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Development of Functional Yoghurt with Walnut Extract and Guava Pulp

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ABSTRACT: This study involves the preparation of functional yogurt using walnut and guava pulp, with an analysis of its physicochemical properties conducted from day 0 to day 21. A notable reduction in pH and a rise in titratable acidity were observed during the storage period. A slight reduction in fat and total solids content is observed from day 0 to day 21 for functional yogurt. Although the difference was not significant ($P \geq 0.05$) until the 7th day of storage, a significant difference ($P \leq 0.05$) was seen on the 14th and 21st days of storage. A consistent reduction in syneresis was observed from the control group to the other three treatments. A substantial variation ($P \leq 0.05$) in total phenolic content was detected from day zero to day 21 of storage. A significant ($P \leq 0.05$) reduction in DPPH radical scavenging activity was observed from day 0 (47.65±1.14) to day 21 (29.58±0.91) for functional yoghurt. The progressive decline in the antioxidant capacity of functional yogurt throughout the storage duration may be ascribed to physicochemical alterations that transpire during this period, resulting in the depletion of both probiotics and phytochemicals.

Keywords: walnut, guava pulp, physicochemical, functional yoghurt.

INTRODUCTION

Consumers' growing focus on health has spurred the development of functional foods, designed to deliver essential nutrients and promote well-being (Shiby and Mishra 2013). Functional foods may be defined as natural or processed foods that contain known or unknown biologically active compounds that provide clinically proven health benefits for the prevention, management, or treatment of chronic diseases in certain, effective, and non-toxic amounts Martirosyan and Singh (2015). Milk and dairy products provide a fundamental component of functional foods. They are designed to meet all dietary requirements over the entirety of human life. Consequently, milk is consistently included in human nutrition and contributes to the equilibrium of essential nutrients. Yogurt's nutritional value is enhanced by its high digestibility and the bioavailability of its protein, calcium, potassium, and B vitamins, making it a useful dietary choice. (Shamloo *et al*., 2012l; Shori and Baba 2013). In addition to improving lactose digestion and gastrointestinal disorders such lactose intolerance, constipation, diarrhea, colon cancer, inflammatory bowel disease, and allergies, it also reinforces the immune system. Yogurt contributes significantly to a healthy diet due to its concentrated nutrients, the body's ability to easily absorb those nutrients, and the presence of beneficial bacteria (Yanni *et al*., 2020). In addition, its unique characteristics can improve the body's ability to absorb and utilize certain nutrients, thereby enhancing their positive impact on health (Meybodi *et al*., 2020).

Fortifying yogurt is a promising strategy for enhancing nutrition in daily diets. Given its global popularity, yogurt can serve as an excellent vehicle for delivering key nutrients, compensating for any deficiencies in its natural composition (Preedy *et al*., 2013).

The International Society for the Study of Fatty Acids and Lipids (2004) states that dietary recommendations for omega-3 fatty acids (2.2g of ALA/day and 0.22g/day of EPA and DHA) can be met by eating foods high in these fatty acids (Gebauer *et al*., 2006). Since the human body cannot produce linoleic and linolenic acids, food should be supplemented with plant extracts containing these fatty acids. Regular consumption of walnuts may have positive effects on human health, cholesterol, anti-inflammation, and weight gain. The addition of fiber to food facilitates a number of physiological actions, such as gastrointestinal health maintenance, intestinal transit time reduction, colon cancer prevention, lowering blood serum levels of total and low-density lipoprotein cholesterol, lowering postprandial blood glucose levels, increasing calcium bioavailability, and strengthening the immune system. A daily consumption of 0.025 to 0.030 kg of fiber is advised for human health benefits as well (Labell, 1990). Vitamins, fiber, fatty acids, tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, and lectins are all abundant in guava. With 80 mg of vitamin C per 100 g of fruit, guava fruit has more vitamin C than citrus fruits. It also has significant levels of vitamin A. Pectin, a dietary fiber, is also abundant in guava fruits (Joseph and Priya 2011).

Walnut kernels boast the highest antioxidant activity among 25 common foods, a result of their abundant phenolic compounds (Gunduc and El 2003). These walnut phenolics are potent antioxidants, effectively neutralizing free radicals. Notably, a 50-gram serving of walnuts—roughly a handful—contains more total phenolics than a glass of apple juice (240 mL), 43 grams of milk chocolate, or a 150-mL glass of red wine (Colaric *et al*., 2005).

In light of this, the goal of this study is to develop functional yoghurt using the pulp of walnuts (*Juglans regia*) and guava (*Psidium gujava*) and to analyze the physicochemical properties.

MATERIALS AND METHODS

Experimental design. Yoghurt samples were prepared with different level of walnut extract as 0% -WY, 10% - WYI, 20% -WYII and 30%- WYIII with 6 percent sugar levels.

Preparation of walnut-extract. The kernels of premium walnuts were steeped in water heated to 60°C for two hours after shelling. Some husks separated spontaneously at this time, but the rest were removed with a high-pressure water jet to clean the deeper layers thoroughly. Crude walnut milk was made by blanching the kernels of walnuts in boiling water for 10 minutes and then homogenizing them with deionized water in a 1:4.8 ratio when the temperature was 60°C. The next step was to strain the raw walnut milk through a 200-mesh sieve.

On the basis of sensory evaluation, 20% walnut extract in yoghurt was standardized at acceptable level and subjected to further studies. Similarly, WYII yoghurt was incorporated with different level of guava pulp.

(1) Control yoghurt (CY) = Plain yoghurt with 6% sugar without walnut and guava pulp

(1) T1 = 20% walnut extract and 5% guava pulp with 6% sugar

(2) T2 = 20% walnut extract and 10% guava pulp with 6% sugar

(3) T3 = 2% walnut extract and 15% guava pulp with 6% sugar

Skim milk powder at the rate of 5.760 per cent (w/v), sugar at the rate of 6 per cent (w/v) and walnut extract at the rate of 20 percent were added to milk and homogenized at 2500 psi. The contents were mixed well and pasteurized at 90°C for 30 minutes, cooled to 42°C. Guava pulp was added at three different levels *viz.*, 5, 10 and 15 per cent and mixed well and inoculated with 2% of yoghurt cultures containing *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus* and incubated at 42°C for 4 to 5 hours and finally stored at 4 to 5°C .

Flow diagram of walnut extract and guava pulp enriched yoghurt

Skim milk
↓
Milk preheating (45 ±2 °C)
↓
Addition of cream (2% fat), skim milk powder (5.760 per cent) and Sugar (6%)
↓
Walnut extract (20%)
(Standardized to 4% fat)
↓
Homogenization at 65°C (2500 psi)
↓
Pasteurization (85°C for 30 min)
↓
Cooling (42°C)
↓
Addition of guava pulp (at the rate of 5, 10 and 15%)
↓
Inoculation (2 per cent yoghurt culture)
↓
Incubating at 42°C /4-5 hrs till it reaches the pH 4.5
↓
Stored at 5°C

Physicochemical properties. The functional yoghurt underwent physico-chemical analysis using standard procedures at storage intervals of 0, 7, 14, and 21 days. The pH was measured using a digital pH meter (Hanna H-2211). Acidity was assessed according to the methodology outlined in IS:SP:18 (part XI)-1981. The syneresis of yoghurt, defined as the release of whey, was quantified using the method outlined by Achanta *et al*. (2007). Fat was estimated according to the procedure outlined in IS:SP:18 (Part XI) – 1981. The total solids content was assessed following the AOAC (1990), 15th edition methodology. The total phenolic content was assessed using the modified method outlined by Shetty *et al*. (1995). The DPPH radical scavenging activities of yogurt samples were determined using the method of Cheung *et al.* (2003) with slight modifications

RESULTS AND DISCUSSIONS

Table 1 and 2 revealed that the values of pH, titratable acidity, syneresis, fat, total solids, phenolic content, DPPH radical scavenging activity for control and functional yoghurt.

pH and titratable acidity. Statistical analysis indicated that the incorporation of guava pulp exceeding 10 percent, in conjunction with 20 percent walnut extract, significantly ($P<0.05$) decreased the pH value of the functional yogurt. The decrease in pH of functional yoghurt can be ascribed to the elevated activity of starter cultures and the inclusion of guava pulp. The findings of Ayar and Gurlin (2014); Jayalalitha *et al.* (2015) support the notion that the addition of fruits influences the pH of yogurt, with the lowest average pH value observed in yogurt supplemented with fruits such as blueberry and mango pulp.

A significant difference ($P\leq0.05$) was observed between T3 and the other two treatments, likely attributable to continuing fermentation by the starter culture, particularly *Lactobacillus delbreuckii* ssp bulgaricus, which typically leads to a substantial reduction in the pH of functional yoghurt. The increase in activity can be ascribed to the elevated phenolic content from the incorporation of guava pulp in T3 (15 percent guava pulp), which may enhance the metabolic activity of beneficial microorganisms.

Syneresis (ml) of yoghurt prepared with walnut extract and guava pulp. A static decrease in syneresis was observed from the control (CY) to the other three treatments, likely due to the high pectin content in guava pulp, which possesses gelling properties that reduce syneresis. The increase in guava pulp content in yogurt resulted in greater water absorption and a reduction in syneresis. This aligns closely with the findings of Jayalalitha *et al*. (2015); Kumar *et al*. (2015), who indicated that the enhancement of total solids in yogurt through the addition of soy or flaxseed resulted in a reduction of whey syneresis. Sarmini *et al*. (2014) reported that the addition of soy and jackfruit increased the fiber content in soy jack yogurt and decreased syneresis. Bulut *et al.* (2021) examined the impact of fortifying yoghurt with extracts of rhubarb (RE), grape seed (GSE), thyme (TE), green tea (GTE), and mint (ME) on its physicochemical, rheological, textural, and sensory properties during refrigerated storage. Their findings indicated that mint-extract-fortified yoghurt was the most preferred, receiving the highest overall sensory score

Fat and Total solids. All three treatments of functional yoghurt exhibited a reduction in fat content. No significant difference ($P\geq0.05$) was observed between the control (CY) and the three treatments of functional yoghurt, namely T_1, T_2, and T_3. Fruit typically has a low fat content. Therefore, the incorporation of fruit pulp as a substitute for fat would have reduced the fat percentage in guava yogurt. Sengupta *et al.* (2014) reported that watermelon juice yogurt had a significantly lower fat content (3.40 ± 0.02 percent) compared to fresh cow milk plain yogurt (3.67 ± 0.02 percent), suggesting that the addition of fruit juice reduced the fat percentage in the yogurt. Sawant *et al*. (2015) reported that the inclusion of fruit pulp such as pineapple and jackfruit pulp did not produce a significant difference in fat percentage between the control and treatment samples.

There was a decrease in total solids content in all the three treatments of functional yoghurt and no significant ($P\geq0.05$) difference was observed between control (CY) and treatments. As the fat content of fruit is generally low, addition of fruit pulp might have decreased the fat per cent of guava yoghurt and consequently decrease the total solids.

Total phenolic content. Latest research findings indicate that phenolic phytochemicals exhibit significant antioxidant activity and has therapeutic capabilities, including anti-diabetic and anti-hypertensive effects. The current analysis indicates a rising trend in phenolic content throughout all three treatments of functional yogurt compared to plain yoghurt. This may be attributed to the incorporation of walnut extract and guava pulp, which are rich in phenolic components, including phenolic acids and polyphenols, typically present in plants.

Zainoldin and Baba (2009) indicated that fruit-enriched yogurt had a higher total phenolic content than plain yogurt. Yogurt is frequently enhanced with fruits, vegetables, herbal extracts, seeds, and nuts, all of which are valuable sources of health-promoting phenolics (Giugliano, 2000).

Swarnalatha *et al*. (2021) reported a study on the preparation Set yoghurt by addition of different levels of 10:90, 20:80, 30:70 (v/v) of tender coconut water (TCW) and milk respectively and conclude that, fortification of yoghurt with 10:90 TCW– milk blends with 0.15% of modified starch using 1% culture improved the textural properties when compared with the control yoghurt. Okur (2022) reported that, of all the samples, the yogurt with 5% walnuts (sample E) contained the most total phenolic compounds, measuring 1027.50 mg GAE/kg

DPPH radical scavenging activity (%). Statistical study demonstrated that DPPH radical scavenging activity increased in all three functional yogurt treatments compared to plain yoghurt. The antioxidant activity of control yoghurt could be related to the presence and activity of probiotics in yoghurt. According to Li-chen Wu *et al*. (2006), the greater antioxidant activity of both white and red dragon fruit yogurt is a favorable property that may improve the therapeutic value of yoghurt. According to Ye *et al.* (2013), the rise in antioxidant activity in yogurt with walnuts is primarily due to the increase in bioactive ingredients.

The elevated antioxidant levels observed in walnut-fortified yogurt samples can be explained by the high polyphenolic content of walnuts. It has been previously established that yogurt's antioxidant activity can be enhanced by adding different components (Baba *et al.*, 2018; Ujiroghene *et al.*, 2019; Anuyahong *et al.*, 2020). According to Okur (2022), the yogurt with 5% walnuts, exhibited the highest antioxidant activity of 19.95 mM TE) among the samples. Sengul (2014) found that the overall antioxidant activity increased with increasing strawberry concentration. By raising the strawberry concentration from 8% to 16%, the total antioxidant activity increased from 78.42 to 82.42 percent.

Table 1: Physico-chemical Properties of fresh Yoghurt prepared with 20% walnut extract and Guava pulp.

Parameters	CY	T_1	T_2	T_3
pH	$4.65^{a} \pm 0.14$	$4.54^{a} \pm 0.15$	$4.42^{a} \pm 0.18$	$4.05^{b} \pm 0.04$
Titratable acidity	$0.85^{a} \pm 0.01$	$0.95^{a} \pm 0.01$	$1.15^{a} \pm 0.07$	$1.27^{b} \pm 0.06$
Syneresis(ml/100g)	$12.25^{d} \pm 0.17$	$8.27^{c} \pm 0.05$	$8.32^{b} \pm 0.05$	$8.12^{a} \pm 0.04$
Fat(g/100g)	4.12±0.04	4.15±0.03	4.09±0.02	3.92±0.05
Total solids(g/100g)	22.15±0.10	25.12±0.12	25.43±0.09	25.42±0.12
Phenolic content mg GAE/g	$26.12^{a} \pm 0.11$	$37.14^{b} \pm 0.16$	$39.17^{c} \pm 0.17$	$43.48^{d} \pm 0.17$
DPPH radical scavenging activity(%)	$24.66^{a} \pm 1.18$	$45.18^{b} \pm 1.17$	$47.65^{c} \pm 1.14$	$55.94^{d} \pm 2.12$

Different superscripts in a row differ significantly ($P \leq 0.05$)

Table 2: Changes in Physicochemical Properties of functional Yoghurt at Refrigerated Storage at 5°C (Mean ± SE).

Parameters	Particulars	0 day	7th day	14 th day	21st day
pH	control yoghurt	4.65 ± 0.14^{Aa}	4.42 ± 0.12^{Ba}	3.95 ± 0.06^{Ca}	3.75 ± 0.05^{Da}
	T_2	4.42 ± 0.18^{Ab}	4.25 ± 0.12^{Bb}	3.85 ± 0.02^{Cb}	3.52 ± 0.01^{Db}
Titrable acidity	control yoghurt	0.85 ± 0.01^{Aa}	0.98 ± 0.01^{Ba}	1.15 ± 0.02^{Ca}	1.18 ± 0.01^{Da}
	T_2	1.15 ± 0.07^{Ab}	1.27 ± 0.04^{Bb}	1.35 ± 0.01^{Cb}	$1.42 \pm 0.0\ 2^{Db}$
Syneresis value	control yoghurt	$12.25^{Ab} \pm 0.17$	$13.32^{Bb} \pm 0.22$	$14.28^{Cb} \pm 0.15$	$15.15^{Db} \pm 0.18$
	T_2	$8.32^{Aa} \pm 0.15$	$8.58^{Aa} \pm 0.21$	$8.75^{Aa} \pm 0.22$	$10.12^{Ba} \pm 0.07$
Fat	control yoghurt	$4.12^{Ca} \pm 0.04$	$4.05^{Ca} \pm 0.02$	$3.85^{Ba} \pm 0.01$	$3.58^{Aa} \pm 0.02$
	T_2	$4.09^{Ca} \pm 0.02$	$4.01^{Ca} \pm 0.02$	$3.81^{Ba} \pm 0.02$	$3.55^{Aa} \pm 0.01$
Total solids	control yoghurt	$22.10^{C} \pm 0.12$	$22.15^{C} \pm 0.05$	$21.08^{Ba} \pm 0.08$	$20.82^{Aa} \pm 0.20$
	T_2	$25.09^{C} \pm 0.05$	$25.03^{C} \pm 0.12$	$24.75^{Bb} \pm 0.11$	$24.60^{Ab} \pm 0.02$
Phenolic content (mgGAE/g)	control yoghurt	$26.12^{Da} \pm 0.11$	$24.14^{Ca} \pm 0.05$	$22.17^{Ba} \pm 0.07$	$20.12^{Aa} \pm 0.05$
	T_2	$39.17^{Db} \pm 0.12$	$37.14^{Cb} \pm 0.12$	$34.15^{Bb} \pm 0.14$	$32.10^{Ab} \pm 0.11$
DPPH radical scavenging activity(%)	control yoghurt	$24.66^{Da} \pm 1.18$	$22.31^{Ca} \pm 1.13$	$20.18^{Ba} \pm 1.02$	$19.15^{Aa} \pm 0.09$
	T_2	$47.65^{Db} \pm 1.14$	$45.12^{Cb} \pm 0.83$	$38.67^{Bb} \pm 0.89$	$29.58^{Ab} \pm 0.91$

T_2: 20% Walnut extract with 10% guava pulp yoghurt; Different uppercase superscripts in row differ significantly ($p \leq 0.05$); Different lowercase superscripts in column differ significantly ($p \leq 0.05$)

CONCLUSIONS

Walnut and guava-enriched yogurt offers a promising product with significant health benefits. The combination of walnuts, rich in healthy fats, proteins, and antioxidants, with guava, a nutrient-dense fruit packed with vitamins, minerals, and dietary fiber, enhances the nutritional profile of yogurt. It is to be noted that, inclusion of dietary fibre, antioxidant and vitamin c rich fruits and omega 3 rich oil is expected to enhance the nutritional and therapeutic values of yoghurt and as such encourage consumption of yoghurt. The inclusion of walnuts and guava also appeals to consumers seeking functional foods that support overall well-being. Based on the physicochemical and functional attributes, it is concluded that functional yoghurt developed had a keeping quality of 14 days, while the control had a shelf life of only 7 days.

FUTURE SCOPE

Continued research in these areas will allow walnut and guava-enriched yogurt to completely fulfill its promise as a nutritious and commercially successful product. To support future health claims, further study into the health advantages of walnut and guava-enriched yogurt is essential. Studies on how this combination affects gut, heart, and immunity may be useful. It has great potential in health food markets, especially in places where these ingredients are popular.

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