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ORIGINAL ARTICLE

Preparation of whey based savory beverage with enhanced bio-accessible zinc

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Abstract Zinc is an essential micronutrient for numerous catalytic, structural and regulatory functions in human body. However, its direct fortification in the food matrix poses the challenges of decreased bio-accessibility by forming insoluble sediments. Complexing zinc with polysaccharides has been reported as a possible intervention to address this issue by keeping the zinc in soluble form. Present investigation was undertaken to transform *paneer* whey containing complexed zinc into a sensorially acceptable beverage by varying its pH from 3.5 to 5.5, common salt concentration from 0.5 to 1.5% and spices concentration at 0.2 and 0.4%. Changes in complexed zinc concentration, apparent viscosity, instrumental color parameters and sensory attributes were determined. Complexed zinc concentration increased ($p<0.05$) with increasing pH, decreasing salt and increasing spices concentration. Whey beverage having 4.5 pH, 1.0% salt and 0.4% spices concentration was most preferred by the sensory panelists. In-vitro digestion of optimized whey beverage revealed that bio-accessibility of zinc was significantly higher ($p<0.05$) in complex form than free from.

Keywords Compexed zinc · *Paneer* whey · Whey beverage · In-vitro bio-accessibility

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Introduction

Zinc is an essential micronutrient for normal human health and development. However, about 17% of the global population and 30% population living in South Asian region are zinc deficient (Maxfield and Crane 2020). Emergence of covid-19 cases in the present scenario has further emphasized on the importance of zinc as an important micronutrient towards boosting immunity and fighting against infectious diseases (Skalny et al. 2020). This has increased the focus on zinc fortification in different food products. However, zinc fortification in the food products is rather challenging. Dong et al. (2018) reported that zinc fortification by the direct addition of zinc salts in the food products does not correspond to increased zinc bio-accessibility and even causes irritation in our gastrointestinal tract. Different strategies were explored to address this issue. Raya et al. (2016) explored zinc nanoparticles as an effective approach for increasing the intestinal absorption and bioavailability of zinc. The authors reported that feeding of zinc oxide nanoparticles supplemented biscuits to zinc deficient rats resulted into rapid increase in their body growth rate, appetite and hair growth, and attributed this to the ultra-small size of zinc nanoparticles as compared to the regular bulky zinc oxide. Lule et al. (2020) utilized human origin *Lactobacilli* strains (SR4 and LGG) for their ability to accumulate zinc in their biomass and deliver it in a highly bioavailable form. The authors reported that the zinc chelated with SR4 and LGG has a bioavailability of 57 and 48%, while that of inorganic ($ZnSO_4$) and organic (Zinc gluconate) forms of zinc had a bioavailability of 15.6 and 21.7%, respectively. Similarly, binding zinc with other nutrients have been reported to increase its bioavailability. Shilpashree et al. (2020) reported that binding zinc with whey proteins increased its bio-accessibility. Caetano-Silva et al. (2018) reported that iron complexed with whey protein hydrolysate had higher bio-accessibility as compared to free iron. Similarly, Dong et al. (2018) reported that binding zinc with *Athelia rolfsii* exopolysaccharide increased its availability in the animal model. On similar lines, zinc binding ability of different polysaccharides in whey was evaluated at our lab and it was found β-glucan exhibited highest zinc binding activity (Prasad et al. 2022). In order to effectively utilize this information for the food industry, the prepared zinc complex needs to be either isolated from whey and then added into a product or the whey (containing the zinc complex) could be transformed into a beverage which is acceptable to the consumers. Among these two, transformation into a beverage appears more suitable than the isolation of zinc complex, as it may include additional techniques which might add to the cost of the product.

Given the easy availability of whey, it has been explored by different workers for preparation of functional beverages (Chavan et al. 2015). Preparation of whey based beverage includes two important steps, viz., a thermal treatment so as to have microbiological safety and addition of flavoring compounds. Whey pH could range from 4.0 to 6.5, depending upon its origin. Whey obtained from the syneresis of fermented product (such as yoghurt) may have pH as low as 4.0, while the pH of whey obtained during rennet casein preparation may approach to a value near to that of milk. In addition, whey contains majority of lactose which was present in milk. Both of these imparts a mild sweet and sour taste to the whey, which needs to be modified to make to acceptable to the consumers. Among the different flavoring sources, spices are known for being rich source of flavoring compounds. They are commonly used for improving the flavor attribute during a beverage preparation. However, use of combination of spices is preferred over single spice so as to avoid the dominance of flavor of single spice and have a proper blend of taste (Prasad et al. 2018; Kumbhare et al. 2021; Badola et al. 2022). Also, Bag and Chattopadhyay (2015) reported about the synergistic activity of combination of herbs and spices against food borne pathogens and pro-oxidants.

Considering this, the present investigation was undertaken to transform the whey containing complexed zinc into an acceptable beverage by simultaneous variation in its pH, and addition of salt and a combination of spice mixture at different levels.

Materials and methods

Materials

Fresh *paneer* whey was produced by acid coagulation (using 1% citric acid solution) of pre-heated buffalo milk (96–98 °C/no hold) to pH 5.2 ± 0.2. Immediately after procuring the whey, it was centrifugally separated to remove fat. The whey had a pH of 5.31 ± 0.12 and had total solid, protein, lactose and ash content of 5.59 ± 0.05%, 0.76 ± 0.02%, 4.29 ± 0.04% and 0.59 ± 0.01%, respectively. Food grade β-glucan, extracted from the cell wall of *Saccharomyces cerevisiae,* was procured from West Coast Pharmaceutical Works (P) Ltd, Gujarat, India (batch no.: BFW 20030). Spices (black pepper and cumin) used in the present investigation were procured from local market of Karnal. All other chemicals, pepsin (CAS No. 9001-75-6) and trypsin (CAS No. 9002-07-7) used during the present work were procured from Sigma Aldrich, India.

Preparation of zinc complex in paneer whey

Freshly skimmed *paneer* whey (1L) was heated to 90 ± 1 °C for 5 min, followed by cooling to 50 °C and filtering through double layered muslin cloth. This was added with 10 g β-glucan and 131.93 mg of $ZnSO_4.7H_2O$ (corresponding to 30 mg/kg of zinc, as per the maximum limit set by the Food Safety and Standards Authority of India; File no. 11/03/Reg/Fortification/2014/pt.III dated 10th May 2018) at 50 ± 1 °C. The contents were subjected to continuous mixing using magnetic stirrer at 1100 rpm for 2 h for zinc complex formation.

Preparation of savory whey beverage

In order to prepare a whey beverage, the contents obtained after complex formation were cooled to 20 ± 1 °C and subjected to different conditions, viz., adjustment to different pH (3.5, 4.5 and 5.5) using 1 N citric acid or 1 N NaOH solution; addition of NaCl (0.5, 1.0 and 1.5%, weight basis) and spices combination (0.2 and 0.4%, weight basis), simultaneously (Fig. 1). The spices combination comprised of black pepper and cumin in the ratio of 1:1. For preparation of beverage samples, first spices were added into the whey followed by common salt addition and finally pH of the whey was adjusted using 1 N citric acid or NaOH solution. Full factorial design was used and a total of 18 combinations of whey beverages were obtained (Table 1), which were evaluated for different physico-chemical (complexed zinc concentration, instrumental color values and viscosity) and sensory attributes. The combination yielding highest sensory acceptability was considered optimum and compared with control whey beverage sample for in vitro bio-accessibility of zinc. Control whey beverage sample for in vitro bio-accessibility of zinc was prepared by

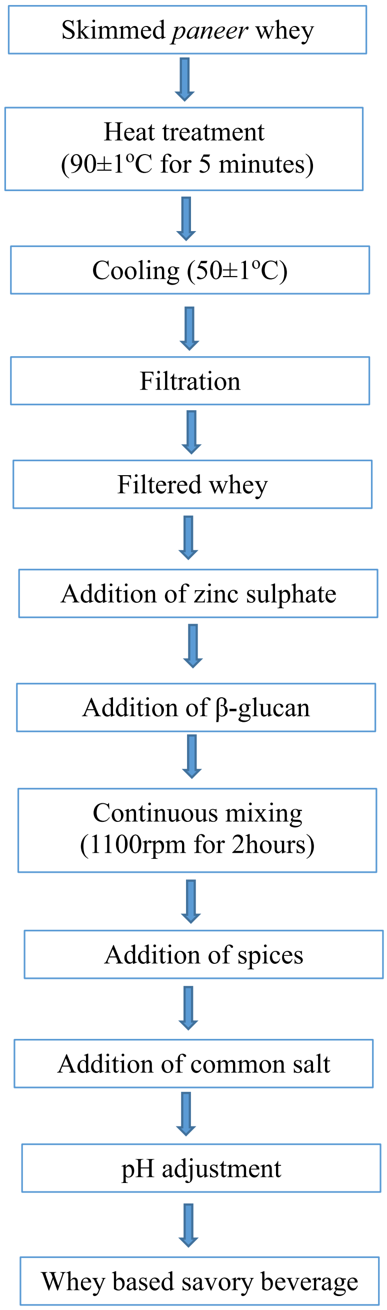

Fig. 1 Flow diagram for preparation of whey based savoy beverage containing complexed zinc

following the same protocol as that of optimized whey beverage, except that no polysaccharide was added during the complex formation step.

Analysis of experimental samples

Complexed zinc concentration

Complexed zinc content in the sample was determined by calculating the amount of zinc which was bound with macro-molecules, viz., polysaccharide and whey proteins, as per the method provided by Patel et al. (2021) with minor modifications. In brief, 15 mL of sample was first centrifuged at 5000 rpm for 20 min and the supernatant was filtered through Whatman filter paper (grade 1). The filtrate was transferred to Amicon ultrafiltration (UF) membrane centrifuge tubes (molecular cut-off: 10 kDa) and centrifuged at 5000 rpm at 20 °C for 30 min. A 10 mL of this permeate was taken in a crucible dish and kept in a muffle furnace (maintained at 550 ± 10 °C) for 6–8 h for ashing the contents. The ash obtained from muffle furnace was dissolved in 2 mL HCl (6 mol/L) and filtered through Whatman filter paper (grade 42). Residues were washed using 10–20 mL (1 M) HNO_3 solution and the volume was made up to 50 mL using double distilled water. This was used for determination of zinc concentration using atomic absorption spectroscope (AA-7000, Shimadzu, Tokyo, Japan) against a standard curve prepared using aqueous solution of zinc sulphate at different concentrations. For determination of 'total' zinc, the sample obtained after the filtration through Whatman filter paper (grade 1) was used. Results are expressed as % complexed zinc, which was calculated using the following formula:

$$\text{Complexed zinc content}\,(\%) = 100 * (\text{total zinc} - \text{free zinc})/\text{total zinc}$$

Apparent viscosity

Apparent viscosity of the sample was determined at 20 °C using rheometer (MCR 52, Anton Paar, Germany) attached to Cone plate CP75-1° (SS) assembly. About 1 mL of sample was placed on the rheometer plate (tempered at 20 °C) and uniformly spread over the plate and the sample flowing out from the plate was gently wiped off before starting the analysis. Viscosity of the sample was determined by maintaining a shear rate of 15 s^{-1} and the results are reported as mPas.

Instrumental color parameters

Instrumental color parameters were determined using Color Flex (M/s. Hunter Associates Laboratory, Inc., Reston VA, USA). Before measurement, the instrument was calibrated using black and white tile provided by the supplier and verified using a green tile with known color values. For determining the color values, the sample container was filled half by the sample and placed gently above the light source. It was them covered by a black coloured metallic container to avoid the interference from the surroundings. Readings were recorded at was daylight 65 mode to obtain the instrumental colour values.

Table 1 Effect of variation in matrix environment on physical attributes of whey beverage

Combination (#)	Whey matrix environment			Apparent viscosity[@] (mPas)	Instrumental color parameters		
	pH	Spices concentration (%, w/w)	Salt concentration (%, w/w)		L* value	a* value	b* value
1	3.5	0.2	0.5	3.35 ± 0.25	38.09 ± 0.18[a]	−4.51 ± 0.19[a]	11.39 ± 0.23[a]
2	3.5	0.2	1.0	3.33 ± 0.16	38.57 ± 0.24[a]	−4.62 ± 0.22[a]	11.43 ± 0.14[a]
3	3.5	0.2	1.5	3.31 ± 0.26	38.06 ± 0.21[a]	−4.46 ± 0.29[a]	11.15 ± 0.16[a]
4	3.5	0.4	0.5	3.39 ± 0.19	36.37 ± 0.19[b]	−1.89 ± 0.31[b]	8.06 ± 0.19[b]
5	3.5	0.4	1.0	3.40 ± 0.17	36.38 ± 0.16[b]	−2.16 ± 0.19[b]	8.04 ± 0.26[b]
6	3.5	0.4	1.5	3.35 ± 0.20	36.45 ± 0.28[b]	−1.85 ± 0.23[b]	8.79 ± 0.20[b]
7	4.5	0.2	0.5	3.36 ± 0.21	39.08 ± 0.16[c]	−5.25 ± 0.14[ac]	12.17 ± 0.21[c]
8	4.5	0.2	1.0	3.38 ± 0.17	39.94 ± 0.24[c]	−5.19 ± 0.25[ac]	12.25 ± 0.23[c]
9	4.5	0.2	1.5	3.36 ± 0.19	39.67 ± 0.21[c]	−4.99 ± 0.13[ac]	12.14 ± 0.29[c]
10	4.5	0.4	0.5	3.37 ± 0.05	37.87 ± 0.15[a]	−2.21 ± 0.20[b]	8.05 ± 0.35[b]
11	4.5	0.4	1.0	3.35 ± 0.11	38.03 ± 0.28[a]	−2.18 ± 0.14[b]	8.26 ± 0.18[b]
12	4.5	0.4	1.5	3.36 ± 0.05	38.15 ± 0.23[a]	−2.49 ± 0.27[b]	8.61 ± 0.26[b]
13	5.5	0.2	0.5	3.34 ± 0.07	39.45 ± 0.16[c]	−5.20 ± 0.20[c]	12.15 ± 0.33[c]
14	5.5	0.2	1.0	3.36 ± 0.08	39.93 ± 0.24[c]	−5.50 ± 0.15[c]	12.65 ± 0.26[c]
15	5.5	0.2	1.5	3.34 ± 0.06	39.69 ± 0.26[c]	−5.66 ± 0.16[c]	12.74 ± 0.18[c]
16	5.5	0.4	0.5	3.35 ± 0.15	37.83 ± 0.19[a]	−3.61 ± 0.26[d]	10.85 ± 0.32[ad]
17	5.5	0.4	1.0	3.34 ± 0.14	37.72 ± 0.22[a]	−3.52 ± 0.14[d]	10.36 ± 0.15[d]
18	5.5	0.4	1.5	3.39 ± 0.20	37.95 ± 0.16[a]	−3.51 ± 0.25[d]	10.59 ± 0.36[a]

@: Apparent viscosity at 15 s^{-1} shear rate

Values are mean ± SE (n = 3)

Values in the same column with different superscript (a, b, c, d) differ significantly ($p < 0.05$) from each other

In-vitro bioaccessiblity of zinc in whey beverages

For in vitro bio-accessibly of zinc in the beverage samples, method provided by Dong et al. (2018) was followed with minor modifications. In brief, 100 mL of sample was mixed with 1L simulated gastric fluid and digested for 2 h at 37 °C (rpm 80). Before mixing with the samples for gastric digestion, pH of gastric fluid was adjusted to 1.2 using 1 N HCl solution. After gastric digestion, pH of contents was adjusted to 6.8 using 1 N NaOH solution, followed by mixing with intestinal fluid and 10 g trypsin for intestinal digestion. Intestinal digestion was done for 3 h at 37 °C (rpm 80). After each digestion process, about 20 mL of miscible fluid was filtered through 0.45 µm membrane filter and used for determination of free zinc as per the previously mentioned method (Sect. 2.3.1). Gastric and intestinal fluid was prepared according to Dong et al. (2018).

Sensory evaluation of whey beverage

The nine-member sensory panel was constituted from the faculty and students of Dairy Technology Division, National Dairy Research Institute, Karnal, India. The sensory panelists were selected based upon their knowledge about the sensory attributes of whey based savory beverages. Freshly prepared beverage sample was transferred to high density poly-ethylene containers (100 mL capacity) and stored in a refrigerator (at 6 ± 2 °C) for overnight. At each testing session, samples were presented with random three-digit codes in the same containers to panel members seated in individual booths. The panellists were asked to shake the sample before evaluation. The samples were evaluated for flavour, body and texture, color and appearance, and overall acceptability using a 9-point hedonic scale, ranging from 'liked extremely' to 'disliked extremely'.

Statistical analysis

All the values obtained during the present investigation are presented as mean ± SEM. The data were statistically analyzed using one way analysis of variance (ANOVA) to evaluate the significant difference between them. Also, Tukey's comparison test was performed to group the significantly different results at 5% level of significance ($p < 0.05$) using SPSS software of M/s. IBM Corporation.

Results and discussion

Optimization of whey matrix for beverage preparation

The whey containing complexed zinc was subjected to different environment as provided in Sect. 2.3. The beverage obtained after these modifications were analysed for different attributes and the results obtained are discussed in this section.

Complexed zinc concentration

The whey sample obtained after the zinc complex formation (i.e., prior to beverage preparation) had 67.06% complexed zinc, which could be attributed to the presence of β-glucan and proteins in the whey. Gupta and Diwan (2017) reported that formation of metal complex by different polysaccharides could be attributed to the presence of free anionic functional groups in them (specifically carboxylic and hydroxyl groups). Similarly, Shilpashree et al. (2020) reported about the complex formation between whey proteins and zinc because of the presence of negatively charged species on whey proteins. Negative charge on the β-glucan could be attributed to the ionisable functional groups (hydroxyl and carboxyl) and non-carbohydrate constituents viz., phosphodiester (in techoic acid), and sulfhydral and carboxyl groups (in proteins) (Sandhya et al. 2018; Maheshwari et al. 2019; Meena et al. 2021a, b). In order to prepare a whey based beverage, the sample containing complexed zinc was subjected to different pH (3.5, 4.5 and 5.5), salt concentration (0.5, 1.0 and 1.5%) and spices concentration (0.2 and 0.4%) in different (#18) combinations. Among these, the combination (#16) having pH 5.5, salt concentration of 0.5% and spices concentration of 0.4% showed highest amount of complexed zinc (72.52%), whereas lowest amount of zinc complex (50.65%) was obtained at pH 3.5, 1.5% NaCl and 0.2% of spices combination (#3). All the studied factors significantly ($p < 0.05$) affected the amount of complexed zinc concentration in the beverage (Fig. 2). Increasing the pH and spice concentration increased the amount of complexed zinc concentration, while increasing salt concentration decreased the same. Increasing pH might have resulted into lesser number of competing H^+ ions with the anionic groups. Similarly, increasing the salt concentration resulted into more of Na^+ ions, which might be competing with the zinc ions for interaction with the anionic groups. The H^+ ions (at low pH) and Na^+ ions (at higher salt level) might be getting attached to the anionic groups present in the whey, thereby decreasing the amount of complexed zinc concentration. Cumin (Srinivasan 2018) and black pepper (Gorgani et al. 2017) are reported to contain terpenes, phenols, and flavonoids, which possess anionic groups. Increasing their

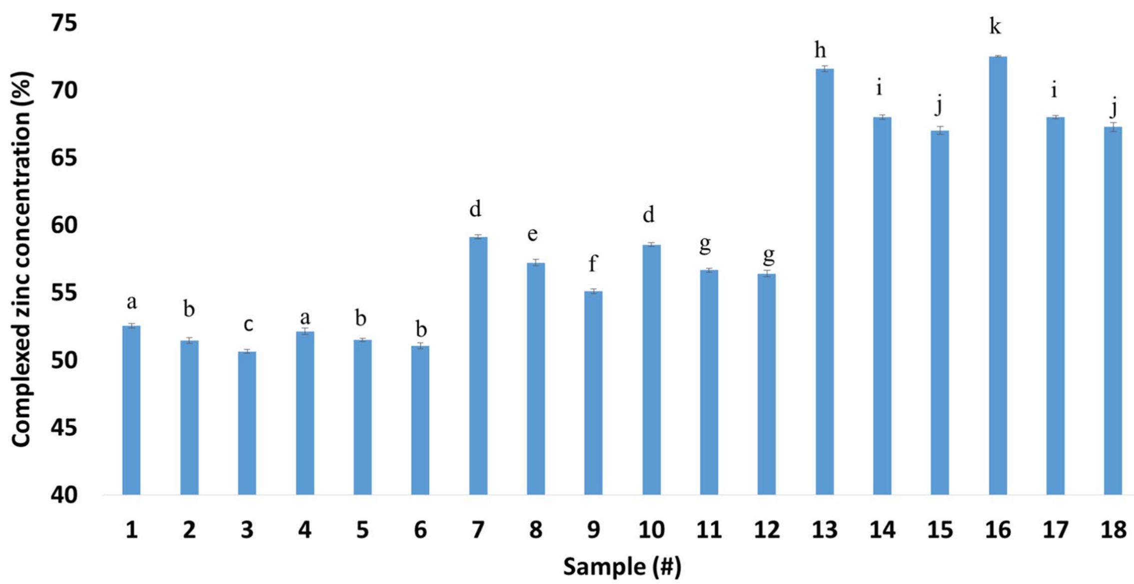

Fig. 2 Effect of variation in matrix environment on complexed zinc concentration in whey beverage. Values are mean ± SE (n = 3); Values in the same column with different superscript (a,b,c,d) differ significantly ($p < 0.05$) from each other. #1: pH 3.5, 0.2% Spices and 0.5% Salt; #2: pH 3.5, 0.2% Spices and 1.0% Salt; #3: pH 3.5, 0.2% Spices and 1.5% Salt; #4: pH 3.5, 0.4% Spices and 0.5% Salt; #5: pH 3.5, 0.4% Spices and 1.0% Salt; #6: pH 3.5, 0.4% Spices and 1.5% Salt; #7: pH 4.5, 0.2% Spices and 0.5% Salt; #8: pH 4.5, 0.2% Spices and 1.0% Salt; #9: pH 4.5, 0.2% Spices and 1.5% Salt; #10: pH 4.5, 0.4% Spices and 0.5% Salt; #11: pH 4.5, 0.4% Spices and 1.0% Salt; #12: pH 4.5, 0.4% Spices and 1.5% Salt; #13: pH 5.5, 0.2% Spices and 0.5% Salt; #14: pH 5.5, 0.2% Spices and 1.0% Salt; #15: pH 5.5, 0.2% Spices and 1.5% Salt; #16: pH 5.5, 0.4% Spices and 0.5% Salt; #17: pH 5.5, 0.4% Spices and 1.0% Salt; #18: pH 5.5, 0.4% Spices and 1.5% Salt

concentration resulted into more number of anionic groups in the matrix and hence higher amount of complexed zinc was obtained at higher spices concentration.

Apparent viscosity

Viscosity is an important physical attribute affecting the flow behaviour and mouthfeel of liquid products. This of particular importance in whey based beverages. Because of low total solids, whey tends to have lower viscosity as compared to other milk based beverages, like flavoured milk, tea, *lassi*, etc. In order to address this issue, whey beverages are often prepared by the addition of hydrocolloids in the form of fruit pulp or concentrate. This not only improves the flavour attribute, but also increases the viscosity and mouthfeel of the product. Apparent viscosity of the sample varied from 3.31 to 3.40 mPas (Table 1). It was observed that viscosity of all the whey beverage was higher than that from whey (1.44mPas at 15 s^{-1} shear rate). This could be attributed to the presence of β-glucan and spices in the sample. β-glucan comprises of glucose as the monomer unit, which are joined by different β linkages (viz., 1 → 3, 1 → 4 and 1 → 6). Presence of these linkages tends to impart hydrophilic character to β-glucan, which results into water binding and viscous nature of β-glucan solutions (Karlsson et al. 2018). Similarly, presence of spices would have increased the viscosity of whey because of presence of proteins and sugars because of their water binding activity. Merah et al. (2020) reported that cumin seeds contain about 20.9–24.7% protein (dry basis) and 8.9–13.2% soluble sugars (dry basis). Pradeep et al. (1993) reported that black pepper comprises of 13.25% protein (dry basis) and 67.75% carbohydrate (dry basis).

Comparison among the different beverage sample revealed no significant effect ($p > 0.05$) of all the studied parameters (pH, salt and spices) on the viscosity of samples (Table 1). The observed results are contrary to Faure et al. (2014), who reported about decreased viscosity of the micronutrient (iron) added β-glucan solution beacause of hydrolysis of β-glucan through Fanton reagents at 85 °C. The observed differences in the viscosity results could be attributed to the difference in the pH and other processing parameters (temperature) required for hydrolysis of β-glucan. Johansson et al. (2006) reported that at very low pH (1–2), complete hydrolysis of β-glucan occurred at high temperature (70 °C), while no hydrolysis was observed at ambient temperature (37 °C). This might be the possible reason for the obtained results in our study, as the whey sample was not subjected to any heat treatment after the formation of zinc complex. This was done as to avoid the detrimental effects of heat treatment on the bio-availability of fortified zinc. Jing et al. (2017) studied the effect of pressure-cooking and microwave heating on the zinc bioavailability in different cereals (rice, barley, buckwheat, wheat and oat) and pulses (chickpea and green gram). The authors reported that decrease in bio-availability of zinc was observed in all the processed cereals and pulses and attributed this to the formation of insoluble precipitates.

Instrumental color parameters

Color and appearance plays an important role in determining the acceptability of any product. Instrumental color values of the samples were evaluated so as to ascertain the changes occurring because of the variation in the whey matrix conditions. Highest lightness (L*) value (39.93 ± 0.24) was observed in the combination (#14) having 5.5 pH, 0.2% spices and 1.0% salt concentration, while the lowest L* value (36.37 ± 0.19) was observed in combination (#4) having 3.5 pH, 0.4% spices and 0.5% salt concentration. Highest greenness (− a*) value (− 5.66 ± 0.16) was observed in the combination (#15) having 5.5 pH, 0.2% spices and 1.5% salt concentration, while lowest greenness value (− 1.89 ± 0.31) was observed in combination (#4) having 3.5 pH, 0.4% spices and 0.5% salt concentration. Yellowness (b*) value was found to be highest (12.74 ± 0.18) in the combination (#15) having 5.5pH, 0.2% spices and 1.5% salt concentration and lowest (8.04 ± 0.26) in combination (#5) having 3.5 pH, 0.4% spices and 1.0% salt concentration. All the instrumental color parameters varied significantly ($p < 0.05$) with variation in the pH, salt and spices concentration in the beverage sample. It was observed that increasing the spices concentration resulted into significant ($p < 0.05$) decrease in the lightness (L*) and yellowness (b*) value, and increase in the redness (a*) of the beverage. On the other hand, lightness (L*) and yellowness (b*) value increased, and redness (a*) value decreased significantly ($p < 0.05$) with an increase in the pH of whey matrix (Table 1). Our results are in agreement with Wang et al. (2020), who reported that increasing the pH resulted into increased yellowness of whey proteins. Changes in the color parameters of whey beverage with the increase in the spices concentration could be attributed to the increased concentration of cuminal, safranal, p-cymene and β-pinene (from cumin) and piperine, piperidine and piperetine (from black pepper) (Shaikh et al. 2006; Sharma et al. 2016). Decreased lightness value of beverage at lower pH could be due to the changes occurring in the spices under acidic pH. Suresh et al. (2007) reported that decreasing the pH resulted into the loss of piperine from 55.8 to 50.2 mg/g black pepper. Similarly, Srinivasan et al. (1992) reported 13–17% and 49–54% loss of piperine at pH 6.1and 5.1, respectively.

Sensory attributes

The aim of this activity was to transform a whey matrix containing complexed zinc into a sensorially acceptable beverage. Sensory attributes are important for determining the consumer acceptability of the product. These attributes could broadly be classified into flavor, color and appearance, and body and texture attribute. Flavor attribute indicates about the taste and aroma of the product, color and appearance indicates about the visual perception, and body and texture indicates about the physical sensation of the product in our mouth. Sensory attributes of the beverages were determined using a panelist having prior experience about the whey based savory beverage and the results are presented in Table 2.

Lowest flavor score (5.01 ± 0.10) was obtained for the sample (#6) having 3.5 pH, 0.4% spices and 1.5% salt concentration, while highest flavor score (7.05 ± 0.11) was obtained for the sample (#11) having 4.5 pH, 0.4% spices and 1.0% salt concentration. Flavor score of the beverage varied significantly ($p < 0.05$) with the variation in pH, and with the addition of salt and spices in whey. It was observed that increasing the pH from 3.5 to 4.5 increased the flavour score of whey beverage, beyond which (from pH 4.5 to 5.5) the scores decreased (Table 2). Further, effect of salt level on the flavour attribute was perceived at pH ≥ 4.5. Increasing the spice level increased the flavour score and higher flavour scores were obtained at 0.4% level.

Highest color and appearance score (7.72 ± 0.10) was obtained for the sample (#15) having 5.5 pH, 0.2% spices and 1.5% salt concentration, while lowest color and appearance score (7.21 ± 0.08) was obtained for the sample (#7) having 4.5 pH, 0.2% spices and 0.5% salt concentration. Although, significant ($p < 0.05$) changes in the hunter color parameters was obtained for different whey beverage samples, but no such significant effect ($p > 0.05$) was observed for color and appearance attribute. Visual images of the whey beverage are presented in Supplementary File 1.

Highest body and texture score (7.69 ± 0.24) was obtained for the sample (#6) having 3.5 pH, 0.4% spices and 1.5% salt concentration, while lowest body and texture score (7.24 ± 0.16) was obtained for the sample (#5) having 3.5 pH, 0.4% spices and 1.0% salt concentration. The changes in body and texture score was found to be significantly not different ($p < 0.05$) from each other. This is in agreement with the results obtained from the viscosity measurement of whey beverages.

Lowest overall acceptability score (5.15 ± 0.16) was obtained for the sample (#5) having 3.5 pH, 0.4% spices and 1.0% salt concentration, while highest overall acceptability score (7.11 ± 0.10) was obtained for the sample (#11) having 4.5 pH, 0.4% spices and 1.0% salt concentration. Similar to flavour scores, overall acceptability scores of the whey beverage samples varied significantly ($p < 0.05$) with the variation in whey matrix conditions.

Table 2 Effect of variation in matrix environment on sensory attributes of whey beverage

Combination (#)	Whey matrix environment			Sensory attributes (scores)			
	pH	Spices concentration (%, w/w)	Salt concentration (%, w/w)	Flavour	Color & appearance	Body & texture	Overall acceptability
1	3.5	0.2	0.5	5.59 ± 0.15^{a}	7.51 ± 0.10	7.39 ± 0.11	5.80 ± 0.18^{a}
2	3.5	0.2	1.0	5.61 ± 0.11^{a}	7.62 ± 0.14	7.43 ± 0.14	5.84 ± 0.14^{a}
3	3.5	0.2	1.5	5.36 ± 0.14^{a}	7.46 ± 0.19	7.35 ± 0.16	5.45 ± 0.22^{ab}
4	3.5	0.4	0.5	5.07 ± 0.19^{b}	7.69 ± 0.14	7.46 ± 0.13	5.31 ± 0.19^{b}
5	3.5	0.4	1.0	5.05 ± 0.16^{b}	7.26 ± 0.10	7.24 ± 0.16	5.15 ± 0.16^{b}
6	3.5	0.4	1.5	5.01 ± 0.10^{b}	7.49 ± 0.13	7.69 ± 0.24	5.18 ± 0.26^{b}
7	4.5	0.2	0.5	6.08 ± 0.20^{c}	7.21 ± 0.08	7.47 ± 0.21	6.23 ± 0.15^{ac}
8	4.5	0.2	1.0	6.54 ± 0.21^{c}	7.25 ± 0.11	7.35 ± 0.23	6.45 ± 0.24^{c}
9	4.5	0.2	1.5	6.27 ± 0.14^{d}	7.36 ± 0.07	7.34 ± 0.19	6.80 ± 0.20^{c}
10	4.5	0.4	0.5	6.57 ± 0.18^{cd}	7.45 ± 0.09	7.35 ± 0.15	6.65 ± 0.18^{c}
11	4.5	0.4	1.0	7.05 ± 0.11^{de}	7.51 ± 0.14	7.26 ± 0.16	7.11 ± 0.10^{d}
12	4.5	0.4	1.5	6.93 ± 0.15^{e}	7.29 ± 0.17	7.61 ± 0.16	7.04 ± 0.14^{cd}
13	5.5	0.2	0.5	6.08 ± 0.22^{c}	7.46 ± 0.16	7.45 ± 0.03	6.22 ± 0.16^{ac}
14	5.5	0.2	1.0	6.34 ± 0.21^{c}	7.56 ± 0.15	7.65 ± 0.06	6.41 ± 0.20^{c}
15	5.5	0.2	1.5	6.27 ± 0.16^{c}	7.72 ± 0.10	7.64 ± 0.02	6.50 ± 0.17^{c}
16	5.5	0.4	0.5	6.50 ± 0.13^{cd}	7.61 ± 0.06	7.45 ± 0.12	6.55 ± 0.23^{c}
17	5.5	0.4	1.0	6.93 ± 0.10^{d}	7.56 ± 0.14	7.36 ± 0.05	6.72 ± 0.11^{c}
18	5.5	0.4	1.5	6.72 ± 0.15^{d}	7.51 ± 0.11	7.59 ± 0.16	6.84 ± 0.26^{c}

Values are mean ± SE (n = 3)

Values in the same column with different superscript (a, b, c, d) differ significantly ($p < 0.05$) from each other

Table 3 In vitro bio-accessibility of zinc in control and experimental whey beverage

Sample	Digestion stage (% complexed zinc)	
	Gastric digestion	Intestinal
Control whey beverage	7.90 ± 0.26^a	6.84 ± 0.22^a
Experimental whey beverage	43.53 ± 0.31^b	36.44 ± 0.18^b

Values are mean ± SE (n = 3)

Values in the same column with different superscript (a, b) differ significantly ($p < 0.05$) from each other

During the optimization of whey matrix environment, it was found that the complexed zinc content decreased with a decrease in pH and increase in the salt concentration, but highest sensory scores were obtained at pH 4.5 and 1.0% salt concentration. Given the fact that the beverage should be acceptable to the consumers, hence the combination having highest sensory acceptability *i.e.,* 4.5 pH, 1.0% salt and 0.4% spices concentration (combination #11) was considered optimum for preparation of whey beverage containing complexed zinc.

In vitro bio-accessibility of complexed zinc

The prime objective of this work was to prepare a whey beverage containing higher amount of bio-accessible zinc. In order to evaluate the bio-accessibility of zinc present in the developed beverage, it was exposed to simulated digestive conditions and compared with the control beverage (containing only zinc but no β-glucan). The results are presented in Table 3. Total amount of zinc present in the optimized beverage was 26.40 mg/kg. After complete digestion, the amount of complexed zinc was found to be 6.84 and 36.44% in control and experimental beverage, respectively. This indicated that presence of β-glucan significantly ($p < 0.05$) improved the retention of zinc in complexed form and increased its bio-accessibility. In a study to explore β-glucan (isolated from *Saccharomyces cerevisiae*) as a potential delivery vehicle for drugs and nutraceuticals, Xie et al. (2016) reported that β-glucan was resistant to digestion against gastric environment and majority (> 70%) of β-glucan bio-absorption occurred at ileum, through the specialized epithelial cells (M cells). Our results are in agreement with Dong et al. (2018), who studied the bio-accessibility of zinc complexed with *Athelia rolfsii* exopolysaccharides and reported that 45.37% of total zinc was present in complexed form after in vitro digestion. Similarly, Javvadi et al. (2018) reported that iron complexed with β-glucan decreased the toxicity induced by excessive iron and improved growth under iron deficient environment. In addition, presence of some amount zinc in complexed form (6.84%) in the control beverage could be attributed to the whey proteins and indicated that whey alone could also serve as matrix for zinc supplementation.

Conclusion

Whey containing complexed zinc was optimized for savory beverage preparation by varying its pH and addition of salt and combination of spices mixture. It was observed that the amount of complexed zinc concentration was negatively affected by the decrease in pH and increase in the salt concentration. However, increasing the spices concentration increased the complexed zinc concentration. On the other hand, sensory attributes of whey beverage were higher at pH 4.5, 1.0% salt and 0.4% spices concentration. Spices and pH variation also resulted into changes in the instrumental color attributes of the whey beverage. Optimized beverage containing complexed zinc had around six times more bio-accessible zinc as compared to the control. The study may find its application in dairy industries by paving the way for whey utilization in the form of micro-nutrient (Zn) fortified beverages.

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Authors' contribution VS and ADW were involved in formal analysis and data acquisition. WP was involved in conceptualization and writing original draft. KK was involved in supervision and resource allocation; SAH was involved in editing the original draft of MS.

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Availability of data and material Studies relating to the present study and mentioned in this MS was derived from public domain resources.

Code availability Not applicable.

Declarations

Conflict of interest The authors have no conflict of interest to disclose with regard to the present work.

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