Characterization and analysis of dhokla with incorporated tomato powder
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Introduction

The development of high-protein foods of plant origin is essential in developing countries because of the shortage and high cost of animal protein. Legumes play an important role in nutrition as they contain proteins, carbohydrates, dietary fibre and several water-soluble vitamins, especially B-complex vitamins and minerals such as calcium and iron [1]. Legume proteins are rich in lysine but deficient in sulphur-containing amino acids, whereas cereal proteins are deficient in lysine but have adequate levels of sulphur-containing amino acids. Consequently, combining grains with legumes provides a better overall balance of essential amino acids than either alone [2]. Bengal gram is a legume widely used in food products throughout India. Durum wheat semolina is mostly used for the preparation of pasta and couscous and also for making bread. Bread made from durum wheat flour is characterized by a relatively slow rate of staling and, consequently, has a longer shelf-life due to its high water-binding capacity [3, 4].

Dhokla is a lactic acid-fermented cake prepared from a batter of coarsely ground rice (Oryza sativa L.) and Bengal gram dhali (Cicer arietinum L.) which is fermented at ambient temperature, steamed in a dish, cut and seasoned [5, 6]. It has an appealing mildly sour taste, colour, flavour and spongy texture and is a source of energy and nutrients [7]. Lactic acid bacteria Leuconostoc mesenteroides, Lactobacillus fermentum and Pediococcus pentosaceus, and yeasts, mainly Pichia silvicola and Saccharomyces cerevisiae, contribute to the fermentation of dhokla batter [5].

Tomatoes (Solanum lycopersicum) are a widely used and versatile fruit and are consumed fresh as well as in processed products because they are rich in several nutrients including vitamin A, vitamin C, potassium, calcium and lycopene [8]. The main antioxidants in tomatoes are carotenoids, ascorbic acid and phenolic compounds [9]. The consumption of tomato and tomato-based products has been associated with a lower risk of developing some cancers such as digestive tract and prostate cancers, which may be due to the presence of lycopene and other antioxidant components [10]. Several food technology studies have been carried out to optimize the processing and storage of tomato products by preventing heat and oxidative damage to the antioxidants [11].

The main objectives of this research were:

- to prepare dhokla with semolina and Bengal gram with incorporated tomato powder,
to perform nutritional analysis (carbohydrate, protein and ash),
• to analyze the relationship between the antioxidant content and antioxidant properties of the prepared dhokla, and
• to examine colour and the colour degradation kinetics and sensory profile characteristics after storage.

Materials and methods

Ingredients
Semolina (Ganesh Grains Ltd, Kolkata, India), sugar (Sakthi Sugar, Chennai, India), Bengal gram flour, refined oil (Purti, Kolkata, India), baking powder (Weikfield Foods Pvt Ltd, Nalagarh, India), curd (Amul, India) and salt (Tata, Mumbai, India) were obtained. Turmeric powder, lemon, mustard seed, cumin seed and curry leaves were purchased from local stores in Jadavpur, Kolkata, India.

Chemicals
Ethanol (Jiangsu Huaxi International Trade Co. Ltd, China), Folin–Ciocalteu reagent, sodium bicarbonate, sodium potassium tartrate, hydrochloric acid (HCl), anthrene, ascorbic acid, glucose (Merck Specialties Pvt. Ltd, Mumbai, India), 2, 2-diphenyl-1 picrylhydrazyl (DPPH), 2, 4-dinitrophenylhydrazine (Sigma-Aldrich, St. Louis, MO, USA), sodium nitrite (NaNO₂), aluminium chloride (AlCl₃), thiourea, bromine (LOBA Chemie, Mumbai, India), sodium hydroxide (NaOH), copper sulphate (CuSO₄) (HiMedia, Mumbai, India), phenolphthalein (RFCL Ltd, New Delhi, India) and bovine serum albumin (5 D Fine-Chem Ltd, Mumbai, India) were obtained.

Dhokla preparation
The dhokla ingredients in wt% were: Bengal gram 10, semolina 20, curd 12, sugar 8, salt 2, lemon juice 5, baking powder 3 and water 40. Bengal gram was first soaked in curd for 1 hour, the other ingredients together with baking powder were added and the mixture was stirred to make a batter. A microwave oven-proof container was greased with refined oil and the batter was poured into it and cooked in a microwave oven (Samsung Combi, India) for 8 min at 450 W. A knife inserted into the dhokla was used to check when it was cooked. The dhokla was then cut into squares. Mustard seeds, cumin seeds, sesame seeds, sugar and lemon juice were heated in oil and poured over the dhokla. Curry leaves were used for garnishing.

Preparation of tomato powder
The tomatoes were washed and sliced. They were then dried in a hot air oven (Mac Pharmatech, Nashik, India) at 60°C for 12 hours and ground to powder in a commercial mixer grinder (Prestige Stylo Mixer Grinder; Prestige, Bangalore, India). The powder was sieved through a BS-60 mesh to obtain particles <0.250 mm in diameter and stored in a freezer (New Brunswick Scientific) at −20°C until analysis.

Preparation of dhokla composite mix
A 100 g sample of dhokla batter was mixed with 1%, 4% and 7% tomato powder. Dhokla without tomato powder was denoted as A and dhokla with 1%, 4% and 7% incorporated tomato powder as B, C and D, respectively.

Evaluation of batter and cooked product
Acidity and pH
A 1 g sample of batter was mixed with 10 ml distilled water. The sample was then titrated with 0.1N NaOH using phenolphthalein indicators to determine the end point [12].

Rheology
Dhokla batter was used for dynamic rheological tests. Dynamic oscillatory tests were performed in a controlled stress rheometer (Physica MCR 51; Anton Paar, Germany). Parallel plates with gaps of 49.986 mm and 0 mm were used and measurements monitored with RheoPlus software (v. 2.65). The temperature was maintained at 25°C with a water circulator device (Neslab RTE 7 refrigerated bath; Thermo Scientific, Waltham, MA, USA). Frequency-sweep tests of dhokla batter were performed at a constant stress of 1% and a frequency ranging between 0 and 35 Hz. Dynamic moduli G’ and G’” were obtained as a function of frequency. G’ is the dynamic elastic or storage modulus, related to material response as a solid, while G’” is the viscous dynamic or loss modulus, related to material response as a fluid.

Antioxidant analysis
Sample preparation
Samples were prepared for antioxidant activity analysis by mixing 1 g sample with 20 ml 80% methanol. The mixture was then sonicated in a sonicator (Trans-o-sonic/D150-IM; Trans-O-Sonic, Mumbai) for 10 min. The sample was next filtered through filter paper and collected in a tube and kept in a refrigerator at 4°C until analyzed.

Antioxidant compounds
Total phenolic content
A 0.2 ml aliquot of sample was added to 1.8 ml distilled water. Next, 0.2 ml Folin-Ciocalteu reagent was added and
mixed by shaking for 5 min. Then, 2 ml 7% sodium carbonate and 0.8 ml distilled water were added. After incubation for 90 min in the dark, the absorbance of the mixture was observed at 750 nm in a spectrophotometer (U 2800; Hitachi, Japan). The results were expressed as mg of gallic acid equivalents (GAE) per gram of sample [13].

**Total flavonoid content**

A 1 ml aliquot of sample, 4 ml distilled water and 0.3 ml NaNO₂ were mixed together. Then, 0.3 ml AlCl₃ and 2 ml 1N NaOH were added. After incubation for 25 min in light, the absorbance of the mixture was observed at 510 nm in the spectrophotometer. The result was expressed as mg of catechin equivalents (CAE) per gram of sample [14].

**Vitamin C content**

A 0.23 ml aliquot of 3% bromine water was added to 4 ml of sample, to which 0.13 ml of 10% thiourea and 1 ml of 2, 4-dinitrophenylhydrazine (DNPH) solution was added to form osazone. All standards samples and blank solutions were kept at 37°C for 3 hours in a thermostatic bath, then cooled in an ice bath for 30 minutes and treated with 5 ml chilled 85% H₂SO₄, with constant stirring. The absorbance of the resultant coloured solution was measured at 521 nm [15]. A standard curve was prepared with different concentrations of ascorbic acid.

**Total antioxidant activity**

A 0.002 g sample of DPPH was mixed with 50 ml of ethanol in a volumetric flask which was kept in the dark in an ice-cold condition. A 0.1 ml sample was mixed with 3.9 ml of prepared DPPH solution and placed in a dark place for 45 min, and absorbance was measured in a spectrophotometer at 515 nm [16]. Total antioxidant activity was calculated as (blank–sample)/blank)*100.

**Colour degradation kinetics**

The colourimetric study was done using a HunterLab colour measurement system (Colour Flex 45/0, D65/10°; Hunter Associates Laboratory, Reston, VA, USA). Samples were placed in optical glass cells 3.5 cm in length. The results were expressed in L* lightness (0: black to 100: white), a* redness (+: green to +: red), and b* yellowness (+: blue to +: yellow) values. A 3.5 cm thick layer of dhokla was covered with the white standard plate (X=79.22; Y=84.10; Z=88.76) for the measurement of diffused reflected light from the cell bottom using a 1.25-inch diaphragm aperture. The colour kinetics of the products were calculated using Eqs. (1) and (2) for a first-order reaction:

\[ \ln(C/C_0) = -kt \]  
\[ \ln(C_0) = -kt \]

where C is the concentration, t is time (day), and k is the first-order reaction rate constant (day⁻¹). Kinetics data were analyzed using linear regression [17].

**Nutritional analysis**

**Carbohydrate content**

A 100 mg sample was measured and hydrolysed with 5 ml 2.5N HCl by keeping it in a boiling water bath for 3 hours. It was then cooled to room temperature. Sodium carbonate was added to neutralize the acid. The volume was made up to 100 ml and then the sample was centrifuged. The supernatant was collected and used for analysis. Glucose was used for the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. ‘0’ served as blank. A 4 ml aliquot of anthrone reagent was added to the sample and heated for 8 min in a boiling water bath. The sample was then cooled rapidly and absorbance was measured at 630 nm [18].

**Protein content**

The protein content of the sample was determined using the Lowry method [19]. A 4.5 ml aliquot of reagent (1 ml of 0.5% CuSO₄.5H₂O) was added to the sample and incubated for 10 min. After incubation, 0.5 ml of reagent (1:1 Folin phenol:water) was added and incubated for 30 min. Absorbance was measured at 660 nm. Bovine serum albumin was used as standard.

**Ash content**

A dhokla sample was put in a weighed crucible and then placed in muffle furnace at 550°C for 4 h until ash was formed. The crucible was weighed and the process repeated until a constant weight of the ash was obtained [15].

**Sensory analysis**

Dhokla samples were coded and given to 40 panel members for sensory scoring. Each set contained one control sample (without fortification) and samples prepared with 1%, 4% and 7% tomato powder. Water was used for mouth rinsing before and after each sample was tested. The dhokla was scored for colour, appearance, taste, aroma and overall acceptability using a numerical scoring system with values ranging from 1 (extremely dislike) to 9 (extremely like).
Statistical analysis
All studies were repeated three times and the mean values calculated. All experimental data were analyzed using analysis of variance (ANOVA). The mean values were compared and grouped by Fisher’s least significant difference test at a significance level of p≤0.05.

Results and discussion

Acidity and pH
Acidity is an important parameter for determining product quality. The acidity of the different dhokla sample is shown in Fig. 1. The acidity of sample 2 (B), 3 (C) and 4 (D) fortified with tomato powder was higher than that of sample 1 (A). Tomato powder is a rich source of citric acid and malic acid [20]. Khazaei et al reported that drying tomato slices increased soluble solids and acidity [21].

Rheology
The rheological property of batter is useful to determine the potential of the ingredient and the quality of the product. The dynamic oscillation test curves showing the storage and loss moduli of the different dhokla batters are shown in Fig. 2. The storage modulus measures elastic responses and stored energy, while the loss modulus determines viscous response and energy dissipated as heat. It was observed that the storage modulus of dhokla batters increased as frequency increased at a constant temperature of 25ºC. The loss moduli of all samples were very low while the storage moduli were higher, indicating that dhokla batters have elastic properties. Sample D had the highest storage modulus, indicating that the batter had the highest elastic properties of all samples. There were no intersecting points between the storage and loss moduli.

Antioxidant properties
The relationship between total phenolic content, flavonoid content and vitamin C and antioxidant activity assessed by DPPH scavenging activity is shown in Fig. 3. The results showed a significant increase in antioxidant compounds after fortification due to the liberation of phenolic compounds as a result of antioxidant activity. The ascorbic acid content of dried tomato was significantly higher than that of fresh tomato. Tomatoes and tomato products are good sources of carotenoids (in particular, lycopene), ascorbic acid (vitamin C), vitamin E, folate, flavonoids and potassium [22]. Comparison of dhokla with and without tomato powder showed that phenol, flavonoid and vitamin C values were 2.34 mg/g, 0.2 mg/g and 8.26 mg/g, respectively, in product A, increased to 5.97 mg/g, 0.58 mg/g and 24.44 mg/g in product C, and were highest at 7.57 mg/g, 0.79 mg/g and 30.22 mg/g in product D. The correlation coefficients (R²) for phenol, flavonoid and vitamin C were above 0.9 at 0.912, 0.906 and 0.907, respectively, which was significant and so follows the first order equation.

Degradation kinetics
A colour degradation kinetics study was performed on stored dhokla. In Fig. 4, C and C0 are replaced by a and a0 and b and b0, respectively. Fig. 4 shows that redness was increased in samples B, C and D due to fortification with tomato powder because tomato contains a high amount of lycopene, carotene and tomatine, which are powerful reddish-yellow natural antioxidants [23]. Greater colour degradation was seen in sample A (without fortification) compared to the three other dhokla samples (with fortification) when they were stored at 4ºC. The correlation coefficient (R²) was 0.762 for sample A, 0.933 for sample C and 0.807 for sample D.
The degradation in colour values indicated that lightness decreased more than redness or yellowness. The decrease in the colour value ‘a’ might be due to carotenoid degradation and non-enzymatic browning with oxidative enzymes such as peroxidase and polyphenolase [17]. Fig. 4 shows that at 4°C, the yellowness of sample D degraded more than that of sample A even though it was fortified with 7% (the highest percentage) tomato powder. The correlation coefficient (R²) was 0.721 for sample D and 0.908 for sample A. The decrease in ‘a’ and ‘b’ values indicated less red and less yellow. Colour changes were affected by both storage time and temperature [24].

Nutritional analysis
Nutritional analysis is important for determining nutritional and calorific values. Table 1 shows that the carbohydrate and protein content of sample A was 19.32% and 8.75%, respectively, due to the presence of legumes and semolina. Legumes include beans, lentils, dried peas and soy and together with nuts and seeds are rich sources of protein [25]. Tomato contains 3.9 g carbohydrate and 0.9 g protein, so when dhokla was fortified with tomato powder the carbohydrate and protein content increased significantly. Brodowski and Geisman reported that tomato pomace is an excellent source of tocopherol (vitamin E) and contains 13% more lysine than soy protein, and so can be used to improve the protein quality of low lysine foods based on cereals [26]. As the ash and fat content of legumes and semolina is very low, their contribution is negligible. The minerals potassium and phosphate constitute about 8% of the dry matter content of tomatoes and affect pH and titratable acidity and thus the taste of tomatoes [27]. The crude protein and ash content of cookies containing 5.0%, 7.5% and 10.0% tomato powder was significantly higher than that of cookies without tomato powder fortification [8].

Sensory analysis
Measuring sensory parameters (colour, flavour, taste, texture and overall acceptability) is important to determine food characteristics and product acceptability. Sensory rating test scores for the taste, colour, appearance and acceptability of the different formulations are compared in Fig. 5. Samples had mean scores ranging from ‘neither like nor dislike’ (5.00) to ‘like very much’ (9.00). Products with high acceptance scores are more successful on the market compared with products with ‘neither like nor dislike’ ratings. Taking this into consideration in our study, assay number 1 shows the lowest value for all the analyses [28].
Colour affects consumer choice [29]. The sample containing 4% tomato powder (C) scored better on colour (8.86) than control sample A (7.8) and sample B containing 1% tomato powder (8.33). The colour score for sample D with 7% tomato powder was decreased at 8.375; the colour became darker as the tomato powder level increased, which was less acceptable. Tripathi and Nath observed that non-enzymatic browning of dried tomato slices may be due to the oxidation and polymerization of ascorbic acid, sugar caramelization and polymerization of lycopene [30]. The same result regarding colour was observed when up to 10% tomato powder was incorporated into cookies [8].

Taste is a very important organoleptic parameter. Tomato has an appetizing, refreshing and pleasing taste. Fig. 5 shows that samples containing tomato powder (B and C) had better scores than sample A. The taste score of sample D (7.8) was significantly lower due to a strong sour taste.

Flavour is very important for the consumer. Sample A scored 7 for flavour. After incorporation of 1% and 4% tomato powder, the flavour score rose to 8.11 and 8.22, while sample D had a decreased score of 7.63 due to a strong tomato smell. Texture is also an important property of food products. Sample A had a texture value of 7.8, which decreased to 7.54 when 1% tomato powder was added, but increased to 8.56 when 4% tomato powder was added, which was the highest texture score among all samples. In terms of overall acceptability, dhokla with 1% and 4% tomato powder was superior to dhokla with 7% tomato powder. The overall acceptability score of sample A was 7.3, while those of samples B and C were higher. Sample C was the best liked dhokla sample.

**Conclusion**

Legumes are inexpensive sources of protein with high nutritional value. The addition of 1%, 4% and 7% tomato powder had a significant impact on the physicochemical, nutritional and sensory properties of dhokla made with semolina and Bengal gram. Fortification also affected the colour of dhokla, with the amount of tomato powder being proportional to redness (‘a’ value). The correlation constant of degradation kinetics follows the first order equation. The incorporation of tomato powder increased the acidity of dhokla from 0.09 to 0.45, while the carbohydrate, protein and ash content increased from 19.32% to 24.33%, 8.75% to 30.51%, and 1.07% to 1.98%, respectively. Antioxidant activity was increased following tomato powder incorporation. Vitamin C, phenolic and flavonoid content increased from 8.26% to 30.22%, 2.34% to 7.57%, and 0.2% to 0.77%, respectively. The results of panel testing showed that dhokla with 4% tomato powder (sample C) had the best sensory, nutritional and physiochemical properties.

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**Conflict of interest**

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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