0.00	0.00	0.00	0.00
12.5	60.85	48.30	50.61	26.42	24.73
25	82.72	68.44	75.41	35.43	34.47
50	88.08	77.56	78.64	41.42	39.02
75	89.20	79.49	82.61	47.47	44.19
100	90.19	82.42	84.60	52.08	47.92
125	90.91	84.42	85.62	54.17	50.09
150	91.47	87.12	88.34	55.65	51.09

* Each value represents the mean value of three replicates. Means with different superscript letters in the same row are significantly different at $p \leq 0.05$. * OSE, onion skin ethanolic extract; CLE, cauliflower leaves aquatic extract; BHT, butylated hydroxytoluene.



*Each value represents the mean value of three replicates. OSE, onion skin ethanolic extract; CLE, cauliflower leaves aquatic extract; BHT, butylated hydroxytoluene.

Figure 2. DPPH radical scavenging activity of Lolypup and Bonboni mixing with OSE and CLE as well as standard (BHT)*

Table 9. IC_{50} (DPPH) of Lolypup and Bonboni mixing with OSE and CLE as well as standard (BHT)*

Tested materials	BHT	OSE	CLE	Lolypup + OSE	Bonboni + CLE
IC_{50} ($\mu\text{g/mL}$)	11.25 ± 0.17^d	15.18 ± 0.81^c	14.14 ± 0.69^c	92.04 ± 0.93^b	142.05 ± 0.78^a

Data represents the mean value of three replicates $\pm$ SD. Means with different superscript letters in the same row are significantly different at $p \leq 0.05$. OSE, onion skin ethanolic extract; CLE, cauliflower leaves aquatic extract; BHT, butylated hydroxytoluene; IC_{50} , half maximal inhibitory concentration.

3.6. Microbial Studies

3.6.1. The Effect of Mixing Plant Parts Extracts on the Total Plate Count of the Suggested Sweet Products Storage At Room Temperature for 60 Days

The incorporation of plant extracts into sweet products has attracted increasing interest due to their potential antimicrobial properties, and this study investigates the total plate count (TPC) of Lolypup and Bonboni sweets, both with and without the addition of Onion Skin Ethanolic Extract (OSE) and Cauliflower Leaves Aquatic Extract (CLE), when stored at room temperature for 60 days. As shown in Table 10, there are significant differences in microbial growth between treated and untreated samples, indicating the effectiveness of these natural additives in reducing bacterial proliferation over time. For example, the TPC in Lolypup + OSE increased from 175 ± 11 cfu/g on Day 0 to only 1232 ± 38 cfu/g on Day 60, compared to 2780 ± 45 cfu/g in the untreated control, while Bonboni + CLE showed a rise from 170 ± 15 cfu/g to just 1390 ± 28 cfu/g, compared to 2820 ± 55 cfu/g in the untreated Bonboni, clearly demonstrating the antimicrobial potential of these plant-based additives. These results are consistent with previous findings; Sagar and Pareek, [104] reported that onion skin extract, due to its high content of phenolic compounds, significantly inhibits the growth of foodborne pathogens, while Zhang et al., [59] found that polyphenols in cauliflower leaves, such as chlorogenic and gallic acids, exhibit strong antimicrobial activity and can prolong food shelf life. The reduction in microbial growth in the treated samples can be attributed to bioactive compounds like flavonoids (e.g., quercetin and kaempferol in onion skin) and polyphenols in cauliflower leaves, which interfere with bacterial cell wall synthesis and metabolic pathways. These findings highlight the potential of OSE and CLE as natural preservatives that can safely enhance the microbiological quality and shelf life of sweet products, offering a promising alternative to synthetic chemical preservatives in line with growing consumer demand for clean-label and health-conscious food options.

Table 10. The effect of mixing plant parts extracts on total plate count (TPC, cfu/g) of sweet products storage at room temperature for 60 days*

Products	Time (Days)				
	0	15	30	45	60
Lolypup	190±15	570±30	1620±36	2050±25	2780±45
Lolypup + OSE	175±11	410±26	970±30	1203±32	1232±38
Bonboni	180±10	550±35	1680±28	2030±30	2820±55
Bonboni + CLE	170±15	390±29	1020±30	1227±27	1390±28

* Each value represents the mean $\pm$ SD (n=3). Means with different superscript letters under the same column were significantly different at $p \leq 0.05$. OSE, onion skin ethanolic extract; CLE, cauliflower leaves aquatic extract

3.6.2. The effect of Mixing Plant Parts Extracts on Total Yeast and Yeast Count of the Suggested Sweet Products Storage At Room Temperature for 60 Days

The incorporation of plant extracts into sweet products

has attracted growing interest due to their promising antimicrobial properties, and this study evaluates the Total Yeast and Mold Count (TYMC) in Lolypup and Bonboni sweets, both with and without the addition of Onion Skin Ethanolic Extract (OSE) and Cauliflower Leaves Aquatic Extract (CLE), stored at room temperature for 60 days. The data presented in Table 11 show considerable differences in fungal growth across treated and untreated products, with TYMC in Lolypup + OSE increasing from 30 ± 4 cfu/g at day 0 to 222 ± 10 cfu/g by day 60, compared to a higher 288 ± 15 cfu/g in the untreated control, and TYMC in Bonboni + CLE rising from 25 ± 2 cfu/g to only 214 ± 8 cfu/g, versus 252 ± 12 cfu/g in the untreated Bonboni, indicating that both plant extracts significantly suppressed fungal proliferation over time. These results are consistent with extensive prior studies demonstrating the antimicrobial potential of plant-derived compounds; for example, Sagar and Pareek, [104] reported the strong antifungal activity of onion skin extract, largely due to its high content of phenolics such as quercetin and kaempferol, while Zhang et al., [59] confirmed the antimicrobial effects of cauliflower leaf polyphenols like chlorogenic and gallic acids in food preservation. The reduction in total yeast and mold count (TYMC) in the treated samples can be directly attributed to these bioactive compounds that interfere with fungal cell structure and metabolic pathways, making them effective natural preservatives. The significant differences in microbial load between treated and control samples not only validate the antimicrobial function of OSE and CLE but also support their use in enhancing the microbiological safety and extending the shelf life of sweet products as clean-label alternatives to synthetic preservatives.

Table 11. The effect of mixing plant parts extracts on total yeast and mold count (TYMC, CFU/g) of sweet products storage at room temperature for 60 days*

Products	Time (Days)				
	0	15	30	45	60
Lolypup	50± 5	110± 10	145± 8	226± 12	288± 15
Lolypup + OSE	30± 4	75± 8	94± 10	140± 15	222± 10
Bonboni	30± 3	112± 5	135± 9	211± 10	252± 12
Bonboni + CLE	25± 2	82± 4	92± 10	134± 8	214± 8

* Each value represents the mean $\pm$ SD (n=3). Means with different superscript letters under the same column were significantly different at $p \leq 0.05$. OSE, onion skin ethanolic extract; CLE, cauliflower leaves aquatic extract

3.7. Sensory Evaluation of Lolypup and Bonboni Mixed with OSE and CLE

Table 12 presents sensory evaluation scores for two food products, Lolypup and Bonboni, both alone and after enrichment with onion skin ethanolic extract (OSE) and cauliflower leaves aquatic extract (CLE), where attributes such as appearance, color, taste, odor, texture, and overall acceptability were rated by ten trained panelists. The data reveal statistically significant improvements ($p \leq 0.05$) in most sensory attributes upon addition of OSE and CLE compared to controls. Specifically, the appearance and color scores were notably enhanced, with Bonboni + CLE achieving the highest ratings (9.12 ± 0.07 for appearance

and 8.35 ± 0.13 for color), attributed to natural pigments like flavonoids and carotenoids in the extracts that improve visual appeal [28,105,106]. Similar enhancements in processed foods have been linked to pigment enrichment and reduced browning [107,108]. Taste and odor scores also increased for Lolypup + OSE (8.37 ± 0.15) and Bonboni + CLE (8.54 ± 0.12), although differences were less pronounced; this aligns with findings that phenolic-rich vegetable extracts can mask off-flavors and add mild aromatic qualities through bioactive compounds such as quercetin and phenolic acids interacting with taste receptors and volatile compounds [70,109,110]. Texture improvements were significant, particularly for Bonboni + CLE (9.17 ± 0.20), likely due to polysaccharides and fibers enhancing mouthfeel, water retention, and structural integrity, as supported by related research on plant extract fortification [111,112,113]. Overall acceptability scores were higher in enriched products (Lolypup + OSE at 9.03 ± 0.09 and Bonboni + CLE at 9.13 ± 0.06), consistent with the trend that antioxidant-rich natural extracts improve sensory quality and consumer preference [95,97]. These positive sensory effects are attributed to the synergistic action of phenolics, pigments, and antioxidants that enhance appearance and flavor while contributing to product stability and shelf-life by mitigating oxidation and reducing rancidity and off-flavors [97,110]. The significant sensory improvements highlight the potential for vegetable by-products as natural food additives in response to growing consumer demand for clean-label, functional foods [114], with added health benefits linked to the antioxidant properties of these extracts reducing risks of chronic diseases [63].

4. Conclusion

This study demonstrated the effective extraction and application of bioactive compounds from agro-industrial food wastes, namely onion skins and cauliflower leaves, to enhance the nutritional and functional qualities of sugar confectionery products, Bonboni and Lolypup. The onion skin ethanolic extract (OSE) was notably rich in phenolics, flavonoids, and terpenoids, whereas the cauliflower leaves aquatic extract (CLA) contained higher levels of carotenoids, polysaccharides, triterpenoids, and lutein. These distinct phytochemical compositions contributed to improved antioxidant activities and antimicrobial effects in the fortified candies, as shown by enhanced free radical scavenging, inhibition of lipid peroxidation, and a significant reduction in microbial growth during 60 days of storage. Although the antioxidant capacities of the enriched sweets were elevated compared to the unfortified controls, they remained lower than synthetic antioxidants such as BHT and α -tocopherol. Sensory evaluations also revealed that the inclusion of these natural extracts positively influenced the products' appearance, color, taste, texture, and overall acceptability, suggesting a favorable consumer response alongside health benefits. Collectively, these findings highlight the potential of utilizing agro-industrial food wastes as sustainable, value-added sources of functional ingredients, supporting circular economy objectives and offering natural alternatives to synthetic additives. This strategy not only improves the quality and health attributes of food products but also promotes waste reduction and environmental sustainability.

Table 12. Sensory evaluation of Lolypup and Bonboni mixing with OSE and CLE

Products	Appearance	Color	Taste	Odor	Texture	Overall acceptability
Lolypup	8.80 ± 0.12^b	7.76 ± 0.15^b	8.33 ± 0.23^a	8.10 ± 0.08^b	8.88 ± 0.21^b	8.52 ± 0.23^b
Lolypup + OSE	8.98 ± 0.10^{ab}	8.15 ± 0.09^a	8.37 ± 0.15^a	8.49 ± 0.06^a	8.84 ± 0.10^b	9.03 ± 0.09^a
Bonboni	8.91 ± 0.12^b	7.81 ± 0.21^b	8.49 ± 0.10^a	8.21 ± 0.12^{ab}	9.12 ± 0.09^a	8.77 ± 0.11^b
Bonboni + CLE	9.12 ± 0.07^a	8.35 ± 0.13^a	8.54 ± 0.12^a	8.73 ± 0.11^a	9.17 ± 0.20^a	9.13 ± 0.06^a

Data represents the mean value of ten panelist's $\pm$ SD. Means with different superscript letters are different significantly at $P \leq 0.05$

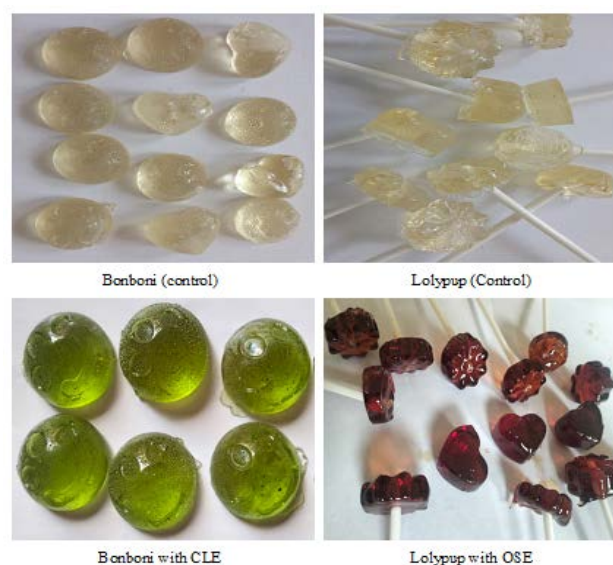


Figure 3. Sensory evaluation of control Lolypup and Bonboni, and mixing with natural plant parts extract (onion skin ethanol extract, OSE and cauliflower aquatic leaves extract, CLE).

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Conflict of Interests

Authors declared no competing of interest whatsoever

Ethical Considerations

The ethical issues of the current work was reviewed and approved by the Scientific Research Ethics Committee, Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt (Approval # 12- SREC- 01-2023).

Authors' Contribution

Yousif Elhassaneen contributed to the preparation and development of the study protocol, oversaw the practical experimental phase, retrieved conceptual information, reviewed and validated the results and statistical analyses, and assisted in preparing and reviewing the manuscript. Olfat Abd El-Moez conducted the practical experiments, collected, tabulated, and interpreted the results, retrieved the necessary information and concepts, and prepared the manuscript draft. Mai Garib and Tarek Abd El-Rahman assisted in preparing the study protocol, supervised the practical experiments, retrieved conceptual information, validated the study results, and contributed to drafting the manuscript.

Abbreviations

AA, antioxidant activity; AAC, antioxidant activity coefficient; Abs, absorbance; ADHD, attention-deficit hyperactivity disorder; AOX, antioxidant value; BCB, β - Carotene Bleaching; BD, BHT, butylated hydroxytoluene; CA, catechine; CLA, cauliflower leaves aquatic extract; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FAO, Food Agriculture Organization; FDA, Food Drug Administration; GA, gallic acid; IC₅₀, half maximal inhibitory concentration; ORR, oxidation rate ratio; OSE, onion skin ethanolic extract; ROS, reactive oxygen species; SDGs, Sustainable Development Goals; TPC, total plate count; TYMC, total yeast and mold count.

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