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Influence of Using Wheatgrass Juice on The Nutritional Value and Characteristics of Set Yogurt

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ABSTRACT

Fermented milk is widely recognized for its nutritional benefits, yet yogurt is often considered lacking in phenolic and antioxidant compounds. This study sought to enhance yogurt's nutritional profile by incorporating wheatgrass juice, a natural source of phenols and ascorbic acid, known for its antioxidant properties. This approach transforms yogurt into a functional food, combining probiotics and antioxidants through the addition of wheatgrass juice.Different ratios of wheatgrass juice (2, 4 and 6%) were used, chemical, physical, and rheological analyses were carried out and sensory properties were tested. The results showed that fortifying yogurt with wheatgrass juice led to an increase in its content of phenols and ascorbic acid, which led to a significant increase in antioxidant properties compared to plain yogurt. The higher the wheatgrass juice concentration, the higher the antioxidant value. The

concentration of 6% wheatgrass juice led to the highest total phenolic and ascorbic acid values. The results also showed that the fortified yogurt with 2% wheatgrass juice had the highest acceptance compared to all other treatments, as it showed excellent sensory quality. The production of yogurt fortified with wheatgrass juice can be considered a good candidate as an improved product, with a high nutritional, and high degree of acceptability by Egyptian consumers

Keywords: Fermented milk, Wheatgrass Juice, Yogurt, Antioxidant

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INTRODUCTION

Nowadays, the food industry is focused on developing healthier goods that are more in line with modern customer demands. Sprouting grains have emerged on the market in recent years as a new culinary element. The higher nutritious content, lesser amount of antinutrients, superior source of bioactive molecules, and sweeter flavor than non-sprouted grains make them a possible innovative element for the actual food industry (**Ding and Feng, 2019).**

According to **the Code of Federal Regulations of the United States Food & Drug Administration (FDA, 1996)**, yogurt can be defined as a food produced by culturing one or more of the optional dairy ingredients namely, cream, milk, partially skimmed milk, and skim milk, used alone or in combination with a characteristic bacterial culture that contains lactic acid producing bacteria, *Lactobacillus bulgaricus and Streptococcus thermophilus*. The global consumption of yogurt has been on the rise due to its recognized nutritional value, therapeutic effects, and functional properties(**McKinley, 2005)**. The use of different fruits and additives in fruit yogurt production has improved its nutritional and sensory properties **(Cakmakcı *et al*., 2012)**. Commonly utilized fruits in yogurt production include peaches, cherries, apricots, papaya, cactus pear, and blueberries **(Arslan and Ozel, 2012)**.

Yogurts added with natural extracts, such as grape and olive pomaces, have been shown to have not only higher antioxidant and antimicrobial effects, but also similar or higher shelf life and sensory acceptance, compared to yogurts added with synthetic preservatives **(Aliakbarian *et al*., 2015; Caleja *et al*., 2016; Hernández-Carrión *et al*., 2015)**. Additionally, the chemical compounds found in these natural extracts appear to exhibit relative stability, maintaining antioxidant activity even after undergoing in vitro simulated digestion. This suggests their potential suitability for incorporation into protein-rich foods **(Rashidinejad, *et al*., 2015).**

Sprouting wheat seeds is a highly nutritious cereal that has health benefits. It has a lot of fiber, protein, vitamins, and minerals. Sprouting increases the nutritional value and makes it simpler to digest. Including sprouted wheat in the diet can aid with immunity, growth and development, and weight management **(Peñaranda *et al*., 2021).**

Wheatgrass Juice (WGJ) is an extract squeezed from the mature sprouts of wheat seeds and it is the richest source of chlorophyll, active enzymes, vitamins A, B, C, E, and K, calcium, potassium, iron, magnesium, sodium, sulfur and at least seventeen amino acids **(Walters, 1992)**

Furthermore, a consistent consumption of fruits and vegetables is associated with a decreased risk of diseases, including cancer and cardiovascular conditions. This health benefit is attributed to the presence of natural antioxidants in fruits and vegetables, as highlighted by **Jang et al., 2010.** As reported by **Kulkarni et al. in 2006**, WGJ exhibits high antioxidant activity, attributed in part to the presence of antioxidants such as phenolic compounds and various flavonoids. The efficacy of phenolics and flavonoids in removing superoxide radicals in vivo has been demonstrated, leading to a reduction in cell damage caused by oxidative stress.Wheatgrass juice antioxidants have a wide range of biological activities, including the prevention of oxidative damage to DNA and lipids, the inhibition of carcinogen formation, the stimulation of gap junction communication, the inhibition of cancer cell proliferation, the promotion of cellular differentiation and apoptosis, and the activation of innate and adaptive immune functions **(Chiu *et al*., 2005; Wheat and Currie, 2008)**

Moreover, it is suggested that cancer patients undergoing chemotherapy can benefit from drinking wheatgrass juice, as it helps improve blood levels and potentially reduces the need for blood-building medications . Additionally, the use of chlorophyll-rich wheatgrass juice has shown to be beneficial in treating skin infections, as well as colon and skin ulcers, as mentioned by **Mujoriya and Bodla in 2011**.Wheatgrass juice is an ideal option for inclusion in mouthwashes to prevent pyorrhea and sore throats due to its anti-microbial qualities (**Hassan and Siddique, 2022**). In line with this, the objective of the present study was to develop an enriched wheatgrass juice yogurt, aiming to create a functional product with inherent bioactive properties.

MATERIAL AND METHODS

Materials

Fresh cow milk was sourced from a local farm in Damanhour, Beheria governorate, while wheat seeds were acquired from the Ministry of Agriculture in Egypt. The commercial starter culture, consisting of *Streptococcus thermophilus and Lactobacillus delbrueckii subsp. Bulgaricus,* was obtained from obtained from CHR HANSEN- Denmark).

Preparation of wheatgrass juice

Wheat seeds (*Triticum aestivum*) underwent a cleaning process with tap water to eliminate undesired particles and reduce microbial content on the surface. Following this, the seeds were soaked overnight to promote germination, subsequently drained, and planted in soil. Harvesting occurred in the autumn, precisely on the 10th day after sowing when the grass had achieved a height of 7 cm. Using scissors, the grass was cut, and wheatgrass juice was extracted through a masticating juicer. The resulting product underwent filtration for refinement.

Chemical composition of wheatgrass juice

Moisture; protein; fat; ash; Vitamin C; Chlorophyll; Total Soluble Solids; Total phenolic content; Total flavonoid content; Antioxidant activity and carbohydrate content of wheatgrass juice were determined according to the methods described by **AOAC (2016**). All determinations were performed in triplicates and the mean values were reported in Table 1.

Yogurt manufacture

The yogurt was made according to **Helal and Tagliazucchi (2018)** with some modifications. In each of the three replications, Wheatgrass juice was included in Cow milk with different ratios (2, 4, and 6 %) and mixed well. The control without the addition of wheatgrass juice was also made to compare the results. Heat treatments were applied (90°C for 3 minutes). Then, all the mixtures were cooled to 45 °C and inoculated with 3% (w/v) of yogurt starter culture. After incubating the samples at 45°C for 4 hours during the fermentation phase, they were cooled and stored at 4°C and subjected to different analyses on the first day of storage.

Table 1. Proximate chemical composition of wheatgrass Juice

Parameters	Mean Value ± SD (per 100 g)
Moisture (g)	**94.00±2.8**
Carbohydrate (g)	**3.123±0.2**
Fat (g)	**0.08±0.001**
Crude Protein (g)	**2.00±0.1**
Ash (mg)	**877±21**
Chlorophyll (mg)	**48.00±2.3**
Ascorbic acid (mg)	**3.30±0.04**
pH	**5.99±0.02**
Total Soluble Solids	**4.44±0.12**
Antioxidant Activity (%)	**93.50±7**
Total phenolic (mg gallic acid)	**33.00±0.07**
Acidity	**0.55±0.01**

Yogurt Physiochemical Analysis

Proximate chemical composition

The proximate chemical composition of various yogurt treatments was assessed using standard methods outlined by **AOAC (2000)**. The analysis covered moisture content, ash, protein, crude fiber, and total solids. Carbohydrate content was calculated by difference, as described by **Ihekoronye and Ngoddy (1985)** using the formula CHO = 100 - % (ash +

protein + fat + crude fiber + moisture). Additionally, total energy was calculated using the formula: Total Energy (calories/g) = Fat percent x 9.3 + Protein percent x 4.1 + Carbohydrate percent x 4.1.

Titratable acidity (expressed as % lactic acid) using titration methods (AOAC, 2000).

pH analysis

A pH meter (Mettler, Toledo, DELTA-320 pH, Shanghai, China) was used to monitor the pH values of yogurt samples.

Total ascorbic acid determination

Ascorbic acid in wheatgrass juice and yogurt was determined according to AOAC method (**AOAC, 2000**).

Determination of total phenolic content

The total phenolic content (TPC) was determined using the Foline-Ciocalteu colorimetric method **by Singleton *et al*. (1999)** with some modifications. The sample (0.1 mL) was mixed with 2 ml of 2% sodium carbonate. After 2 minutes, 0.1 mL of 50% Foline-Ciocalteu reagent was added, and the solution was allowed to stand for 30 minutes at room temperature. The samples were measured at 750 nm versus a blank using a spectrophotometer. The results were expressed as mg of gallic acid equivalent (GAE)/ mL of sample

Antioxidant activity determination

The DPPH assay was conducted according to the method of **Blois (1958)** .0.1 ml of extract was mixed with 2.9 ml of 0.1 Mm DPPH in methanol. The mixtures were shaken vigorously and allowed to stand in the dark at room temperature for 20 minutes, after which the absorbance was read at 517 nm using a spectrophotometer. The scavenging capacity of the sample was calculated using the following equation:

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Scavenging activity % = [1- (A sample – A blank) /(A control – A blank)]*100

Rheological Analysis

Textural Profile Analysis

The texture profile analysis of yogurt samples was determined using a texture analyzer (TA-RT-KI) equipped with load cell 10000 g of and a cylindrical probe (TA18). Before TPA analysis, the samples were left at 25 ºC. TPA was performed by compressing twice using the probe to make a 5mm penetration with 2 mm/s pretest speed and 1 mm/s test speed. Hardness, springiness, and cohesiveness were determined from TPA by using software. All measurements were carried out in triplicate for each sample.

Syneresis

Wheying-off was determined according to **Modha and Pal (2011)** by placing 10 mL of beverage in a clean 15 mL graduated capped tube and leaving it undisturbed in the refrigerator (5±1 C) for 24 hours. The amount of separated whey can be recorded and represented as a percentage. wheying-off.

Sensory Evaluation

The sensory evaluation was based on a hedonic scale according to **Peryam and Pilgrim (1957)** and conducted with 25 nontrained judges (students). Descriptive-sensory analysis with 10 points of the scale (1 =dislike extremely, 5 =either like or dislike, 10 =like extremely) was applied to assess flavor, color, consistency/texture, and overall acceptability of all yogurt samples on the first day of cold storage.

Microbial properties

Testing for total coliform group, mold, and yeast was according to standard methods for the examination of dairy products using the Violet Red Bile Agar medium (VRBA) and Potato Dextrose Agar medium (PDA), respectively **(Marth, 1978)**.

Statistical Analysis

Data are presented as mean±SD for three replicates of each sample. Univariate Analysis of Variance (ANOVA) was conducted using StatGraphics 16.1.11 (StatPoint Technologies, Inc., Virginia, USA) for multiple comparisons, with statistical significance set at $p<0.05$.

RESULTS AND DISCUSSION

Nutritional composition of yogurt with wheatgrass juice

Table 2 shows the different chemical parameters of yogurt with different percentages of wheatgrass juice. Regarding the high moisture content in wheatgrass juice (94±2.8%); the moisture content of yogurt from the different percentages of wheatgrass juice was significantly higher than the control because incorporation of wheatgrass juice.

In a similar way fat, protein, ash, carbohydrate, and energy content of yogurt from different percentages of wheatgrass juice was significant ($p\leq0.05$) lower than the control, this might be due to lower fat, protein, ash, carbohydrate, and energy content in wheatgrass juice as previously shown in Table 1.

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Table 2. Physiochemical Properties of yogurt samples enriched with wheatgrass juice

Parameters	Control (0% wheatgrass juice)	2% wheatgrass juice	4% wheatgrass juice	6% wheatgrass juice
Moisture (%)	84.70±0.20[b]	87.65±0.14[a]	88.31±0.30[a]	88.56±0.05[a]
Carbohydrates (%)	7.76±0.32[a]	5.08±0.16[b]	4.83±0.11[bc]	4.79±0.08[c]
Protein (%)	3.56±0.06[a]	3.46±0.05[a]	3.24±0.04[b]	3.08±0.08[b]
Fat (%)	3.01±0.01[a]	2.91±0.02[a]	2.80±0.07[ab]	2.74±0.03[b]
Ash (%)	0.724±0.001[a]	0.710±0.006[ab]	0.703±0.001[b]	0.695±0.002[b]
Energy(kcal)	75.65±0.22[a]	62.44±0.05[b]	59.49±0.30[bc]	58.46±0.14[c]
pH	4.72±0.006[a]	4.68±0.017[a]	4.66±0.010[a]	4.65±0.038[a]
Acidity	0.71±0.010 [b]	0.72±0.006[b]	0.74±0.004[ab]	0.76±0.004[a]
TS	15.30±0.30[a]	12.21±0.20[b]	11.68±0.10[c]	11.41±0.10[c]

Data are Means±SD (n = 3), [a-d] Significant differences within the same raw are shown by different letters at $p<0.05$

Antioxidant activity of control and yogurt enriched with wheatgrass juice

The ascorbic acid, phenolic contents, and antioxidant activity values of yogurt samples are presented in **Table 3.** There were significant differences in the ascorbic acid, phenolic contents, and antioxidant activity of the samples ($P <0.05$).

The ascorbic acid content of yogurt varied from 0.78±0.12 to 0.93±0.15mg/ 100g for the control and yogurt contained 6% wheatgrass juice, respectively. it may be due to the highest ascorbic acid in wheatgrass juice.

Total Phenolic contents ranged between (0.67±0.14 and 1.64±0.104 mg EGA /100g). As expected, yogurt with a higher ratio of wheatgrass juice resulted in higher total phenolic content. The control treatment showed the lowest phenolic content.

As a result of the higher ascorbic acid content and phenolic content in wheatgrass juice **Chomchan et al, (2016)**, the fortification of wheatgrass juice positively affected the antioxidant activity of yogurt. The highest value of antioxidant activity was found in the highest concentration of wheatgrass juice treatment (6%). On the other hand, the control treatment showed the lowest value.

Table 3 Antioxidant activity of yogurt samples enriched with wheatgrass juice

Sample	Ascorbic acid (mg/ 100g)	TPC (mg EGA /100g)	Antioxidant Activity (%)
Control (0% wheatgrass juice)	$0.78±0.12^{d}$	$0.67±0.14^{d}$	$0.19±0.01^{d}$
2% wheatgrass juice	$0.81±0.12^{cd}$	$0.96±0.07^{c}$	$1.13±0.01^{c}$
4% wheatgrass juice	$0.85±0.08^{bc}$	$1.31±0.10^{b}$	$2.04±0.03^{b}$
6% wheatgrass juice	$0.93±0.15^{ab}$	$1.64±0.10^{a}$	$3.04±0.14^{a}$

Data are Means±SD (n = 3), $^{a\text{-}d}$ Significant differences within the same column are shown by different letters at $p<0.05$

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Rheological properties

Texture Profile Analysis

Results for texture profile analysis parameters such as hardness, cohesiveness, and springiness are presented in Table 4.

Table. 4. Texture profile analysis of yogurt samples enriched with wheatgrass juice

Treatment	Hardness (g)	Springiness (mm)	Cohesiveness
Control (0% wheatgrass juice)	**27±0.76[a]**	**2.52±0.08[a]**	**0.36±0.02[a]**
2% wheatgrass juice	**24±2.00[a]**	**2.12±0.15[b]**	**0.32±0.01[b]**
4% wheatgrass juice	**18±0.17[b]**	**1.88±0.07[c]**	**0.27±0.02[c]**
6% wheatgrass juice	**12±0.10[c]**	**1.47±0.05[d]**	**0.22±0.03[d]**

Data are Means±SD (n = 3), [a-d] Significant differences within the same column are shown by different letters at $p<0.05$

Hardness, defined as the maximum force during the first compression cycle (**Gauche et al., 2009**), exhibited a significant decrease in yogurt formulations with the addition of wheatgrass juice compared to the control sample. Moreover, the reduction in hardness was more pronounced with higher concentrations of wheatgrass juice added to the yogurt, as indicated in Table 4.The highest level of hardness was related to the control (27.00 ±0.76 g) while the lowest hardness level was attributed to the sample which contained the highest level of wheatgrass (12±0.1 g). Similar results were reported by **(Hashemi Gahruie et al. 2019)** who found that the addition level of wheat germ (0, 10, 20, and 30% based on dry matter) to yogurt samples Hardness (g) were 23 ±0.71, 20.25±1.06, 14.75±0.35 g, respectively.

Springiness, which reflects the speed at which a sample returns to its original condition after the application of deformative forces, was assessed in this study. The control treatment exhibited the highest springiness value. However, the introduction of wheatgrass juice resulted in a decrease in springiness, with this reduction becoming more prominent as the ratio of wheatgrass juice increased. This trend aligns with the findings reported by **Helal et al. (2018).**Cohesiveness is defined as the ratio of the positive force area during the second penetration to that of the first penetration. It may be measured as the rate at which the material is disintegrated under mechanical action. Tensile strength is a manifestation of cohesiveness. The cohesiveness indicates the ability of the product to hold together (**Chandra and Shamasundar, 2015**).

Similar to the hardness and springiness, Control the highest Cohesiveness (**2.52±0.08)**, while the addition of wheatgrass juice decreased the Cohesiveness with increasing its ratio recorded 2.12±0.15, 1.88±0.07 and 1.47±0.05 for the concentrations of 2, 4 and 6% of wheatgrass juice, respectively. Consistent with hardness and springiness, the control sample exhibited the highest cohesiveness (2.52±0.08). The addition of wheatgrass juice led to a reduction in cohesiveness, with decreasing values corresponding to increasing wheatgrass juice ratios. Specifically, cohesiveness was recorded as 2.12±0.15, 1.88±0.07, and 1.47±0.05 for concentrations of 2%, 4%, and 6% of wheatgrass juice, respectively.

Syneresis

Syneresis, defined as the formation of a top liquid phase (whey) resulting from the shrinkage of a gel, is a common problem in yogurt. Wheying-off is a negative characteristic of set yogurt and it is defined as the expulsion of whey from the casein network. Spontaneous wheying-off is the separation of whey without the application of any external force that is associated with an unstable gel network.

This can be caused by increased rearrangements of the gel matrix or by mechanical damage to the weak gel network. Manufacturers used stabilizers, such as starch, pectin, and gelatin, to prevent wheying-off **(Nikoofar et al. 2013)**

Common causes of wheying-off include high incubation time, disproportionate whey protein to casein ratio, low solid content, and physical mishandling of the product during storage and distribution.

The most syneresis was observed with the highest concentration of wheatgrass juice (6%) which recorded 5.2±0.4 %, while the lower concentrations were almost similar to control. This result could be attributed to the decreasing total solids content of wheatgrass juice

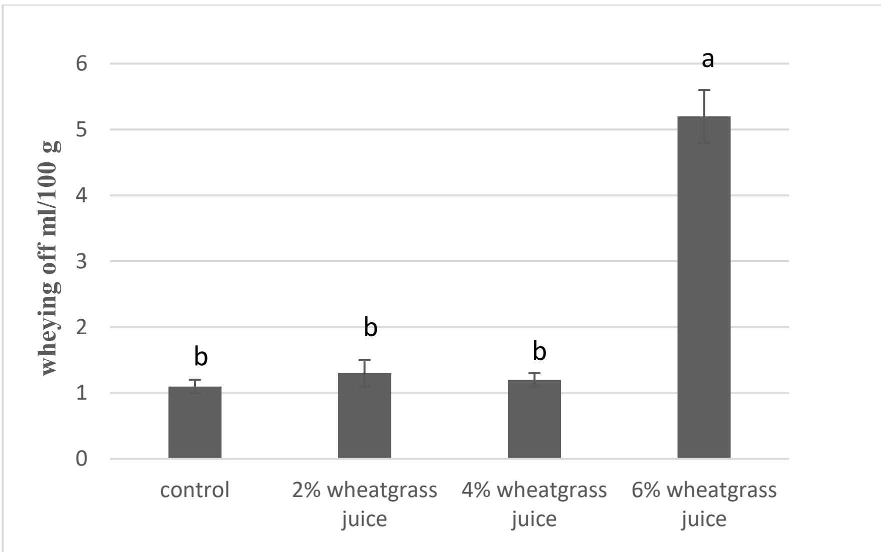

Figure 1. Syneresis values of control and yogurt fortification with different ration of wheatgrass juice, Values are means of three independent samples ± standard deviation (SD). Different letters indicate significantly different values (*P < 0.05*).

Sensory evaluation of yogurt

Sensory evaluation was applied to the different treatments of yogurt and the data is shown in Table 5. The addition of wheatgrass juice led to a lower color evaluation and the results showed a significant difference (P <0.05) compared to the control one; the increase of wheatgrass concentration negatively affected the panelist evaluation. The green and violet colors of most vegetables are contributed by chlorophyll and anthocyanins **Lazcano et al. (2001)**, therefore the degradation of color in wheatgrass juice spreads can most likely be related to the degradation of chlorophyll. The addition of 2% wheatgrass juice on yogurt showed the highest acceptability values among the other concentrations.

Similar to color, the flavor of the control sample had the highest value, followed by the 2% wheatgrass juice concentration. However, in terms of consistency and overall acceptability, the addition of 2% of wheatgrass juice was the most recommended by the panelists after the control treatment. Other levels of wheatgrass juice were not well-received in terms of flavor, consistency, and overall acceptability, with a decreasing trend observed as the wheatgrass juice concentration increased. This suggests that higher concentrations of wheatgrass juice negatively impacted the sensory attributes of flavor, consistency, and overall acceptability.

Table 5. Sensory evaluation of yogurt samples enriched with wheatgrass juice

Sample	Color	Flavor	consistency	Overall acceptability
Control (0% wheatgrass juice)	9.15 ± 0.05^{a}	9.07 ± 0.06^{a}	$9.55\pm0.05^{a,b}$	9.25 ± 0.13^{a}
2% wheatgrass juice	8.50 ± 0.10^{b}	8.66 ± 0.09^{b}	$9.03\pm0.15^{b,c}$	8.73 ± 0.13^{a}
4% wheatgrass juice	7.26 ± 0.08^{c}	7.52 ± 0.03^{c}	8.00 ± 1.00^{c}	7.50 ± 0.05^{b}
6% wheatgrass juice	6.66 ± 0.12^{d}	6.13 ± 0.15^{c}	6.40 ± 0.20^{d}	7.03 ± 0.15^{c}

Data are Means±SD (n = 25), $^{a-d}$ Significant differences within the same column are shown by different letters at p<0.05

Microbial quality

Coliform group and mold and yeast were not detected in all yogurt samples

CONCLUSION

The study investigated the impact of varying ratios of wheatgrass juice (2%, 4%, and 6%) on yogurt, conducting comprehensive chemical, physical, and rheological analyses while evaluating sensory attributes. The addition of wheatgrass juice led to increased phenol and ascorbic acid content in yogurt, resulting in significantly enhanced antioxidant properties compared to plain yogurt. Higher concentrations of wheatgrass juice correlated with greater antioxidant values, with the 6% concentration yielding the highest total phenolic and ascorbic acid levels. In terms of sensory quality, the yogurt enriched with 2% wheatgrass juice outperformed all other treatments, making it the preferred choice. Overall, yogurt fortified with wheatgrass juice emerges as a promising candidate for an enhanced, nutritionally rich product.

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تأثير استخدام عصير عشبة القمح على القيمة الغذائية وخصائص الزبادي الجالس

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الملخص العربي

تعرف الالبان المتخمرة على نطاق واسع بفوائدها الغذائية ، ومع ذلك غالبا ما يعتبر الزبادي مصدرا فقيرا للمركبات الفينولية ومضادات الأكسدة. سعت هذه الدراسة إلى تعزيز الخصائص الغذائية للزبادي من خلال دمج عصير عشبة القمح ، وهو مصدر طبيعي للفينولات وحمض الأسكوربيك ، المعروف بخصائصه المضادة للأكسدة. يحول هذا النهج الزبادي إلى غذاء وظيفي ، يجمع بين البروبيوتيك ومضادات الأكسدة من خلال إضافة عصير عشبة القمح. تم استخدام نسب مختلفة من عصير عشبة القمح (٢ و ٤ و ٦٪) ، وتم إجراء التحليلات الكيميائية والفيزيائية والريولوجية واختبار الخصائص الحسية. أظهرت النتائج أن تقوية الزبادي بعصير عشبة القمح أدى إلى زيادة محتواه من الفينولات وحمض الأسكوربيك ، مما أدى إلى زيادة كبيرة في خصائص مضادات الأكسدة مقارنة بالزبادي العادي. كلما زاد تركيز عصير عشبة القمح ، زادت قيمة مضادات الأكسدة. أدى تركيز ٦٪ من عصير عشبة القمح إلى أعلى إجمالي قيم من الفينولات وحمض الأسكوربيك. أظهرت النتائج أيضا أن الزبادي المدعم بعصير عشبة القمح بنسبة ٢٪ كان له أعلى قبول مقارنة بجميع العلاجات الأخرى ، حيث أظهر جودة حسية ممتازة. يمكن اعتبار إنتاج الزبادي المدعم بعصير عشبة القمح مرشحا جيدا كمنتج محسن ، مع تغذية عالية ، ودرجة عالية من القبول من قبل المستهلكين المصريين.