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Original article

Assessment of angiotensin converting enzyme inhibitory activity and quality attributes of yoghurt enriched with *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris* and *Brassica oleracea*

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ABSTRACT

The new concept of functional foods has led to the varieties in the production of foods that provide not only basic nutrition, but can also warrant good health and longevity. This study deals with the production and evaluation of fortified yogurts' with *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris* and *Brassica oleracea*. The qualitative and quantitative phytochemical analysis of above mentioned plant extracts before using them into the preparation of functional yoghurt was carried out. The sensory evaluation of enriched yogurts with plant extracts carried out using 9 point hedonic scale. Comparative analysis between enriched yogurts and plain yogurt was carried. The results indicated increase in ash contents, water holding capacity, titratable acidity, total soluble solids, total phenolic content, tannin content, and total flavonoid content in fortified yogurt as compared to plain yogurt. In addition to this fortified yogurts showed greater antioxidant and antibacterial activity in contrast to plain yogurt. However, moisture contents, pH and susceptibility to syneresis of yogurt decreases with the addition of plant extracts. Shelf life of plain and fortified yogurt was determined both at room and refrigerated temperature. The results revealed that shelf life of fortified yogurt was greater as compared to plain yogurt. *In silico* analysis was carried out by using the galaxy web software. The results indicated that bioactive compounds including ascorbic acid, sinapinic acid, cinnamaldehyde and linalool acetate present in the flavored yogurts binds with angiotensin converting enzyme. All enriched yogurts showed higher anti-Angiotensin converting enzyme activity as compared to plain-yogurt.

1. Introduction

Yogurt has been a very renowned fermented dairy product that comes with a variety of names and forms. Yogurt's popularity might be attributed to its numerous health advantages and medicinal properties. In addition to this yogurt, also, helps to balance the bacteria in our stomach, allowing us to digest meals more effectively (Tamime and Robinson, 2007).

Yogurt has long been regarded as a product that promotes health and may help the digestive system by health-conscious consumers. Yogurt is currently prepared utilizing a variety of formulations to increase the product's health benefits. Dairy foods have been the driving force behind the production of new commercial items in the market because of the growing demand for functional foods from multicultural consumers (Shori, 2022).

Addition of additives to yoghurt is the easiest yet most effective way to change its characteristics. Both natural and artificial additives are available in the market. The majority of natural additives are plant components (like leaf, seed, fruit, root etc.) or extracts. Plant extracts should be added during processing to improve the functionality of the food rather than being added to the final product. In this way, sensory and chemical aspects of such food are more enhanced (Lai and Roy, 2004).

Spices are employed from earlier times for enhancing the flavor of substances, for increasing the storage life of food and for their therapeutic activities. Variety of spices is capable of showing anti-diabetic, anti-bacterial, analgesic, anti-oxidant and anti-cancer

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effects. Spices are also capable of impeding the bacterial spoilage of food stuff (De et al., 1999). *Cinnamomum verum* (Cinnamon) is commonly utilized as a spice all over the world. It is also utilized in traditional medicine (Lee and Balick, 2005). *Elettaria cardamomum* (Cardamom) seeds are utilized as the condiment or spice in the meals. Cardamoms also possess flavonoid such as quercetin, pelargonidin and luteolin that are responsible for its antioxidant activity. Cardamom efficiently lowers blood pressure and enhances fibrinolysis in patients with stage 1 hypertension while having no effect on blood lipids or fibrinogen levels (Verma et al., 2009).

Vegetables are high in potassium, folate, dietary fibres, antioxidants and bioactive components, provitamin A, and water-soluble vitamins (vitamin C and group B vitamins), all of which can aid in preventing cancer, hypertension, and coronary heart disease, as well as lower the risk of stroke (Yildiz and Ozcan, 2019). *Beta vulgaris* (Beet root) is rich in potent antioxidants and minerals. It can be used to lower blood pressure, prevent cardiovascular disease, maintain proper liver function, and prevent cancer. The incorporation of beetroot juice into yogurt can enhance nutritional values and properties of the yogurt as well as provide many health benefits (Delchier et al., 2016; Nyamete and Mongi, 2017). *Brassica oleracea* (Red cabbage) is a well-known healthful and nutritious vegetable that is consumed over the world. The high phytochemical content, minerals including iron, magnesium etc., vitamins, antioxidants, low protein, cholesterol, and saturated fats content contribute to red cabbage's popularity. Red cabbage is also recognized for having high anthocyanin content (Shalaby and Amin, 2018).

Current industrial technologies have helped in the development of a variety of flavored yoghurt products that utilizes a variety of natural flavorings and coloring agents, such as garden fresh fruits, vegetables, real nuts, and candies like chocolate and coffee chunks, which are attractive and appealing due to the micronutrients and sensorial content (Cruz-Jentoft et al., 2010). One of the most important worldwide industrial advancements right now is the hunt for unique food constituents and flavors with greater health advantages (Netzel et al., 2007).

In silico techniques were applied to characterize the properties and structure of the proteins The ACE proteins have an important physiological role in blood pressure regulation, where it controls angiotensin I conversion to angiotensin II. Conversely ACE inhibition can have an antihypertensive effect as well leading to decrease in blood pressure. ACE inhibitors inhibit the function of angiotensin-converting enzymes, decreasing angiotensin 2 synthesis. The use of ACE inhibitors to treat hypertension is a widely used application (Solanki et al., 2017). Hypertension can be prevented and treated with foods that contain inhibitory peptides of the ACE-1 (Vermeirssen et al., 2004). In animal and human trials, plant based extracts can reduce ACE activity, block angiotensin 2 receptor binding, and show hypotensive action (Patten et al., 2016).

Yogurt's popularity has recently risen as a result of its fortification with vegetables and spices. Vegetables and spices incorporated yoghurts are in high demand these days.

2. Materials and methods

2.1. Collection of samples

Cinnamomum verum, Elettaria cardamomum, Beta vulgaris and Brassica oleracea was purchased from local market of Lahore.

2.2. Spices extract preparation

Cinnamomum verum and *Elettaria cardamomum* was ground into fine powder and properly mixed with distilled H_2O in the different ratio with concentrations (0.1–0.3 g mL^{-1}). The mixture was left for overnight; afterwards that mixture was centrifuged (6000g, 15 min). The supernatant obtained was utilized as aqueous extract in the preparation of bio yogurt (Behrad et al., 2009).

2.3. Vegetable extract preparation

Beta vulgaris and *Brassica oleracea* were cleaned by washing with water in order to remove adherent debris, sand and extraneous material. *Beta vulgaris* and *Brassica oleracea* were sliced into about 2–3 mm thick pieces, and subjected to extraction using juice extractor. The pulp was sieved using a muslin cloth to obtain a transparent filtered juice, and then the filtered juice was pasteurized at 85 °C to destroy enzymes and minimize the microbial load. Different concentrations (0.1–0.3 g ml^{-1}) were used for bio yogurt preparation. The juice was stored in an air-tight container for further use (Malik et al., 2019).

2.4. Preparation of bio yogurt

Buffalo milk was purchased from local market of Lahore and then filtered. Pasteurization was carried for 15 min at the temperature of 85 °C. The milk was then allowed to cool for 20–30 min. 10 ml of all the plant extracts in different concentrations was added into 85 ml of pasteurized milk with 5 g of starter culture. 1 g free fat skim milk powder was added in order to increase the whey content. The mixture was properly mixed before being incubated at 41 °C. Afterward, the pH of the mixture was determined. The same processes were followed to make plain bio yogurt (control), except plant extract was replaced with the same amount of distilled water (Shori and Baba, 2011).

2.5. Sensory evaluation

Sensory evaluation was performed utilizing 9 point hedonic scale described by Ranganna (1986). Sensory evaluation was carried out on the quality attributes like color, taste, flavor and overall acceptability.

2.6. Proximate analysis

Determination of ash content, moisture content, titratable acidity, pH and total soluble solids was carried out (AOAC, 2005).

2.7. Qualitative phytochemical analysis

Qualitative phytochemical analysis including test for protein, carbohydrates, tannins, flavonoids, phylobatannins, steroids, terpenoids, coumarins, quinone, carotenoids, anthraquinones, phytosterol, phenolic compound, glycoside was performed for both plant extracts and yogurts samples (Ajayi et al., 2011; Solihah et al., 2012; Soni and Sosa, 2013; Jyothiprabha and Venkatachalam, 2016; Shaikh and Patil, 2020).

2.8. Quantitative phytochemical analysis

2.8.1. Determination of total phenolic content

Total phenolic content was assessed according to the method of Shetty et al. (1995). In 0.5 ml of each plant extract, 0.6 ml of Folin-Ciocalteu reagent and 1.5 ml of 20 % sodium carbonate were added, and the total volume was raised up to 10 ml. The reaction mixture was filtered after 90 min of standing in the dark for colour development. The blank was run parallel. The absorbance of the solution at 765 nm was measured using an ultraviolet visible spectrophotometer. The total phenolic content of the samples was determined using a standard curve of Gallic acid.

2.8.2. Determination of total flavonoid content (TFC)

TFC was evaluated according to Kalita et al. (2013). 5 g of the each plant extract was dissolved in a small quantity of distilled water, and the make total volume up to 25 ml. The solution was filtered, and 3 ml of the filtrate was added to a flask along with 1.3 ml methanol, 0.1 ml of 10 % aluminum chloride, and 0.1 ml potassium acetate, followed by distilled water to make the final volume 10 ml. The solution was than filtered and allowed to stand in the dark for 15 min for color development. A UV–Vis spectrophotometer was used to measure the mixture's absorbance at 417 nm. The total flavonoid content of the samples was determined using a standard curve of quercetin.

2.8.3. Determination of total tannins content

0.1 ml of the each sample extract was added in 8 ml of distilled water. After that, 1 ml of 35 % Na_2CO_3 solution and 0.5 ml Folin-Ciocalteu reagents were added and mixed properly and allowed to stand at room temperature for 30 min. The blank was run parallel. The absorbance of all the samples was recorded at 725 nm using spectrophotometer (Rani and Mythili, 2014). The total tannin content of the samples was determined using a tannic acid standard curve.

2.9. Determination of antibacterial activity

The antibacterial activity of aqueous extracts of *Cinnamomum verum*, *Elettaria cardamomum*, *Beta vulgaris*, and *Brassica oleracea* and enriched yogurts samples against Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*) bacteria was determined by using disc diffusion method (El Atki et al., 2019).

2.10. Determination of antioxidant activity of extracts

Antioxidant activity was determined according to the method of Villano et al. (2007). Using the DPPH assay, the antioxidant activity of all extracts was evaluated.

2.10.1. Susceptibility to syneresis [S] and water holding capacity [WHC]

Syneresis of various yoghurt samples were evaluated according to the method of Abd El-Salam et al. (1991). Following formula was used to estimate syneresis susceptibility

$$S = V_1/V_2 \times 100$$

where V_1 is the volume of whey collected after drainage and V_2 is the volume of yogurt sample.

Water holding capacity of various yoghurt samples were evaluated according to the method of Abd El-Salam et al. (1991). The following equation was used to determine WHC:

$$WHC(\%) = 1 - W_1/W_2 \times 100$$

where W_1 is the weight of whey after centrifugation and W_2 is the weight of yogurt (Abd El-Salam et al., 1991).

2.10.2. Determination of shelf life

Each modified and control yoghurt sample was placed at room temperature and refrigerated temperature in order to determine the shelf life of yoghurt. The samples were evaluated regularly until the yoghurts expired in terms of its flavor, color, and appearance (Deb et al., 2013).

2.10.3. FTIR analysis of yoghurt samples

FTIR analysis of plant extract and yogurt samples was performed using IRTracer-100 according to the method of Jaafer et al. (2020).

2.10.4. In silico analysis

*In silico a*nalysis of bioactive compounds of *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris* and *Brassica oleracea* and their docking with the angiotensin converting enzyme was carried out by using the galaxy web software (Solanki et al., 2017). The different ligands were retrieved the PubChem database (https://pubchem.ncbi.nlm.nih.gov) and the ACE protein was retrieved from the PDB database (https://www.rcsb.org) with the accession no. 5AMB. The Pymol software was used to refine the protein structure. Finally the different ligands were docked with the ACE protein using the GalaxyWeb server (https://galaxy.seoklab.org).

2.10.5. Statistical analysis

All the data was arranged and statistical analysis (Post Hoc multiple comparison test under one way ANOVA and standard deviation) was performed by using SPSS (version 32.0) software.

3. Results

3.1. Sensory evaluation

In present study control yoghurt and *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, Brassica oleracea* enriched yoghurts were prepared**.** Sensory evaluation of control and *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, Brassica oleracea* enriched yoghurts for parameters like taste, color, texture, odor and overall acceptance was performed. Sensory evaluation was carried out by trained panelists using a 9 point hedonic scale ranging from 1 (extremely dislike) to 9 (extremely like). Results indicated that there were differences in sensory scores of all types of yoghurt. Out of all the concentrations prepared, Cinnamon (0.1 %), cardamom (0.3 %), red cabbage (0.2 %) and beetroot (0.2 %) were most accepted by the consumers in terms of its sensory evaluation (Fig. 1).

In terms of sensory rating, among the four distinct varieties of flavored yoghurts, cardamom enriched yoghurt is the best fortified product because of its sweet and fruity flavor and aroma while red cabbage and beetroot incorporated yoghurts were most accepted in terms of its color. Cinnamon yoghurt was not much preferred because of sightly bitter flavor, although its color and odor were considered good by consumers.

3.2. Qualitative analysis

In present study qualitative phytochemical analysis of all the extracts (Table S1) and enriched yoghurt samples were carried out (Table S2). All the four extracts show positive results for carbohydrate and glycoside tests. The obtained results showed the presence of flavonoids, coumarin, tannins, terpenoids, saponin, glycoside, and phenolic compounds in cinnamon extracts. While the ninhydrin test for protein is positive for all except cinnamon but cinnamon enriched yoghurt showed positive result for ninhydrin test. Cardamom showed positive results for proteins, carbohydrates, tannins, flavonoids, phylobatanins, saponins, glycosides, phenolic compounds, quinones and coumarins.

The aqueous extract of Red cabbage juice showed the presences of all the phytochemicals except anthraquinone and carotenoids while beetroot show positive results for all the tests except for carotenoids test. Qualitative test results of all the enriched yoghurt samples were same as their extracts except cinnamon enriched yoghurt that showed positive result for ninhydrin test.

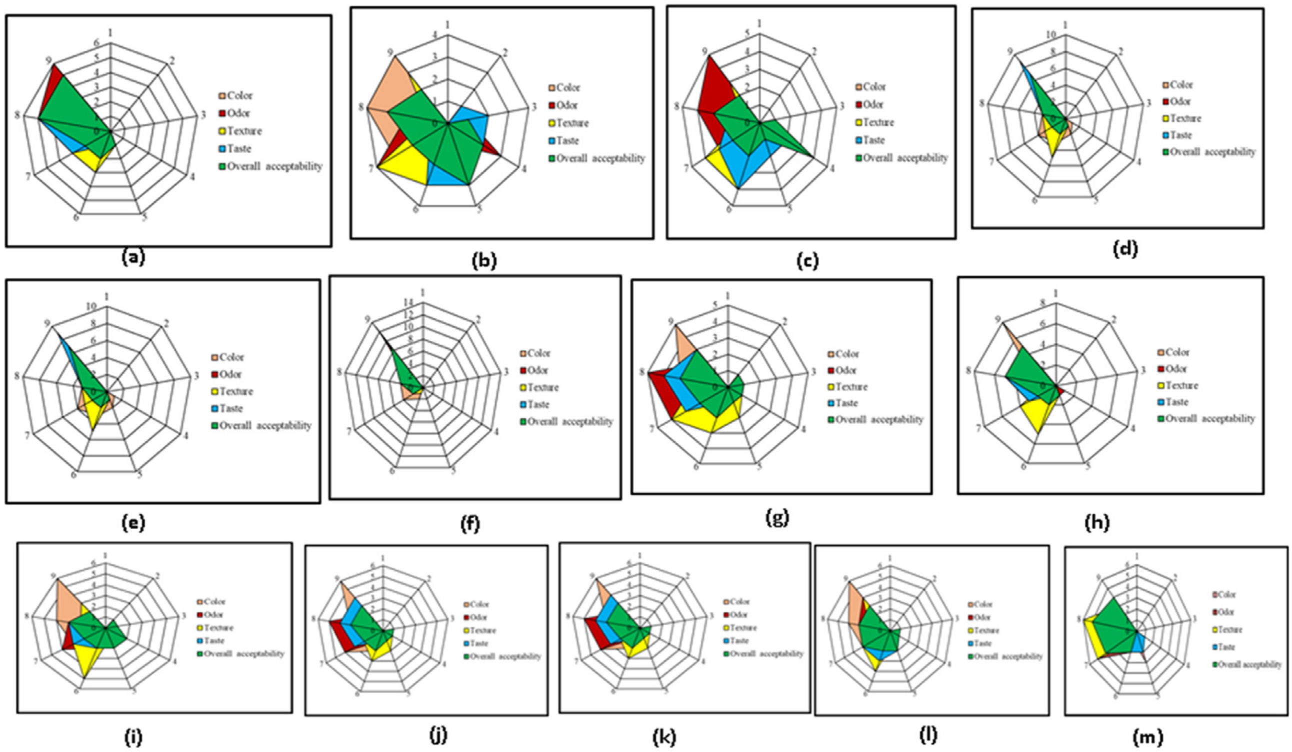


Fig. 1. Sensory evaluation of different yoghurt samples (a) Cinnamon yoghurt (0.1%) (b) Cinnamon yoghurt (0.2%) (c) Cinnamon yoghurt (0.3%) (d) Cardamom yoghurt (0.1%) (e) Cardamom yoghurt (0.2%) (f) Cardamom yoghurt (0.3%) (g) Red cabbage yoghurt (0.1%) (h) Red cabbage yoghurt (0.2%) (i) Red cabbage yoghurt (0.3%) (j) Beetroot yoghurt (0.1%) (k) Beetroot yoghurt (0.2%) (l) Beetroot yoghurt (0.3%) (m) Control yoghurt.

3.3. Quantitative analysis

3.3.1. Determination of total phenolic content (TPC) of different extracts and yoghurt samples

Total phenolic contents (TPC) of extracts of *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, Brassica oleracea* were evaluated (Fig. 2a) and out of all the four extracts beetroot showed highest total phenolic content. In addition to this, Total phenolic contents (TPC) of control yoghurt and *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, Brassica oleracea* enriched yoghurt were also evaluated (Fig. 2b). Beetroot yoghurt showed highest total phenolic content among all four yoghurt samples. However, the mixture of yoghurts showed the highest TPC. The incorporation of yoghurt with *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris*, and *Brassica oleracea* increased the TPC of yoghurt.

3.3.2. Determination of total tannin content of different extracts and yoghurt samples

Total tannin content of extracts of *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris*, and *Brassica oleracea* incorporated yoghurt were evaluated (Fig. 2c). Beetroot showed highest tannin content followed by red cabbage, cinnamon and cardamom respectively. Total tannin content of plain yoghurt and *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, Brassica oleracea* enriched yoghurt were also evaluated (Fig. 2d). Among the spices enriched yoghurts, cinnamon enriched yoghurt showed highest tannin content as compared to others. Similarly among the vegetable enriched yogurts beetroot enriched yoghurt showed highest tannin content in contrast to red cabbage enriched yoghurt. The incorporation of yoghurt with *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, Brassica oleracea* increase the total tannin content of yoghurt.

3.3.3. Determination of total flavonoid content (TFC) of different extracts and yoghurt samples

Total flavonoid content (TFC) of *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, Brassica oleracea* extracts were evaluated (Fig. 3a). Beetroot extracts showed highest flavonoid content as compared to all other extracts samples. Total flavonoid content of plain yoghurt and *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, Brassica oleracea* enriched yoghurt were also evaluated (Fig. 3b). Beetroot enriched yoghurt showed highest flavonoid content and cardamom enriched yoghurt showed the least flavonoid content. The incorporation of *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, Brassica oleracea* increase the TFC of yoghurt.

3.3.4. Determination of antioxidant activity of different plant extracts and yoghurt samples

Antioxidant activity of extracts of *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, Brassica oleracea* (Fig. 3c) and plain yoghurt and *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, Brassica oleracea* incorporated yoghurt were evaluated (Fig. 3d). The results indicated that as the volume of extracts and yoghurt samples increases, the antioxidant activity also increases. The cardamom extract showed the highest antioxidant content (95 %) out of the all extract samples. Yogurt enriched with *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, Brassica oleracea* samples showed higher antioxidant activity in contrast to plain yogurt. The DPPH radical scavenging activity of plain yogurt was 56.28 %. The results indicated that by increasing the concentration of extracts, the antioxidant activity also increases.

3.4. Ash content of yogurt samples

Ash contents of plain and enriched yoghurt samples were determined (Fig. 4a). The incorporation of extracts slightly increases the

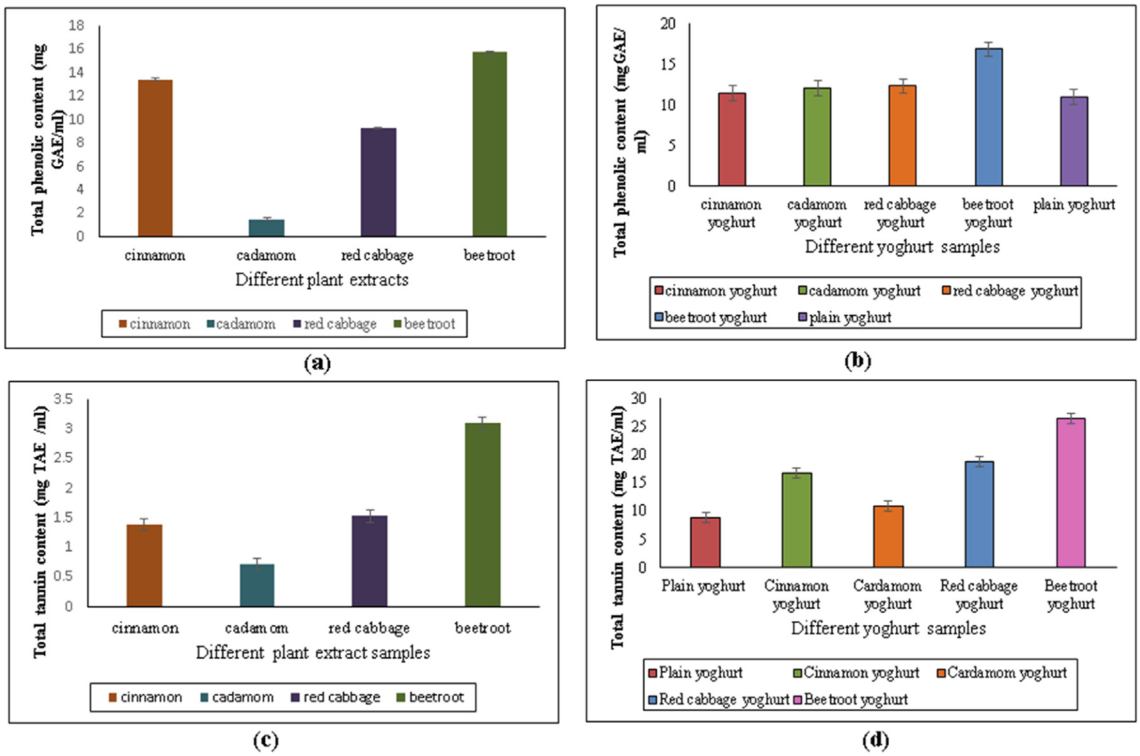


Fig. 2. (a) Total phenolic content of different plant extracts (b) Total phenolic content of plain and enriched yoghurt samples (c) Total tannin content of different extracts (d) Total tannin content of plain and enriched yoghurt samples.

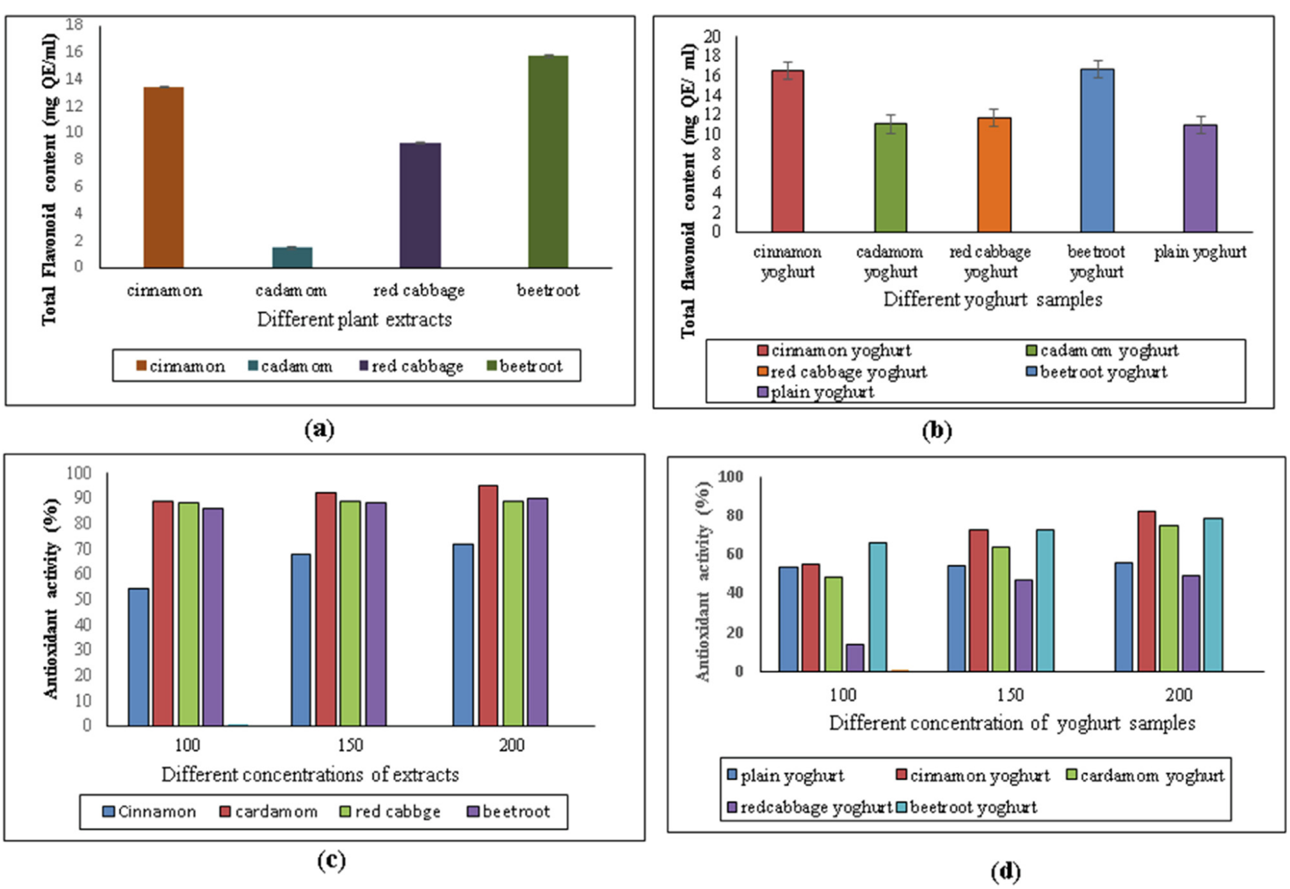


Fig. 3. (a) Total flavonoid content of different plant extracts (b) Total flavonoid content of plain and enriched yoghurt samples (c) Antioxidant activity of different extracts (d) Antioxidant activity of plain and enriched yoghurt samples.

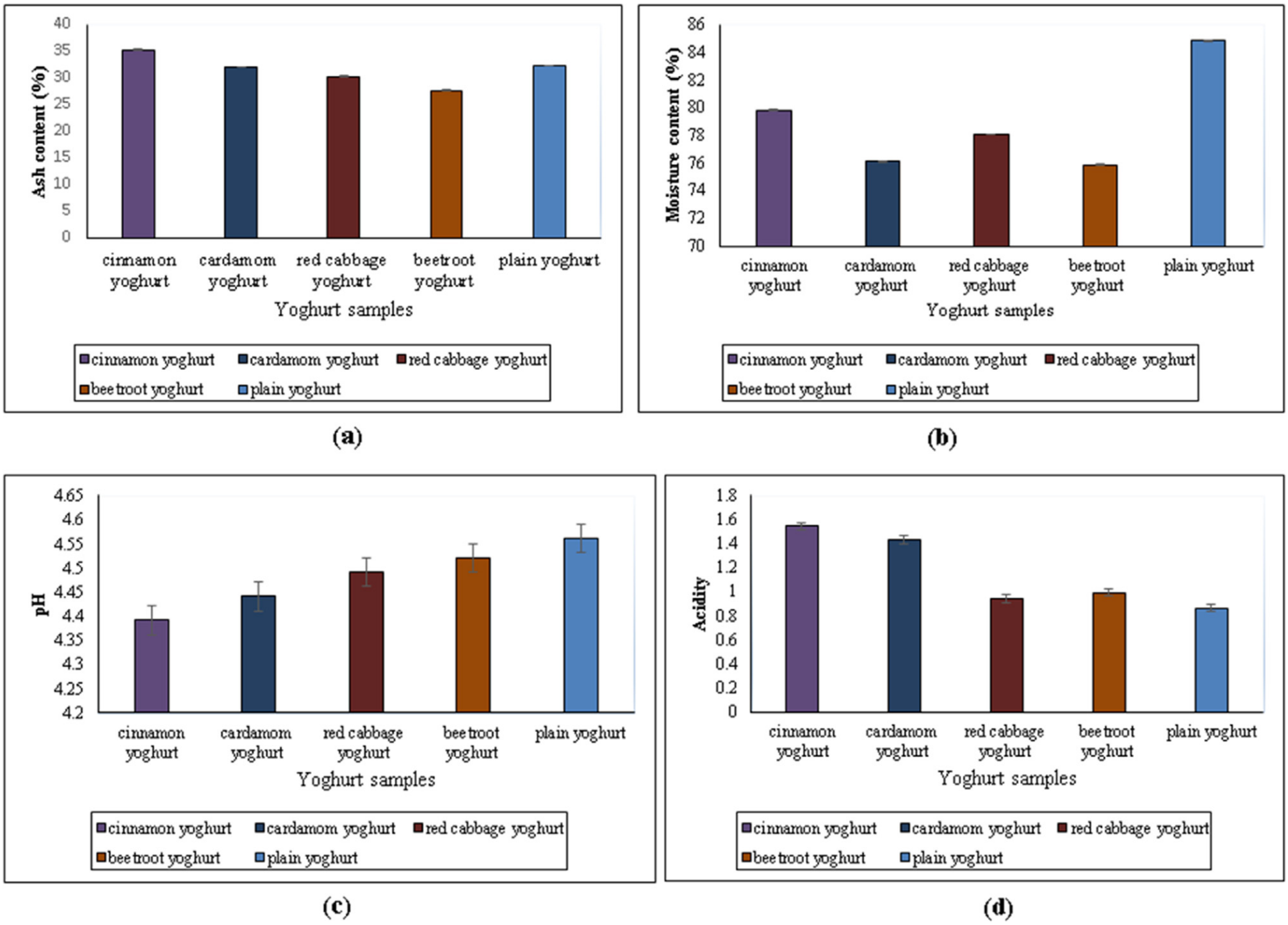


Fig. 4. (a) Ash content of different yoghurt samples (b) moisture content of different yoghurt samples (c) pH of different yoghurt samples (d) titratable acidity of different yoghurt samples.

ash content of yoghurt samples. The ash content of plain yoghurt measured was 0.46 %. The results indicated highest ash contents were found in beetroot and red cabbage enriched yogurt samples (0.96 %) and lowest in cinnamon enriched yoghurt samples (0.68 %).

3.5. Moisture content of yogurt samples

Moisture content of plain and enriched yoghurt samples were determined (Fig. 4b). The moisture content of plain yoghurt was 84.86 %. The results indicated highest moisture contents were found in cinnamon enriched yogurt samples and lowest in beetroot enriched yoghurt samples.

3.6. Determination of pH of yogurt samples

The pH of the plain and different enriched yoghurt samples were evaluated (Fig. 4c). The results exhibited that all enriched yoghurt samples have lower pH values and a higher acidity as compared to control yoghurt sample.

3.7. Determination of titratable acidity

Titratable acidity of control and different extract incorporated yoghurt samples were evaluated (Fig. 4d). The incorporation of extract slightly increases the titratable acidity of yoghurt samples. The results indicated that the acidity of plain yoghurt was 0.86 while the acidity of cinnamon enriched yoghurt was 1.54. Cinnamon enriched yoghurt indicated the highest value of acidity when compared to other yoghurt samples.

3.8. Determination of total soluble solids

The TSS of control and different enriched yoghurt samples were evaluated (Fig. 5a). The incorporation of extracts slightly increases the total soluble solids. Plain yoghurt had the TSS of 11.20° brix. The TSS of cinnamon, cardamom, red cabbage and beetroot enriched yoghurt samples were 13.09, 11.80, 13.09 and 12.80 respectively.

3.9. Susceptibility to syneresis (S) and water holding capacity (WHC)

Syneresis of plain and different enriched yoghurt samples were evaluated (Fig. 5b). The plain yogurt sample showed a higher syneresis (54.90 %) than enriched yogurt samples. The syneresis value of cinnamon, cardamom, red cabbage and beetroot enriched yoghurt samples were 48.2, 49.9, 52, 53.4 % respectively that were all lower than the value of control yoghurt sample. Also, water holding capacity of plain and enriched yoghurt samples were evaluated (Fig. 5c). The results showed that WHC of enriched yoghurt samples were higher than the plain yoghurt (32.43 %).

3.10. Determination of antibacterial activity of different plant extracts and enriched yoghurt samples

Antibacterial activity of *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris* and *Brassica oleracea* extracts at different con-

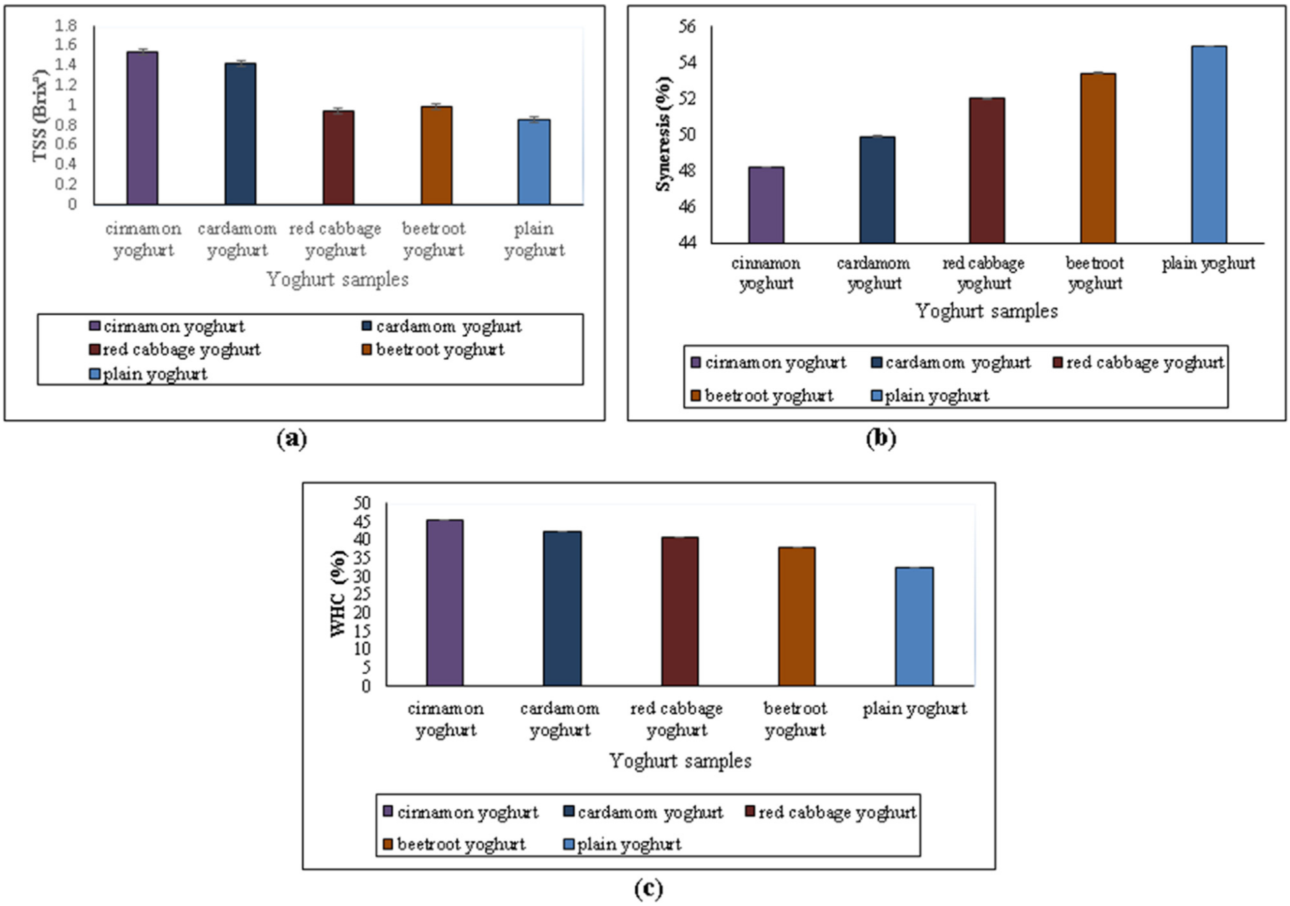


Fig. 5. (a) TSS of different yoghurt samples (b) syneresis of different yoghurt samples (c) water holding capacity of different yoghurt samples.

centrations (i.e. 50–200 μl) against *E.coli* and *S. aureus* (Fig. 6a and b) were evaluated. The results indicated that as the concentration of extracts increases, the zones of inhibition also increases. Among all four extracts beetroot extract showed the largest zone of inhibition against *E. coli* and *S. aureus* while cardamom extract showed the least. Antibacterial activity of plain and enriched yoghurt samples against *E. coli* and *S. aureus* were determined (Fig. 7c and d). The results showed that as the concentration of different enriched yoghurt samples increases, the zones of inhibition also increase. Among the enriched yoghurt samples red cabbage enriched yoghurt showed highest anti-bacterial activity against *E. coli* while cardamom enriched yoghurt showed highest activity against *S. aureus*.

3.11. Determination of shelf life

Shelf life of all the prepared yoghurt samples was evaluated at room temperature as well as at refrigerated temperature. Color, taste, odor and texture were noted at different time intervals. Addition of spices and vegetable extracts enhances the shelf life of yoghurt samples. At room temperature plain yoghurt was spoiled after 36 h while extracts incorporated yoghurt samples were deteriorated after 48 h (Tables S3–S7). At refrigerated temperature, control yoghurt spoiled after 21 days while extracts incorporated yoghurt samples remain edible for 28 days. So, incorporation of extracts enhances the shelf life of yoghurt (Tables S8–S12).

3.12. FTIR analysis

The functional groups of the components were separated using the FTIR after the plant extract was passed through it. FTIR analysis of extracts of *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris and Brassica oleracea* (Fig S1a–d) and yoghurts incorporated with *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris and Brassica oleracea* was conducted using SHIMATZU IR-tracer-100 (Fig S2a–f). The peaks observed in the FTIR spectra were compared with the different ligand spectra found in the PubChem database. These were compared and identified as potential ligands found in the various extracts.

3.12.1. In silico analysis

The results of *in silico* analysis indicates a bioactive compound known as Ascorbic acid present in the *Beta vulgaris* binds with the binding site of ACE protein (Fig. 7, Table 1). Different hydrogen bonds and hydrophobic interactions were formed between ligand structure and protein. Out of the predicted 10 models (data not given) by the GalaxyWeb server, model 1 was chosen as it has been considered as the best model as it shows different binding interactions. In model 1, three hydrogen bonds were formed while 3 amino acid residues were involved in hydrophobic interaction with angiotensin converting enzyme. Hydrogen bonds are depicted with dashed line and hydrophobic interactions are shown as arcs.

A bioactive compound known as Sinapinic acid present in the *Brassica oleracea* binds with the binding site of ACE protein (Fig. 8, Table 1). Two hydrogen bonds were formed while eight amino acids were involved in hydrophobic interactions.

A bio active compound cinnamaldehyde present in *Cinnamomum verum* binds with the binding site of angiotensin converting enzyme (Fig. 9, Table 1). In model 1, one amino acid was involved in making hydrogen bond while 7amino acids were involved in hydrophobic interactions.

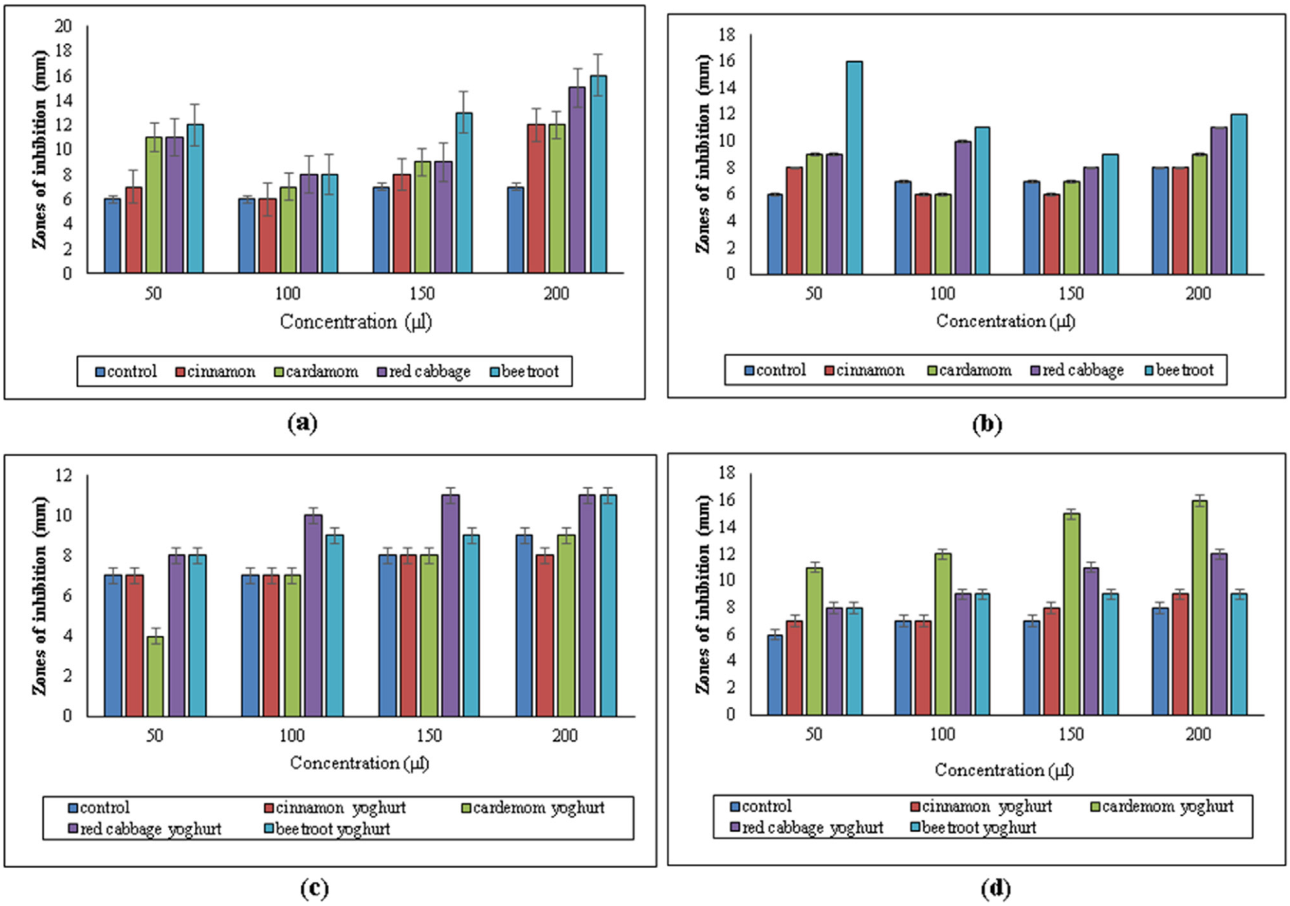


Fig. 6. Evaluation of antibacterial activity (a) different plant extracts against *E.coli* (b) different plant extracts against *S. aureus* (c) plain and different enriched yoghurt samples against *E.coli* (d) plain and different enriched yoghurt samples against *S. aureus.*

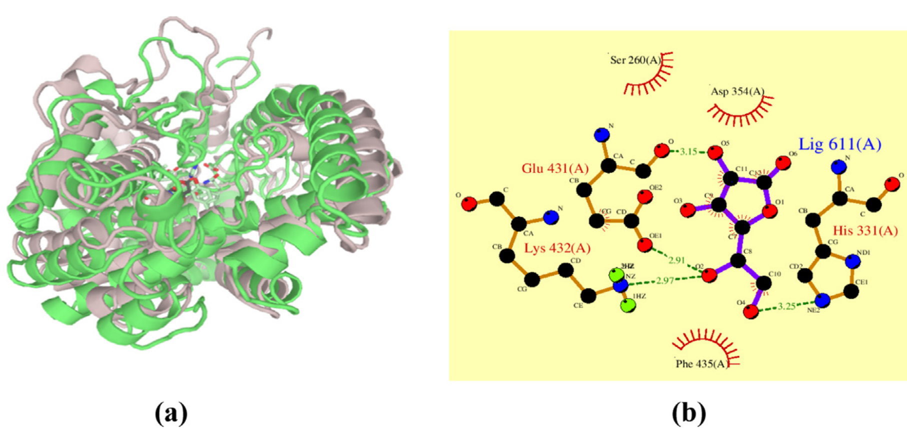


Fig. 7. (a) Binding of angiotensin converting enzyme with ascorbic acid (b) model 1.

A bioactive compound, linalool acetate present in the *Elettaria cardamomum* binds with the binding site of ACE protein (Fig. 10, Table 1). In model 1, three amino acids were involved in making hydrogen bonds, while 10 amino acid residues were involved in hydrophobic interactions.

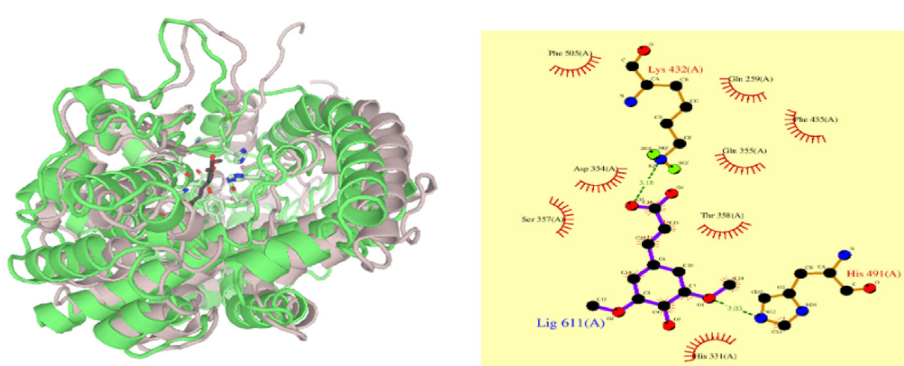
4. Discussion

Yogurt is world's most well-known fermented dairy products, not just for its nutritional value but also for its health benefits. Yoghurt as a dairy product is more nutritious as compared to milk. It is easily digestible by the people having lactose intolerance and has more medicinal and therapeutic worth than milk. Yoghurt is highly consumed all over the world (Amal et al., 2016). In the present study, different spices and vegetable enriched yoghurts were prepared and assessed for quality as the incorporation of *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, Brassica oleracea* is the most economical and efficient way of improving the yoghurt's therapeutic attributes. Addition of spices (*Cinnamomum verum, Elettaria cardamomum*) and vegetables extracts (*Beta vul-*

Table 1
Binding of different ligands with ACE proteins.

Ligand	ACE AA	Types of bond	Length (Ä)	Ligand-AA binding sites
Ascorbic acid	His 331	HB	3.25	O4-NE2
	Glu 431	HB	2.91, 3.15	OE1-O2, O-O5
	Lys 432	HB	2.97	O2-NZ
	Ser 260	HI		
	Asp 354	HI		
	Phe 435	HI		
Sinapinic acid	Lys 432	HB	3.18	NZ-O5
	His 491	HB	3.03	NE2-O1
	His 331	HI		
	Thr 358	HI		
	Asp 354	HI		
	Ser 357	HI		
	Gln 355	HI		
	Phe 435	HI		
	Gln 259	HI		
	Phe 505	HI		
Cinnamaldehyde	Ser 400	HB	3.06	O1-OG
	Met 353	HI		
	Val 279	HI		
	Met 283	HI		
	Leu 410	HI		
	Val 399	HI		
	Leu 356	HI		
	His 360	HI		
Linalool acetate	Thr 358	HB	2.68	OD1-O2
	Gln 259	HB	3.10	NE2-O1
	Gln 355	HB	2.84	NE2-O2
	Ser 357	HI		
	Glu 431	HI		
	Phe 435	HI		
	Tyr 501	HI		
	Tyr 498	HI		
	His 491	HI		
	Phe 505	HI		
	His 331	HI		
	Ser 260	HI		
	Asp 354	HI		

HB – Hydrogen bonding; HI – Hydrophobic interaction; O – Oxygen; N – Nitrogen; G – Gamma; D – Delta; E – epsilon, Z – Zeta.



(a) (b)

Fig. 8. (a) Binding of angiotensin converting enzyme with sinapinic acid (b) model 1.

garis, Brassica oleracea) enhances the sensory attributes like texture, appearance, flavor, odor and color.

In the present study different enriched yoghurts were prepared. In order to produce yoghurt, milk was first pasteurized at the 85 °C for 15 min because high temperature treatment was necessary for the prevention from microbes and this is in accordance with Anjum et al. (2007) who also reported high temperature treatment for the elimination of microorganism.. Pasteurization is necessary because spoilage microbes were unable to interfere with probiotic strains due to heat treatment. Moreover, pasteurization helped to prepare texture and appearance of yoghurt more appealing as high temperature allowed milk proteins to become denatured and coagulate to form semi solid gel like material. So, separation of water during storage would be decreased resulting in good shelf life. The milk is chilled after pasteurization and then inoculated with a previous yoghurt sample as it contains yoghurt starter cultures including LAB, that are responsible for converting milk into gel curd. The function of the starter cultures is to ferment lactose in order to make lactic acid. Lactic acid causes the pH of the milk to drop, causing it to coagulate or form the smooth jelly that is the property of yoghurt. Lactose, a sugar found in milk, is converted to lactic acid in yoghurt by bacteria.

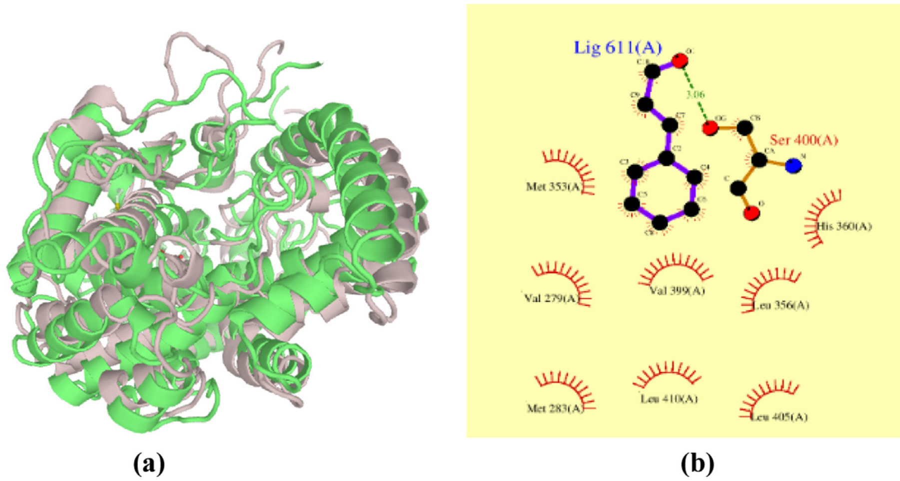


Fig. 9. (a) Binding of angiotensin converting enzyme with cinnamaldehyde (b) model 1.

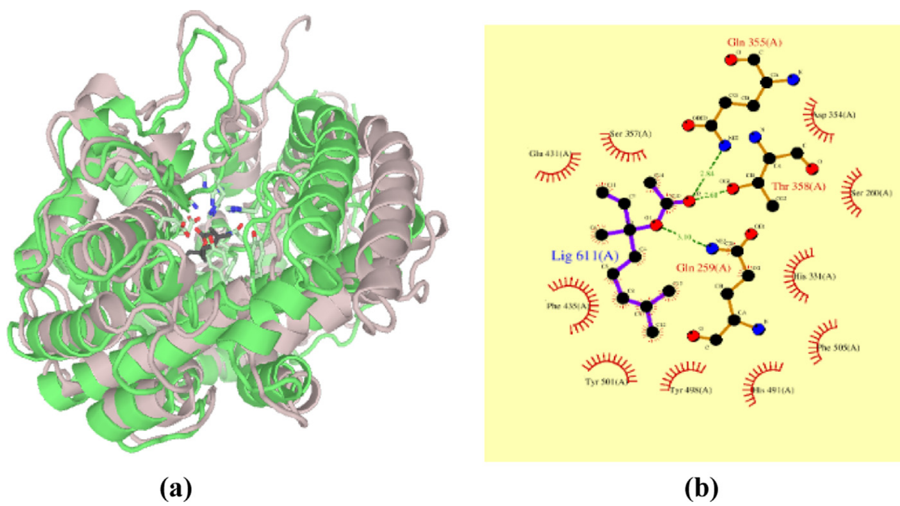


Fig. 10. (a) Binding of angiotensin converting enzyme with linalool acetate (b) model 1.

In the present study, 1 g free fat skim milk powder was used during yoghurt preparation to increase the whey content. Powdered milk is added to get a better firmness in yoghurt. This increases the volume of milk solids, so the resulting yogurt is thicker (Guyot and Kulozik, 2011). After adding small amount of previously available yoghurt, skim milk powder and different extracts the yoghurt was incubated at 41 °C. An explanation for this aspect might be that the incubation temperature is very significant for the quality of yoghurt. For yoghurt bacteria to reproduce properly, the temperature must be between 37 and 45 °C. Bacteria are inactivated by high temperatures, while low temperatures prevent growth. Yoghurt was then stored at the temperature of about 10℃ to cease the process of fermentation and to stop further acid development. Low temperature also helps in setting the appearance and texture of yoghurt (Katsiari et al. 2002).

In the current research four different types of yoghurts were prepared by using different concentration of spices and vegetables extracts. The yogurt samples containing vegetables and spices extracts had significantly higher flavor score, in contrast to the control yoghurt. Sensory evaluation of different types of yoghurt was performed to get the best one. Out of the four different types of flavored yoghurts, cardamom incorporated yoghurt is considered as the best fortified product in terms of its sensory evaluation because of its sweet flavor and good fruity smell. The reason might be that cardamom contains 1,8-cineole, which gives cardamom its specific odor while alpha-terpinyl is a major flavor component (Kubo et al., 1991). Red cabbage and beetroot incorporated yoghurts were most accepted in terms of its color. The purplish color of red cabbage is caused by a type of pigment molecule known as anthocyanins (Shalaby and Amin, 2018) while the red color of beetroot was due to red and yellow pigment called betalains. Betanin is the most abundant betacyanin in beetroot, accounting for 75–95 % of the red pigment (Ozcan et al., 2021). Cinnamon enriched yoghurt was not much preferred because of its slightly bitter and woody taste that is due to the presence of compound known as cinnamaldehyde that gives cinnamon its flavor and odor (Gong et al., 2020) although its' color and odor were considered good by consumers.

In the current study, qualitative and quantitative phytochemical analysis of *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, Brassica oleracea* extracts and enriched yoghurt samples were carried out. Flavonoids and tannins possess anti-oxidant qualities, whereas saponins have anti-cancer characteristics and lower cholesterol levels. From the results of the phytochemical analysis, it was noted that *Cinnamomum verum* aqueous extract contains all the phytochemicals which were tested except for proteins, anthraquinones, phylobatanins and carotenoids and these results were in line with the results of Raja and Ravindranadh

(2014) who also noted the absence of proteins, anthraquinones, phylobatanins and carotenoids in the aqueous extract of cinnamon. Cinnamon contains almost no protein or fat but contains trace amounts of many other vitamins and other nutrients that are beneficial for human health.

Phytochemical analysis of the *Elettaria cardamomum* aqueous extract indicated the presence of all the phytochemicals except steroids, phytosterols, anthraquinone and carotenoids. These results were in agreement with the results of Lakshmi et al. (2022) who also reported the absence of terpenoids, steroid and phytosterols.

The aqueous extract of red cabbage juice showed the presences of all the phytochemicals except anthraquinone and carotenoids and these results were similar with the results of Chauhan et al. (2016) who noted the presence of phytochemicals such as alkaloid, glycosides, steroids, flavonoids, saponin, tannin, terpenoids and phytosterols in aqueous extract of red cabbage as well. Red cabbage extract is rich in phytochemical compound which may help in treating many diseases. It has the potential to cure many diseases. Phytochemicals such as glycosides, steroids, flavonoids, saponin, tannin, terpenoids, and phytosterols were found in red cabbage, increasing its therapeutic potential and allowing it to be used to treat a variety of ailments. Beetroot showed positive results for all the tests except for carotenoids test and these results were in agreement with the results of Harborne (1987) who reported the presence of flavonoid, tannins, saponin and phenolic compounds in beet root extract.

Plants possessed a wide range of chemical substances known as phenolic compounds and flavonoids. TPC of the yoghurt increased by the addition of *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, Brassica oleracea* extracts and these results were in line with the findings of Helal and Tagliazucchi (2018) who reported that addition of *Cinnamomum verum* powder to yoghurt boosted the TPC and antioxidant activity when compared to plain yoghurt. Phenolic compound also provide anti-fungal and antimicrobial properties of food so yoghurt shelf life and quality also enhanced. Synthetic phenolic compounds are costly as well as damaging to health so the spices and vegetables are good natural source of phenolic and antioxidant compounds. The antioxidant activity is highly associated with total phenolic content (Helal and Tagliazucchi 2018).

Flavonoids are polyphenolic compounds found in various fruits, vegetable and other plant based products. Flavonoids are linked with many therapeutic effects such as they are responsible for preventing cancer, heart diseases etc. In this work, Total flavonoid content (TFC) of extracts and enriched yoghurt samples were evaluated. TFC of plain yoghurt was very less but the incorporation of different extracts increases the flavonoid content to some extent. This is in agreement with Abou El Samh et al. (2013) who noted that plain yoghurt did not have any flavonoid content but enrichment of yoghurt with different fruits and vegetables enhanced the flavonoid content of yoghurt.

Plants also contain chemicals such tannin. Tannins are polyphenols that give food its astringent flavor and have anti-carcinogenic and antibacterial characteristics. In current study, tannin content of several spices and vegetables extracts as well as enriched yogurts was evaluated. Beetroot showed highest tannin content followed by red cabbage, cinnamon and cardamom respectively. Total tannin contents of plain yoghurt and *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, and Brassica oleracea* enriched yoghurt were also evaluated. The incorporation of yoghurt with *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, Brassica oleracea* increase the total tannin content of yoghurt. This is in accordance with Ogori et al. (2017) who described that plain yoghurt did not have any tannin content but enrichment of yoghurt with different fruits and vegetables enhanced the tannin content of yoghurt.

According to the results, adding *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, Brassica oleracea* aqueous extract to yoghurt dramatically dropped the pH and raised the acidity compared to the control. These findings are also similar with those of Shalaby and Amin (2018). This is because the infusion of these extracts increased the metabolic activity of starter lactic acid bacteria in yoghurt thus, raising the acidity. The current research showed that titratable acidity of yoghurt increased by the addition of *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, Brassica oleracea* extracts. This result is in accordance with Yildiz and Ozcan (2019) who described that titratable acidity of vegetable enriched yoghurt was more than plain yoghurt. This is due to the reason that extracts provide different nutrients and minerals to starter culture. The starter cultures can convert more sugars to lactic acid that enhanced the overall acidity level. The increase in overall acidity indicates the amount of lactic acid contained in the yoghurt during fermentation. On the other hand, pH of yoghurt decreased because acidity increased.

In present research, total soluble solids of yoghurt increased with the addition of cinnamon, cardamom, red cabbage and beetroot extracts and these results are similar to the outcomes of Saldana et al. (2018) who also observed that addition of plant extracts increases the TSS. Determination of TSS is important because it determines the quality of yoghurt. Higher TSS ensures the longer shelf life of products as amount of dissolved sugars in the products absorb the moisture thus, preventing the bacterial growth by limiting the aqueous medium.

In present study, ash and moisture content of enriched yoghurt samples as well as plain yoghurt was determined. The incorporation of extracts slightly increases the ash content of yoghurt samples. The highest ash contents were found in beetroot and red cabbage enriched yogurt samples and lowest in cinnamon enriched yoghurt samples. The highest moisture contents were found in cinnamon enriched yogurt samples and lowest in beetroot enriched yoghurt samples and these results agrees with observation of Ihemeje et al. (2015) who found that the moisture content below 90 % was most preferable. The presence of higher moisture content affects the texture and mouth feel. The low ash content and high moisture content of yoghurt may be due to milk dilution before fermentation (Ahn et al., 2014).

Syneresis is among the most essential yoghurt quality attributes. A high amount of syneresis indicates that the yoghurt is of poor quality. Both vegetable concentration and storage period had a considerable impact on yoghurt syneresis. The current results indicates that plain yogurt showed a higher syneresis than enriched yogurt samples, Yogurt containing different extracts had significantly lower syneresis. Many researches regard syneresis as one of the most essential characteristics reflecting the quality of yoghurt during preservation. In addition to this water holding capacity was determined for all yogurt samples containing extracts as compared to the control yoghurt sample. The results indicated that WHC of enriched yoghurt samples were higher than the plain yoghurt and these results agree with finding of Dabija et al. (2018).

The antibacterial potential of *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris, Brassica oleracea* aqueous extract at different concentrations (50–200 μl) against *E. coli* and *S. aureus* were evaluated. The results showed that the zones of inhibition increases as the concentration of extracts increases. Among the all four extracts beetroot extract showed the highest antibacterial potential against *Escherichia coli* and Staphylococcus aureus while cardamom extract showed the least. Antibacterial activity of plain as well as enriched yoghurt samples against *Escherichia coli* and Staphylococcus aureus were also evaluated. The results indicated that as the concentration of different enriched yoghurt samples increases, the zones of inhibition also increases. Among the enriched yoghurt samples red cabbage enriched yoghurt showed highest antibacterial potential against *E. coli* while cardamom enriched yoghurt showed highest activity against Staphylococcus aureus. The findings of this study was in accordance with those of Arora and Kaur (2007), who reported that aqueous extracts of *E. cardamomum* (green cardamom) fruits were efficient against many human pathogenic microorganisms. Furthermore, it was claimed that aqueous cardamom extract can be utilized as a natural potential source in various pharmaceutical formulations to guard against a variety of chronic diseases, particularly obesity and diabetes. The results of present study were encouraging as cinnamon, cardamom, red cabbage and beetroot possessed antibacterial properties against *E. coli* and *S. aureus.*

The shelf life of plain, spices and vegetables enriched yoghurts was determined at room temperature as well at refrigerated temperature. The shelf life of yoghurt increased by the incorporation of different spices and vegetable extracts and this is in line with EL-Ahwal et al. (2019) who also found an increase in shelf life of plain yoghurt by incorporating it with cinnamon at refrigerated temperature as compared to room temperature. Control yoghurt deteriorated after 36 h of storage at room temperature while spices and vegetable incorporated yoghurts remain edible for about 48 h, plain yogurt remain edible for about 21 days but spices and vegetable incorporated yoghurt remain good for 28 days at refrigerated temperature.. Yoghurt kept at refrigerator has longer shelf life as compared to yoghurt stored at room temperature. When produced properly and stored in the refrigerator, yoghurt has a shelf life of ten to twenty days. Molds, yeasts, and slow-growing bacteria can ruin yoghurt if it has been stored for longer period of time but by the addition of spices and vegetables extracts, the shelf life increases because these extracts work as the natural preservatives and has effectively delayed contamination in the yogurt sample (Srivastava et al., 2015).

ACE inhibitors are first-line medications for the treatment of hypertension, and they are especially useful in preventing diabetes and high blood pressure-related kidney problems. Even in people with generally normal blood pressure, ACE inhibitors are sometimes utilized in the treatment of heart failure as well as to avoid diabetic neuropathy. After oral treatment, angiotensin converting enzyme inhibitory peptides can cause antihypertensive effects (Abdel-Hamid et al., 2017).

The *in silico* method has enabled us to reveal the binding of bioactive compounds to its respective targets. The results have clearly showed that the bioactive compounds like (sinapinic acid, ascorbic acid, cinnamaldehyde and linalool acetate) present in the flavored yoghurts enriched with *Beta vulgaris, Brassica oleracea, Cinnamomum verum* and *Elettaria cardamomum* binds with angiotensin converting enzyme. All enriched yogurt samples showed higher anti-ACE activity because bioactive compounds present in *Cinnamomum verum, Elettaria cardamomum, Beta vulgaris and Brassica oleracea* enhances the ACE inhibitory activity of plain yoghurt. The *In silico* studies reveal that ascorbic acid, sinapinic acid, cinnamaldehyde and linalool acetate due to their unique structures are able to gain access into the active site of ACE, establish chemical interactions simultaneously with amino acids and zinc atom and causing alterations in the 3-D structure of catalytic protein thus proving to be a successful ACE inhibitor.

5. Conclusion

From the current study it was concluded that there were significant improvements in nutrient composition, physicochemical as well as sensory properties of yoghurt samples enriched with cinnamon, cardamom, red cabbage and beetroot extracts. Modern medicine has numerous adverse effects and is not entirely safe to consume. As a result, it is preferable to consume natural foods that have no adverse effects and are relatively safe for human consumption. *In silico analysis* results suggested that plant extracts and enriched yoghurt samples contain bioactive compounds with significant therapeutic health effects and thus could be used to treat and manage hypertension.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2023.103556.

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