International Journal of Social Sciences Bulletin
Volume 2, Issue 4, 2024 ISSN: (E) 3007-1917 (P) 3007-1909

ENHANCING ANTIOXIDANT CONTENT AND CONSUMER APPEAL IN GREEK YOGURT FORTIFIED WITH GRAPE SEED AND STRAWBERRY PULP

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Received	Revised	Accepted	Published
03 September, 2024	03 October, 2024	22 October, 2024	08 November, 2024

ABSTRACT

This study developed Greek yogurt fortified with grape seed extract and strawberry pulp to improve its nutritional and sensory properties. Goat milk was used as the base and carefully processed to maintain optimal quality. To improve the yogurt's antioxidant properties, flavor, and smell, phenolic-rich grape seed extract and natural strawberry flavor were added. Detailed chemical, physical, and sensory examinations showed that yogurt can be creamy and well-balanced. These results demonstrate the usefulness of fortification as an approach to increasing health efficiency and the desirability of the Yogurt product to the consumer's growing appetite for functional foods.

Keywords: *Goat Milk, Greek yogurt, Grape seeds, antioxidants, polyphenols.*

INTRODUCTION

Greek yogurt has also antioxidants and polyphenols that are beneficial to the body, in addition to the protein and probiotics. Antioxidants scavenge free radicals and are needed in a healthy system where oxidative stress is omnipresent. These resilient compounds serve to enhance the endurance of the human body, augment certain areas such as gut health, and provide anti-inflammatory effects to the body. Nonetheless, adding polyphenol-rich ingredients, such as Grape Seed Extract (GSE) or strawberry pulp, would further increase the existing benefits of Greek yogurt, making it a desirable food for customers seeking for antioxidant-rich product. Pigni and Sereno suggest that strawberries contain anthocyanins, polysaccharides, and other antioxidants, vitamins, and minerals as well as fiber which are the key ingredients necessary to provide the body with nutrients. Ellagic acid, quercetin, catechin, pelargonidin-3-glucoside are flavonoid that are vital in strawberries. These compounds protect the body against oxidative stress that leads to free radicals which are known to cause chronic diseases such as cardiovascular diseases, cancer and inflammatory disorders after being neutralized. For example:

Ellagic Acid: This polyphenol has strong antioxidant activity and has been studied for its anti-carcinogenic properties, specifically through the inhibition of cancer cell proliferation and induction of apoptosis in specific cancer cell lines. It also possesses anti-inflammatory properties by blocking pathways which generate inflammation mediators (Fig 1.).

Quercetin: A strong antioxidant with anti-inflammatory activity, quercetin has possible cardiovascular health benefits owing to its ability to lower raised blood pressure and help maintain

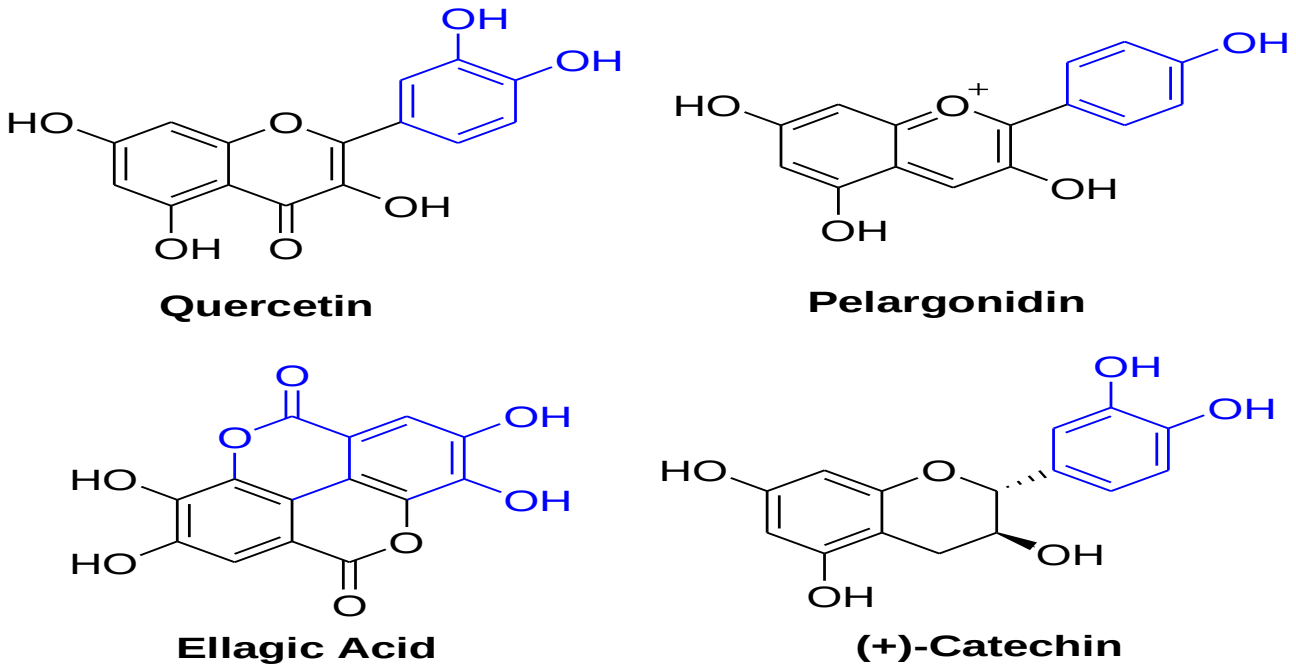
vascular integrity. It is also associated with immune-modulating activity, which may further prevent cells from undergoing damage.

Catechin: This antioxidant is one of the flavonoids present in strawberries and grape seeds. Besides preventing heart disease and alleviating inflammation in the body, it is believed to be beneficial for weight loss by regulating lipid metabolism.

Pelargonidin-3-glucoside: Responsible for the red pigmentation in strawberries, this pigment is claimed to help protect cardiovascular and heart health. It has been found to lower lipid levels, decrease oxidative stress, and exhibit anti-inflammatory properties.

Health Benefits of Hitting Towards Polyphenols-Rich Fusion: The combination of GSE and strawberry pulp in Greek yogurt does not only boost yogurt's antioxidant profile but could also be beneficial in enhancing its anti-inflammatory, cardioprotective, and anti-cancer properties. For instance, specific polyphenols present in GSE, proanthocyanidins to be specific, have been reported to possess the potential of free radical scavenging where they reduce oxidative stress and its consequences in the form of heart diseases and other degenerative diseases (Tulipani *et al*, 2011; Chouchouli *et al*, 2013). This study intends to make Greek yogurt a functional food by having the practices of this region of the world combining GSE and strawberry pulp to create a health product that utilizes functional polyphenols and antioxidant properties. The improved antioxidant profile also adds to the nutrition aspect of the yogurt, after all, improved antioxidant status is an added texture with additional taste and color, which modern consumers are looking for.

Fig. 1 Plant-Derived Polyphenols: Quercetin, Pelargonidin, Ellagic Acid, and (+)-Catechin"



MATERIALS AND METHODS

This study was carried out in the Food Science Laboratory in Department of Chemistry, University of Engineering and Technology, Lahore and Food Science Laboratory of Pakistan Council of Scientific and Industrial Research. Goat milk was sourced from a local dairy farm in Mughalpura, Lahore, Pakistan, stored at 4°C for 12 hours, and then transported to the laboratory. Grape seeds from various grape varieties (green, red, and black grapes) were isolated after purchasing the grapes from markets in Fatehgarh and Singhpura Mandi in Lahore, as well as Al-Madina in Saudi Arabia. Fresh strawberries from the Fatehgarh market in Lahore were used to obtain a natural strawberry flavor for sensory evaluation. Five liters of goat milk were boiled at approximately 100.5°C

to eliminate pathogenic microorganisms, then cooled, and its physicochemical properties, including pH, fat content, and protein content, were analyzed to ensure alignment with the desired composition for Greek yogurt production.

Greek yogurt was produced using boiled goat milk, which was cooled to an optimal temperature for inoculation with starter cultures. Leftover Greek yogurt was used as a natural starter culture. After inoculation, the milk was incubated at a controlled temperature for approximately 5 to 6 hours, allowing fermentation to proceed until the desired pH of around 5.0 was reached. During fermentation, Lactobacillus bulgaricus and Streptococcus thermophilus bacteria converted lactose to lactic acid, promoting the coagulation of milk proteins and creating the characteristic texture and flavor of Greek yogurt. After fermentation, the yogurt was strained using a muslin cloth to separate the whey from the curd, resulting in a thicker, protein-rich yogurt ideal for fortification. Red and black grapes were washed, deseeded, and air-dried to remove moisture. The seeds were ground into a fine powder using a mortar and pestle to ensure uniform particle size. For extract preparation, 2 g of grape seed powder was mixed with 20 ml of distilled water in a flask and shaken overnight on a rotary shaker to facilitate extraction. The mixture was then filtered, and the clear extract was used for yogurt fortification and further analysis.

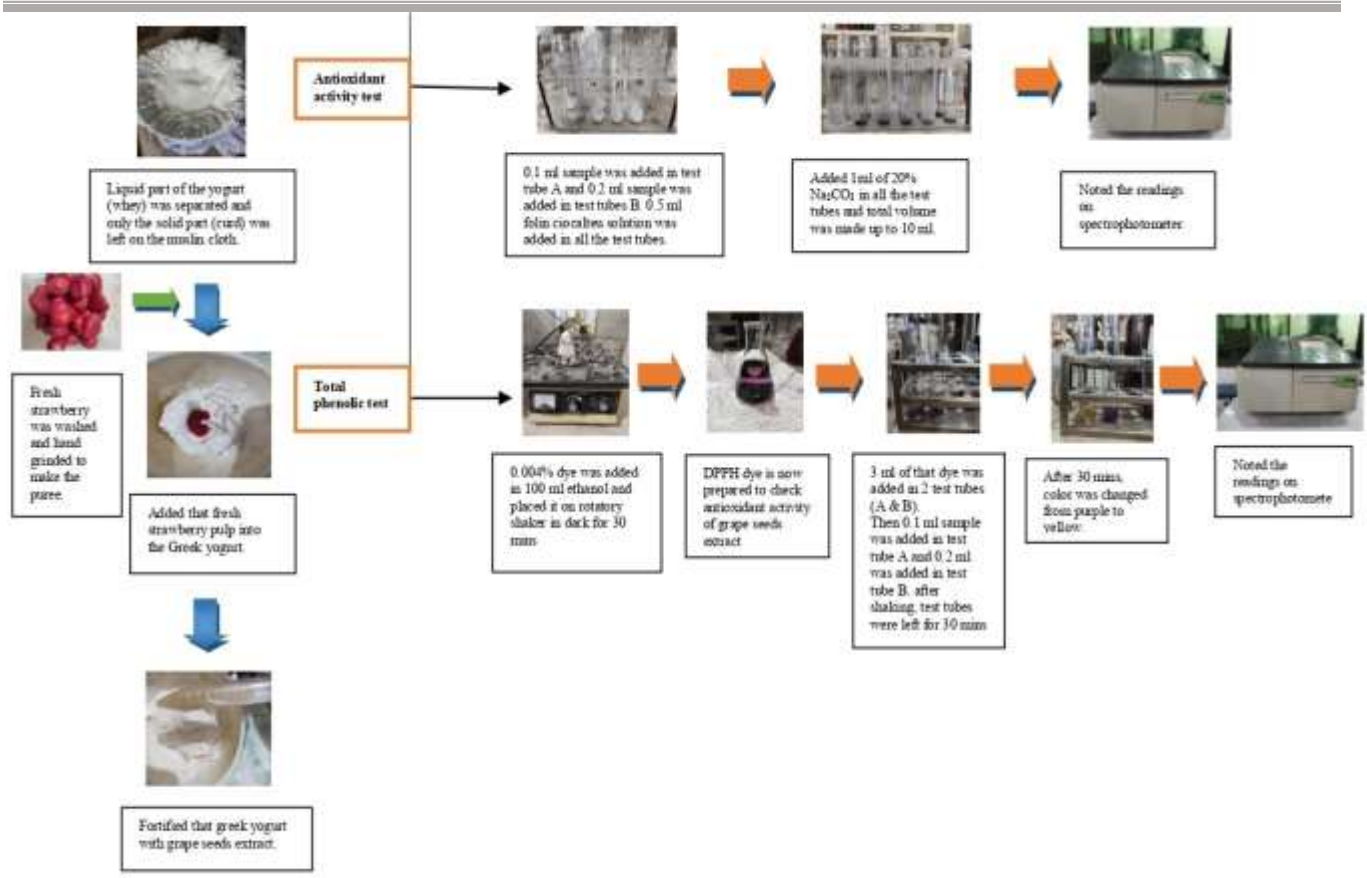


Fig. 2 Experimental setup followed during the present study

Chemical and Physical Analysis

Antioxidant Activity Test

To measure antioxidant activity, a 0.004% DPPH solution was prepared by dissolving DPPH in 100 ml of ethanol. This solution was shaken in the dark for 30 minutes to ensure full dissolution. Next, 3 ml of the DPPH solution was added to two test tubes. For the evaluation of the antioxidant activity of the sample yogurt, 0.1 ml of the yogurt sample was placed into one test tube (Sample A), and 0.2 ml was placed into the second test tube (Sample B). The tubes were agitated and subsequently stored in the dark for thirty minutes. The color change from purple to yellow indicated a reduction in DPPH radicals, and absorbance was measured at 517 nm using a spectrophotometer (FWTL/018).

Total Phenolic Content (TPC) Test

To find out TPC, test tubes containing yogurt samples were prepared in sterile conditions. One tube was filled with a 0.1 ml yogurt sample, while a 0.2 ml yogurt sample was used to fill the other tube. In each tube, 0.5 ml of Folin-Ciocalteu Reagent and 1 ml of 20% sodium carbonate solution were also added, the final volume being made up to 10 ml with distilled water. The samples were kept in the dark at room temperature for an hour before a spectrophotometer was used to take readings to measure absorbance at 765 nanometers using ethanol blank which was also the control. To enhance the accuracy of the quantification, a calibration curve for gallic acid for which the TPC of samples was expressed in terms of milligrams of Gallic acid equivalents per gram of dry weight of the sample (mg GAE/g DW) was prepared.

Sensory Evaluation

A sensory evaluation was conducted to assess the organoleptic properties of fortified Greek yogurt, focusing on consistency, taste, and flavor. Thirty panelists, including students and faculty from the Department of Chemistry at the University of Engineering and Technology, Lahore, participated in

the evaluation. Samples were presented individually to each panelist, along with a plate and spoon. Panelists rated the yogurt based on color, texture, aroma, taste, and overall acceptability using a 9-point hedonic scale, with 9 representing "like extremely" and 1 representing "dislike extremely.

RESULTS AND ANALYSIS

The goat milk used in the preparation of the yogurt contained 3.8% fat and 3.5% protein, which were both in the desired levels for Greek yogurt making. Microbial analysis indicated a total count of 2.5 x 10^5 CFU/ml, which was within the acceptable limits establishing the milk for further processing. In this case, they were starter cultures taken from the remaining Greek yogurt that contained Lactobacillus bulgaricus and Streptococcus thermophilus. The 42-degree temperature was maintained for 5-6 hours during the inoculation of the milk which enabled the fermentation process. A creamy yogurt with a sour taste was obtained during the fermentation, consistent with the expected organoleptic properties of Greek yogurt. The epiphytic extracts of the grape seed powder have been evaluated for total phenolic concentration and antioxidant activity. For this study, the measuring technique employed the Folin-Ciocalteu method, where the phenolic content was verified as 191.66 mg GAE/g. The DPPH assay showed an 82.505% inhibition level in the antioxidant activity indicating grape seed extract's high antioxidant activity and efficiency in yogurt fortification. The grape seed powder phenolic content was assessed after the preparation of its aqueous extract. For each sample absorbance values were obtained by a spectrophotometer and recorded. The amount of phenolic content in grapes was quantified using the gavel acid equivalent measure from the curve with $y=0.0996x$ and a coefficient of 0.9999. This TPC value is relatively high for grape seeds and was reported at 191.66 mg GAE/g. It was observed that the absorbance of the solutions increased linearly with the concentration of the grape seed powder; thus, the concentration and the absorbance were related.

Sample 1A: Exhibited the lowest concentration and absorbance, representing a low-concentration sample.

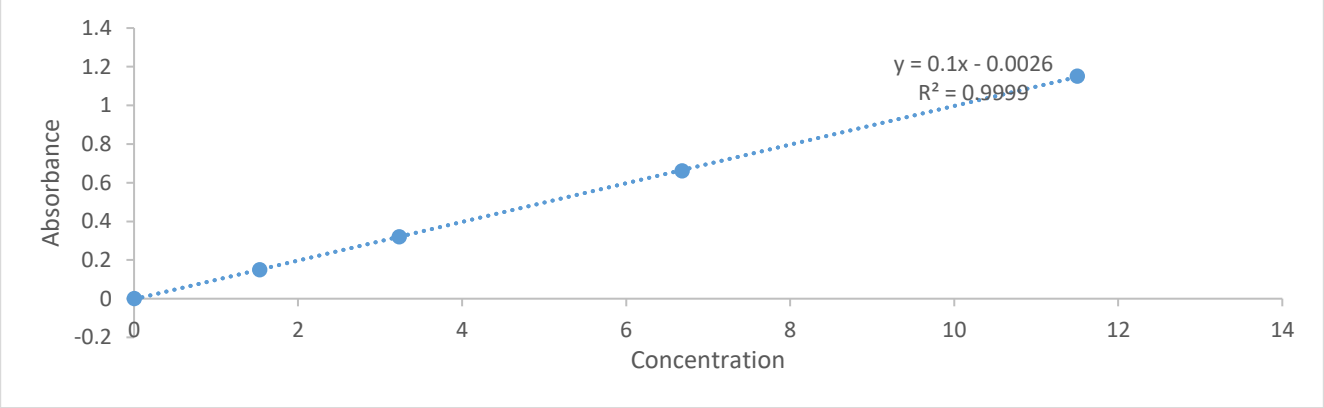
Sample 2A: Showed moderate concentration and absorbance, representing a mid-concentration sample.

Sample 3A: Indicated higher concentration and absorbance.

Sample 4A: Exhibited the highest concentration and absorbance, potentially indicating a saturated solution.

This progression in absorbance values across samples reflects the direct relationship between grape seed powder.

Fig. 3 Absorbance Curve of Grape Seed Powder Extract at Varying Concentrations



Concentration and Phenolic content

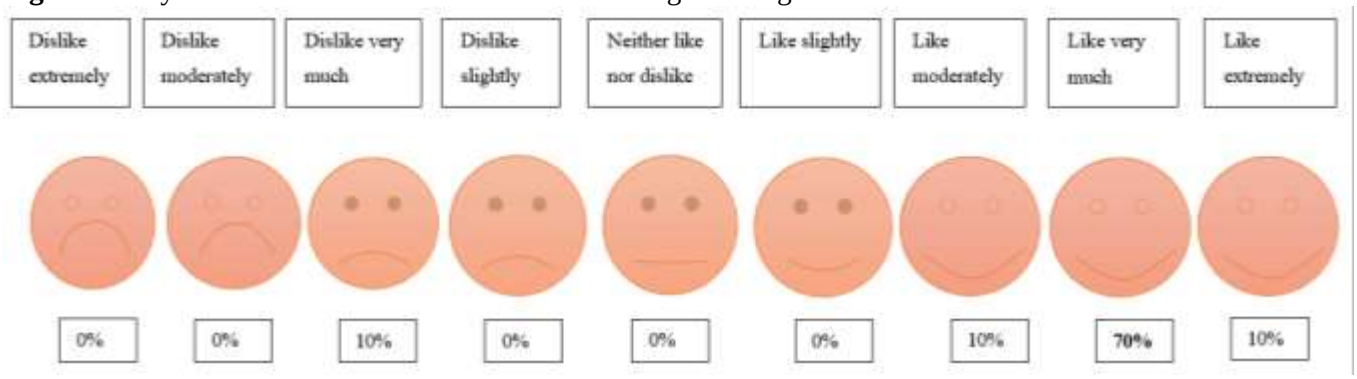
The pH of the fortified Greek yogurt was measured at 4.2, which is suitable for yogurt. The total lactobacillus count was 2.5 x 10*5 CFU/ml, ensuring adequate probiotic viability.

Total Phenolic Content in Fortified Yogurt

Using the Folin-Ciocalteu assay, the fortified Greek yogurt exhibited a total phenolic content of 191.66 mg GAE/100g, indicating the successful transfer of

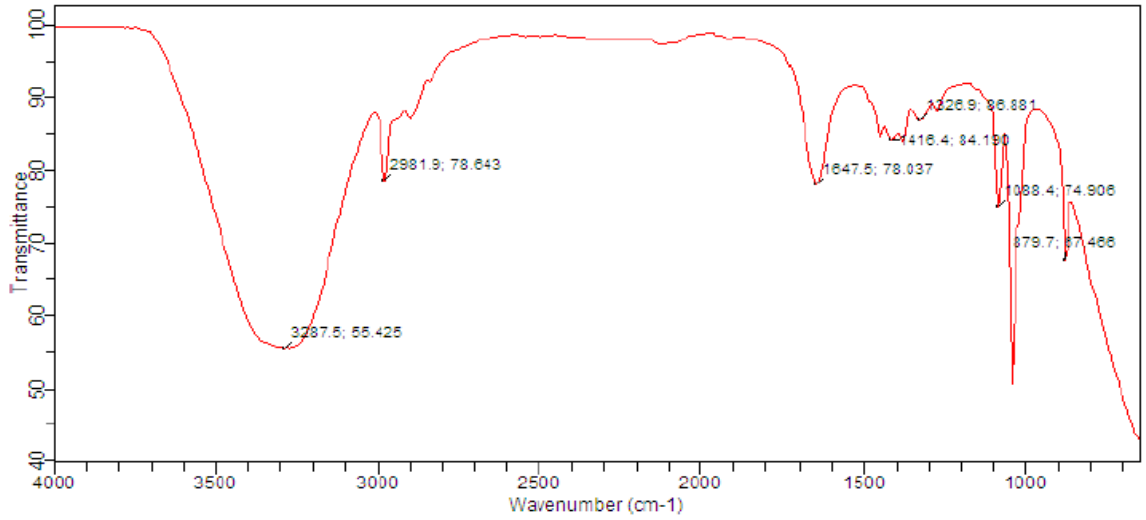
phenolic compounds from the grape seed extract to the yogurt.

Fig. 4 Sensory Evaluation Results of Fortified Greek Yogurt Using a 9-Point Hedonic Scale

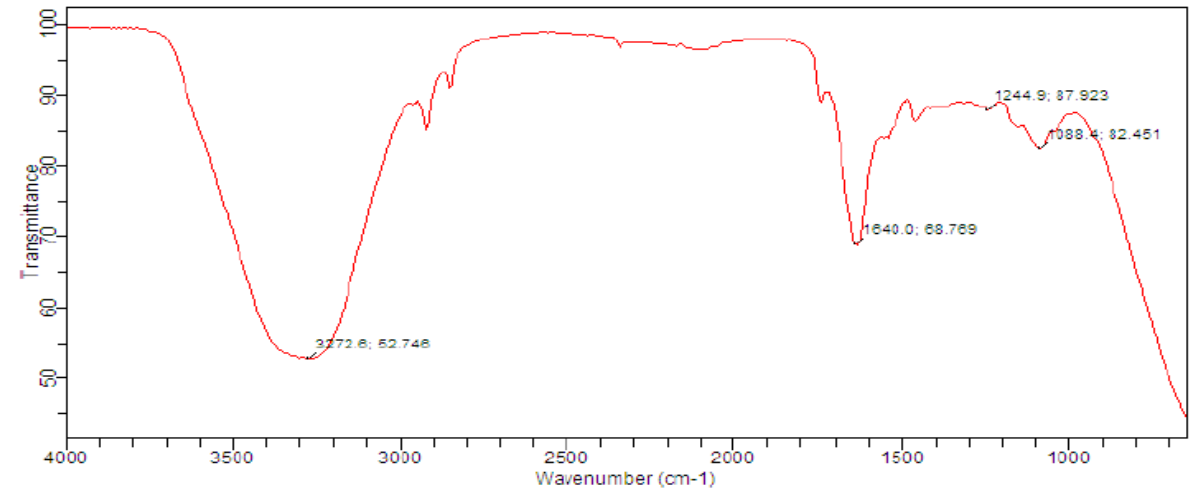


The sensory evaluation assessed the yogurt's texture, taste, and overall acceptability. Both the fortified yogurt and control samples had a smooth and creamy texture. Panelists described the fortified yogurt's taste as pleasant, with a balanced blend of sweetness from the strawberry pulp and tartness from the yogurt. The addition of grape seed extract did not negatively affect the sensory attributes. Approximately 70% of evaluators rated the yogurt "8" on the hedonic scale, indicating they "liked the product very much," while 10% rated it "9," showing extreme liking. A small percentage (10%) rated it "7," indicating moderate liking, and 10% rated it "3," showing they "disliked the product very much. Panelists who favored the product were typically health-conscious individuals or gym enthusiasts who appreciated the protein and antioxidant-enriched profile of the yogurt. This feedback suggests that fortified yogurt appeals particularly to consumers interested in functional, nutrient-dense foods.

The antioxidant activity of the fortified yogurt was determined using the DPPH assay, which revealed a 70% inhibition of DPPH radicals. The significant antioxidant activity can be attributed to the presence of grape seed extract, which is rich in polyphenols and other bioactive compounds. Absorbance measurements were taken at 517 nm, and the fortified yogurt demonstrated superior antioxidant potential with 82.505% inhibition, confirming its efficacy as a functional food with health-promoting benefits.



(a)



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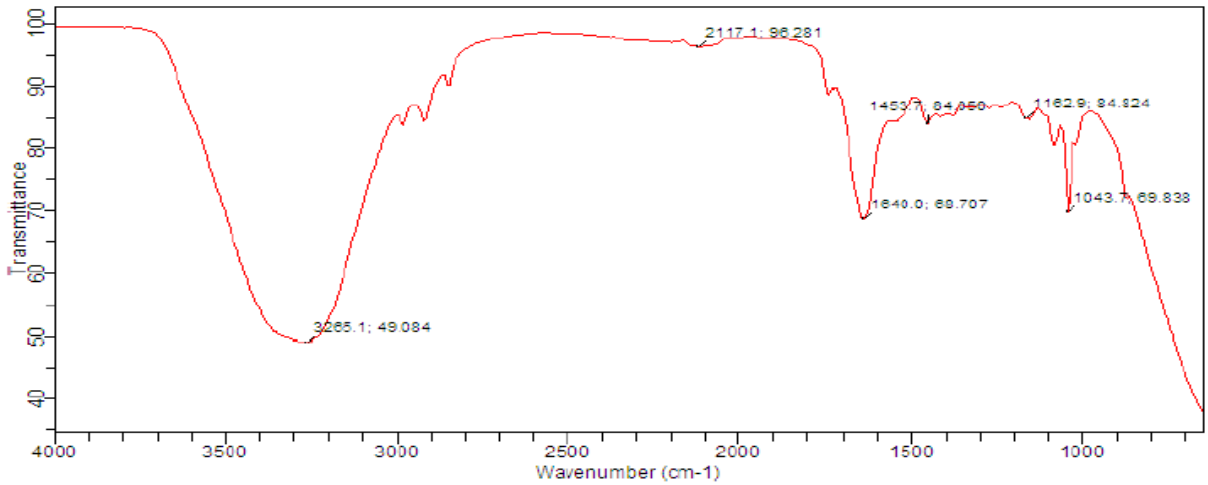

Fig 5. (a) FTIR analysis of grape seed extract (b) FTIR analysis of yogurt (c) FTIR analysis of yogurt fortified with grape seed extract

DISCUSSION

This research primarily aimed at improving the nutritional quality of the enriched Greek yogurt by incorporating GSE and strawberry pulp which are both high in polyphenols. The enriched yogurt provided a very encouraging Total Phenolic Content (TPC) of 191.66 mg GAE/100g which highlights the success of the reinforcement process. Polyphenols which are water-soluble phytochemicals with antioxidant properties are essential in curbing oxidative stress to reduce the likelihood of chronic diseases such as cardiovascular diseases, cancer, and neurodegenerative diseases. An inhibition percentage of 82.505% was observed in the DPPH assay and this also implies that the fortified yogurt had high antioxidant potential which was due to polyphenolic components in GSE including proanthocyanidins and catechins which scavenge free radicals and have protective effects (Peng *et al.*, 2010). Past research is consistent with these results where videos observed that GSE enhances the total phenolic content and antioxidant activity of yogurt products and this activity is persistent over time (Chouchouli *et al.*, 2013). Very high antioxidant levels have also been reported in yogurts enriched with other fruit extracts like hawthorn and wild strawberry which support the idea of incorporating plant extracts in enhancing dairy products (Ramchandani *et al.*, 2010). It was important to improve the nutritional content of the yogurt, but at the same time, the target market needed not to reject the product due to its sensory characteristics. The

sensory analysis showed that the yogurt's flavor, texture, or appearance was not adversely affected by the addition of GSE and strawberry pulp. The incorporation of strawberry pulp may have contributed to the overall flavor. GSE might have had some unpleasant bitterness but would have been balanced out by the strawberry pulp which has a mild sweetness. Other studies have reported that fruit extracts typically enhance the organoleptic properties of milk products to some extent, with the greatest improvements seen in taste and mouthfeel (Pacifico *et al.,* 2011). In this study, 80% of the panelists rated fortified yogurt as high on the hedonic scale and thus would be accepted by consumers. This also suggests that the product could be appealing to consumers. It also highlights that a balance was struck between functional advantages and sensory characteristics in this formulation which can be useful for muscle-conscious consumers.

The enthusiastic consumer acceptance of fortified Greek-style yogurt along with the increased interest in functional foods which offer more health benefits than merely nutrition is expected growth. There is also rising enthusiasm towards including functional foods with natural antioxidants such as GSE and strawberry pulp, which are believed to contribute to minimizing the risk of chronic diseases. In functional foods, GSE is largely included for its antioxidative properties which are cardiovascular protective and possess anti-cancer benefits (Jaster *et al.,* 2018). The inclusion of strawberry pulp is yet another health booster, which contains bioactive substances promoting heart health and diminishing inflammation (Pacifico *et al.,* 2011). The use of goat milk as a base further increases the acceptability of the yogurt in lactose intolerant consumers and also those with cow milk protein allergy as goat milk has better digestibility. This feature makes goat milk yogurt more appealing to the market's niche sectors that have restrictive diets. Goat milk yogurt with GSE and SRP is hypothesized to have improved antioxidant properties without deteriorating sensory qualities. There's a charge in TPC and antioxidant activity which reinforces the concept of fortification. Such findings correspond to other studies that have been able to prove that botanicals do increase the nutritional value of dairy products while the sensory qualities have not changed (Karaaslan *et al,* 2011). While this study demonstrates the value of fortifying yogurt with GSE and strawberry pulp, further studies could evaluate the effects of such yogurts over long periods of time. Broader sensory evaluations may also help to understand the preferences of more consumers. Finally, other possible fortifying agents, like fruit extracts or fibers, should be studied to improve the yogurt's functional characteristics and expand its market.

CONCLUSION

This research shows here that grape se seeds extract and strawberry pulp were successfully added on nongreasy functional protein, and its various production processes of development and its all features must be seen as a break-thought functional food concept. Due to the fortification process, the thickness of the yogurt was increased and its antioxidant properties are remarked to be better than the unforteuraed version. Improvements made in textures, flavors, and the activity of antioxidants indicate a better health benefit as well as better future prospects for sales as the tastes are better. Further work should be done with different stimulation methods and look for the best composition of ingredients and also the effects on consumers after a long time sales. The results of this study add substantial knowledge to the existing scholarly literature in food science and nutrition and have an important meaning for practitioners looking to create healthy functional food products from the market perspective.

Co-author's contribution: SN executed all the experiments. HA supervised grape seed extraction and analysis of Total Phenolic content and its antioxidant properties.

All authors have substantial contributions to the final manuscript and approved this submission.

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