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Research article

Improving physicochemical, rheometry and sensory attributes of fortified beverages using jujube alcoholic/aqueous extract loaded Gellan-Protein macrocarriers

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ABSTRACT

The use of phenolic bioactive substances in beverages is introduced by novel techniques as a functional food product. Gel beads from jujube extract were prepared by extrusion method using encapsulation and coated by whey protein isolate and soy protein isolate and thus, a functional beverage was prepared from these beads. There were three types of beads, including Gellan, Gellan/whey protein isolate and Gellan/soy protein isolate. The pH, acidity, Brix, turbidity, viscosity and sensory properties were evaluated. Observing the increase in pH is the result of the release of small amounts of fruit extract, the effect of which can be seen in the inverse relationship of acidity next to pH. The results demonstrate that the highest viscosity is related to protein beverages, especially Gellan gum/SPI beads' beverage. Hence, the highest turbidity in Gellan gum/SPI beads' beverage was visible on the 14th day (66.6 NTU). Thereby, there is potential for these Gellan beads beverages with suitable sensory scores to be wholly utilized and developed with the aim of this study. Along with it, this new beverage can attract the opinion of a wide range of consumers. Therewith, the industrialization of such types of products helps to improve the consumer market.

1. Introduction

Antioxidant and phenolic compounds need to be protected against environmental factors, for which delivery systems have been proposed as a solution [1]. Biopolymers or proteins in these systems protect these bioactive compounds as walls. Jujube fruit (Ziziphus Spp.) is also preferred since it has a high potential of phenolics [2–4]. Preparation of beads in macro form is a significant solution for the protection of plant extracts, based on which new products can be produced [5].

Gellan gum (GG) is an anionic (linear) polysaccharide and is biocompatible, hydrophilic and biodegradable. It is obtained from Pseudomonas elodea and is made from D-glucose, D-glucose, D-glucuronic acid and L-rhamnose units [6,7]. Polysaccharides such as

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carrageenan, alginate and gellan can fabricate strong gels under slow cooling [8]. Whey protein is one of the functional and animal proteins that is a by-product in cheese production. This protein contains compounds including minerals, milk fat, lactose, etc., and causes gelling properties [9–11]. Soy proteins (SP) can be used together with hydrocolloids as a carrier to enhance the delivery of bioactive substances due to their biodegradability, high availability, non-toxicity, and thermal stability.

Also, this protein causes water retention and emulsifying properties. In addition, some research showed that combining it with polysaccharides can increase the water-holding capacity of soy protein. However, the use of soy proteins in the coating is still limited and using them together with gellan is a new coating [12]. Among the foodstuffs, drinks occupy an important part in the markets, which can be provided to the consumer in an enriched form (phenolic extracts, plant and animal proteins, etc.). Enriching beverages with protein has always been of interest to athletes and some general consumers [13,14]. According to the stated cases, trying to make different functional beverages with the aim of providing nutritional value, attractiveness for the consumer, proper functional and sensory characteristics is a priority.

According to the studies of others, research has been carried out in the field of enrichment of products with bioactive compounds, including beverages, as well as in the presence of proteins and sometimes hydrocolloids. Khoshdouni Farahani et al. [15] in their research, prepared beads from other hydrocolloids and proteins, and they were used in beverages to check their properties. In some studies, the value of plant proteins in functional foods has been mentioned [16] or permeate (containing kumquat) in whey beverages [9]. The impact of Persian gum on the quantitative characteristics of kefir drink by Beirami-Serizkani et al. [17] represents a change in viscosity.

Hydrogels were used to protect some polyphenol-rich materials (in which the curcumin solution in the oil is the dispersed phase and the gellan aqueous solution is the continuous phase) to prepare seeds that contain curcumin [18]. Research has proven that gellan beads significantly help delay the release of encapsulated substances [19]. As well as, alginate-soy protein hydrogels were prepared to evaluate the survival of probiotics in mango juice and the results showed the survival of probiotic cells in them well [20]. The value of phenolic compounds and the provision of their storage conditions in capsules or beads through their use in various products, such as beverages, can lead to improving their efficiency. The aim of the present research is to produce a new functional beverage with Gellan beads along with proteins (whey and soy proteins) which are enriched with jujube extract and its effect on physicochemical properties, viscosity and sensory properties.

2. Material and methods

2.1. Materials

Gellan gum (molecular weight = 500000 Da) and calcium chloride dehydrate (CaCl2.2H2O) were purchased from CP Kelco, Atlanta, GA (USA) and Merck Co., Darmstadt (Germany), respectively. Whey protein isolate (95.6 % protein) and soy protein isolate (90 % protein) were purchased from Davisco Foods Intl., Inc., Eden Prairie, MN, USA and Shandong Yuxin BioTech Co., Ltd (non-GMO), China, respectively. Jujube fruits are supplied by a local market of medicinal plants (Bojnourd, khorasanrazavi, Iran). Also, ethanol was from Merck (Darmstadt, Germany) and all reagents were analytical grade.

2.2. Extract of jujube fruit

2.2.1. Alcoholic extract preparation

Drying the fresh jujube led to obtaining Jujube fruit powder (room temperature) and it subsequently dried in an oven (40 °C) [21]. Ethanol (80 %) was added to Jujube powder and shook at 37 °C. Finally, the extract was separated from the plant by centrifugation (Heidolph, Laborota 4003, Germany) [3,21,22].

2.2.2. Aqueous extract preparation

A mixture of pre-jujube pulp was prepared and shaken (180 rpm). Then the resulting extract was centrifuged and separated (Heidolph, Laborota 4003, Germany) [23]. In the last step, the two extracts were mixed and stored in the refrigerator [24].

2.3. Preparation of three types of Gellan beads

Gellan gum solution (2 %) was made by deionized water and an aqueous solution of WPI (20 %) and SPI (8 %) were heated at 40 °C and continued until 78 °C to denature and then the solutions were cooled. Final polymer solutions were made from Gellan gum solution with WPI (5:1) and SPI (3.5:1) solutions, as a separate and complex solution to form beads. Then, Jujube extract (1:4) was added to biopolymer and protein solutions. To form beads, a syringe pump was used to drop these solutions into an ionic solution (calcium chloride) [25,26].

2.4. Preparation of Gellan beads beverages

Sensory properties and viscosity and also, physicochemical characteristics (pH, brix, acidity and turbidity) of beverages containing beads during storage were checked out. The beverage produced by sugar (110 g/l) (Merk, Germany), stabilizer (0.3 %) (Merk, Germany), citric acid (1 g/l) (Merk, Germany) and benzoate (100–150 ppm) (Merk, Germany). This study had three samples: beverage with Gellan gum + jujube extract, beverage with Gellan gum/WPI + jujube extract, beverage with Gellan gum/SPI + jujube extract.

The flavor and color were used according to favorites. The produced beverages were kept in glassy bottles for 14 days in the refrigerator and the evaluations were done on one, seven and fourteen days.

2.5. pH measurement

The pH of the beverages was analyzed by a digital pH meter (Heidolph, Germany) which was calibrated with buffers (4 and 7) [27].

2.6. The acidity measurements of beverages

In an erlenmeyer flask, 20 g of fruit juice was mixed with 250 ml of distilled water. 0.1 N sodium hydroxide solution was used to create a stable pale pink color by phenolphthalein reagent [27,28]. The titratable acidity was announced as g citric acid/100 g sample (equation (1)):

$$A = V \times 0.064 \times 100/m \quad (1)$$

where, V consumption volume of sodium hydroxide (0.1 N) in the milliliters specimen, A was titratable acidity (g citric acid/100 g) of specimen and m was weight of specimen (grams).

2.7. Measurement of water-soluble solids (Brix)

Some drops of the beverages were placed on the Japan refractometer prism (ATAGO) and to read the concentration of water-soluble solids at 20 °C. Brix is displayed automatically and after each sample, the prism of the device is washed [15].

2.8. Turbidity measurement of beverages

The turbidity of beverages was evaluated by a Turbidimeter (2100AN Laboratory Turbidimeter, Hach, USA) and was calculated on NTU units. The beverage is filled in special vials of the device up to the mark line, which is accompanied by the passage of the light of the device through the glass vial [29].

2.9. Viscosity measurement

A Brookfield viscometer (DV-II$^+$-RV, Brookfield Engineering Laboratories, INC., USA) by spindle No.2 (speeds 60 and 100 rpm) was used to investigate apparent viscosity of beverages. In this way, the beverage is placed in a beaker under the spindle of the viscometer and the parameters of the device are adjusted and then run [29,30].

2.10. Sensory evaluation

Sensory characteristics of the beverages (color, aroma, taste, texture and overall acceptance) were checked using a 5-point hedonic scale and ten trained evaluators were used to evaluate the samples [31].

2.11. Statistical analysis

SPSS 26 (SPSS Inc., Chicago, IL, USA) statistics software was used for statistical analysis of the result of three repetitions with a significant difference of $P < 0.05$ and Duncan's multiple range tests were carried out for mean analysis (mean ± SD) [32].

3. Results and discussion

3.1. The pH and acidity changes over time

The results of measuring changes in pH and acidity of gellan beads' beverages are illustrated in Tables 1 and 2. An increasing trend was found in the pH of all samples during time, although there was a slight decrease on the 7th day, and then this increase was seen. A

Table 1
The pH of Gellan beads' beverages.

Treatments / Days	Gellan gum beads' beverage	Gellan gum/WPI beads' beverage	Gellan gum/SPI beads' beverage
1st day	3.45 ± 0.01^{cd}	3.80 ± 0.05^{a}	3.72 ± 0.02^{b}
7th day	3.40 ± 0.02^{d}	3.76 ± 0.04^{ab}	3.70 ± 0.07^{b}
14th day	3.49 ± 0.01^{c}	3.81 ± 0.03^{a}	3.82 ± 0.02^{a}

Values are mean ± SD from triplicate determinations; Different letters show the statistical significant differences ($P < 0.05$).

significant difference between the Gellan beads' beverages was visible (on 1st day). The pH at maximum content was in the Gellan gum/SPI beads' beverage (3.82) and the lowest in the Gellan gum beads' beverage was 3.49 on the fourteenth day and it was seen a significant difference between Gellan beads' beverages. These sequential numbers were 3.80 (Gellan gum/WPI beads' beverage) and 3.45 (Gellan gum beads' beverage) on the first day. As Table 2 depicts, the trend of acidity in all Gellan beads' beverage is decreasing and a significant difference can be seen between most samples in maintenance. The highest level of acidity was observed in Gellan beads' beverage (1.280 g/one hundred grams of citric acid) and the lowest level of acidity was visible in Gellan gum/SPI beads' beverage at approx 1.216 g/one hundred grams of citric acid. Since the results were close to each other, there was only sometimes a significant difference between the acidity results of each sample on days 1st, 7th and 14th.

Citric acid, as a formulation ingredient, has an effect on the walls of the beads and causes partial separation of wall proteins from the beads (whey and soy proteins) and also causes the partial release of jujube extract to the medium. The mentioned cases can indicate an increase in pH and a slight decrease in acidity. This can be explained in this way that fruit extracts can cause changes in the pH and acidity of the medium and another factor of these changes is related to the effect of protein in the Gellan beads' beverage. In the achievements of Aamer et al. [9], it is mentioned that the use of the paste and the accompanying puree can affect the pH range of such functional beverages. In confirmation of this data, it is stated that the changes in the acidity content are also seen in Yoon, Woodams, & Hang [33], analysis.

From the data of the number of researchers, it has also been acknowledged that gums can make changes in the medium of beverages by increasing the pH and then reducing the acidity [34]. To sum up, the acidity level of the gellan beads beverage was higher than the hydrocolloid (alginate) beads beverage of Khoshdouni Farahani et al. [29]'s research, which can be illustrated that more release of extract from gellan beads occurred.

3.2. The changes in water-soluble solid content (Brix) during the storage period

The final outcomes of the assays for Brix are shown in Table 3. The degree of brix indicates the gram of soluble in one hundred grams of a solution. So, the brix enhanced slightly during time, which was only on the 7th day, and then, the trend was constant. The type of coating on the walls causes changes in the speed of material diffusion. Its impressment can be observed on the 7th day in all the beverage products, however in small amounts.

Overall, the Brix level of all samples was 10.8 on the first day, and this level reached 11 with a slight increase on the 7th and 14th day, which was the same in all of them. Statistical results have reported this difference as significant. Aamer et al. [9] stated in their survey that constant values of Brix of beverages can be observed over storage. Karami et al. [35] also stated that Brix can have a positive impact on changes in viscosity and also improve the consistency of drinking. In Khoshdouni Farahani et al. [15]'s study, it was also stated that a slight increase in brix occurs over time, which is in agreement with the present work.

3.3. Turbidity assessment

The turbidity content (NTU) of Gellan gum beads' beverages was identified and noted in Table 4, in such a way that an increasing trend in the amount of turbidity in the samples was visible. The content of the turbidity in the maximum mode was in Gellan gum/SPI beads' beverage (66.6 NTU) and its lowest state was found in Gellan gum beads' beverage (22.2 NTU) (on the fourteenth day). The highest and lowest levels of turbidity on the first day were also the same, and contents of 47.5 and 12.7 NTU were announced for them, respectively.

Assumptions state that the partial diffusion of the extract core and its wall to the surrounding medium can be done in the long term and a similar trend has been exhibited on all days. Gellan beads have less turbidity in the beverage due to their transparency, while gellan complex beads with protein due to their cloudy color, which is white and milky and caused by the structure, allow less light to transmission through the apparatus and therefore display more turbidity. From one side, the impact of partial release of hydrocolloid and protein in the medium, the size, color and number of double helix community and the trapping of free particles in the network can be seen in the turbidity. Furthermore, observing the increase in intermolecular bonds between the particles in the medium (gum, water, protein and free compounds) and following the trapping of these substances in the gel network, provides more turbidity for the protein-rich beads beverage [36]. Finally, these important factors that have the ability to change the turbidity in drinks (such as protein/gum) have been noted and identified in other studies [37].

Table 2
The acidity of Gellan beads' beverages (gr per 100 gr of citric acid).

Treatments / Days	Gellan gum beads' beverage	Gellan gum/WPI beads' beverage	Gellan gum/SPI beads' beverage
1st day	1.280 ± 0.01^{a}	1.248 ± 0.01^{ab}	1.216 ± 0.01^{abc}
7th day	1.225 ± 0.01^{abc}	1.20 ± 0.05^{bcd}	1.177 ± 0.02^{bcd}
14th day	1.20 ± 0.10^{bcd}	1.155 ± 0.01^{cd}	1.126 ± 0.02^{d}

Values are mean ± SD from triplicate determinations; Different letters show the statistical significant differences ($P < 0.05$).

Table 3
The brix of Gellan beads' beverages (21 °C).

Treatments / Days	Gellan gum beads' beverage	Gellan gum/WPI beads' beverage	Gellan gum/SPI beads' beverage
1st day	10.8 ± 0.02[b]	10.8 ± 0.06[b]	10.8 ± 0.03[b]
7th day	11 ± 0.05[a]	11 ± 0.03[a]	11 ± 0.02[a]
14th day	11 ± 0.04[a]	11 ± 0.01[a]	11 ± 0.06[a]

Values are mean ± SD from triplicate determinations; Different letters show the statistical significant differences ($P < 0.05$).

Table 4
The turbidity of Gellan beads' beverages (NTU).

Treatments / Days	Gellan gum beads' beverage	Gellan gum/WPI beads' beverage	Gellan gum/SPI beads' beverage
1st day	12.7 ± 0.05[j]	15.4 ± 0.15[h]	47.5 ± 0.12[c]
7th day	16.3 ± 0.05[g]	19.2 ± 0.08[f]	58.6 ± 0.04[b]
14th day	22.2 ± 0.10[e]	24.9 ± 0.05[d]	66.6 ± 0.14[a]

Values are mean ± SD from triplicate determinations; Different letters show the statistical significant differences ($P < 0.05$).

3.4. The effect of Gellan beads type beverages on viscosity

Viscosity results in Table 5 show that different gellan beads in beverages are effective in changes in flow behavior and cause significant differences between samples ($P < 0.05$). In terms of content, the highest amount of viscosity is found in Gellan gum/SPI beads' beverage (256 cp) and the lowest viscosity was found in Gellan gum beads' beverage (120 cp) (rotation: 10 rpm). Our studies have acknowledged that Gellan gum/SPI beads' beverage represents a higher viscosity than Gellan gum/WPI beads' beverage and there is a direct correlation between protein beads beverages with higher viscosity. Certainly, the nature of the beads, the concentration of their compounds and the ratio of their use have an impact on the interaction of these materials with the medium, and the possibility of their precipitation can also be seen in the process of viscosity changes. Gellan polysaccharide has the possibility of creating gelling properties and subsequently high consistency of the medium in the form of texturing, which is more visible if proteins are used (gelling properties) [8,28].

Besides, it was mentioned by Plancken et al. [38] that such features are due to the effect of hydrophobic groups of protein compounds. It seems that the composition of the types of gelan beads is such that protein-containing beads, especially soy protein, have more liberation in the medium and lead to an increase in viscosity. Also, since they show heavier beads than non-protein types, therefore, they have a greater impact on changes are evident.

According to previous studies, protein content is one of the factors that has a significant effect on viscosity in beverages [39]. Based on the mentioned formulas, the whey protein in this project has a higher concentration than soy protein, which makes their beads more stable, and therefore, they release fewer substances into the medium, and its effect will be less on viscosity. In the review by Prakash et al. [40] and Vahid-moghadam et al. [41], it is mentioned that the case of using all kinds of hydrocolloids and proteins can change viscosity. As well as, using heating in protein beverages is one of the effective factors in viscosity [42]. Regarding the networking of some gums, it should be stated that the formation of a network and the creation of a stronger structure causes an increase in viscosity. Gums can increase the resistance of the specimen against viscosity by the mechanism of increasing water binding capacity [43]. The results of Table 5 clearly present that there is an indirect relationship between rotation and viscosity and in the majority of the results of different cycles of 10, 30, 60 and 100 rpm, a significant difference can be observed. Making changes in the direction of opening some bonds and directing the number of effective units in the structure is one of the reasons for changes in viscosity. A mechanism for reducing the viscosity is considered in such a way that the increasing effect of the shear rate creates changes in the hydrodynamic forces of the masses and then the molecular network bands in its structure (gums) lead to breakage and finally slow degradation is seen in the samples [44]. Due to the fact that some gums are hydrocolloids with the ability to absorb ions, they have the possibility of binding to protein surfaces (charged) and the interaction between them causes an increase in viscosity [45]. It has also been noted in the opinions of others that the presence of polysaccharides/hydrocolloids in a variety of acidic/fermented beverages increases the

Table 5
The impact of beverages containing Gellan beads on viscosity (cp).

Treatments			
Rotational speed- Time	Gellan gum beads' beverage	Gellan gum/WPI beads' beverage	Gellan gum/SPI beads' beverage
10 rpm	120 ± 0.50[d]	196 ± 0.40[b]	256 ± 0.40[a]
30 rpm	60 ± 0.90[gh]	101.3 ± 0.60[e]	136 ± 0.40[c]
60 rpm	42.5 ± 0.30[j]	70 ± 0.09[fg]	91.3 ± 0.50[e]
100 rpm	40 ± 0.70[j]	58 ± 0.70[h]	76.4 ± 0.40[f]

Values are mean ± SD from triplicate determinations; Different letters show the statistical significant differences ($P < 0.05$).

apparent viscosity.

3.5. Sensory assessment

The results of the assays for sensory assessment of types of gellan beads in beverages are shown in Fig. 1. The assessment of the appearance, color, texture and aroma grades in Gellan beads beverage was implemented by a 5-point hedonic. Trained panelists (10 persons) examined the above-mentioned features. The highest taste score is particularly considered in Gellan gum and Gellan gum/WPI beads' beverage rather than others. In terms of significance ($p < 0.05$), no difference was considered between color, appearance, aroma and texture and it was visible only in the taste and final acceptance score. The difference expressed between Gellan gum and Gellan gum/WPI beads' beverages compared to Gellan gum/SPI beads' beverage was exhibited.

This outcomes were in good agreement with the findings stated in the literature [15]. Based on current results which are in agreement with Aamer et al. [9], greater admissibility in the sensory attributes of functional drinks with the presence of puree/paste along with whey protein leads to an increase in overall acceptability, which Castro et al. [46] also confirmed in his work. There have previously been reportages that using gum (tragacanth) has a positive impact on the taste of drinks like, doogh [47], as well as being widely used in the food industry, since no significant difference was seen between sensory measurements of samples. Another research noted that the presence of Arabic gum in functional yogurt causes to obtain highest acceptability [48]. It has been shown by Akin and Ozcan [49], that the presence of rice/chicken protein in the beverages makes the aroma and appearance attractive. It is also noted that the acceptable smell and taste can be smelled in fermented milk drinks (containing plant proteins). Therefore, the consumer's preference is for soy, pea and rice protein beverages.

4. Conclusion

The choice of biopolymers, including gellan with whey and soy proteins, was preferred to protect the valuable phenolic compounds. The production of an enriched beverage with the aim of using two proteins and a bioactive extract improved the value of the product. The obtained results illustrated acceptable physicochemical/sensory characteristics in gelan beads beverages. The maximum overall acceptance score was assigned to beverages containing Gellan gum alginate/IFPG and Gellan gum/WPI beads. The type of material of the beads completely displays changes in the various properties of the beverage. Gellan gum/SPI beads' beverage had the highest viscosity. As well as the type of gellan beads of the beverage caused changes in acidity and pH values. In general, the data is representative of this subject that the produced gelan beads beverage has been able to create the physical/appearance/sensory properties desired by the consumer. Therefore, Gellan beads are able to suggest as an efficient source of polyphenols for the fortification of beverage systems. Besides, in comparison with other beverages, this new one is attractive and provides health benefits to adults and children.

Ethical approval

This research includes sensory evaluation, which confirms that these experiments were conducted according to established ethical guidelines, and informed consent obtained from the participants. This confirmation complies with all regulations and confirmation that informed consent was obtained.

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Data availability statement

The data may be available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Zahra Khoshdouni Farahani: Writing - original draft, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mohammad Ebrahimzadeh Mousavi:** Writing - review & editing, Visualization, Validation, Supervision, Project administration. **Salam Adnan Ibrahim:** Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Zahra khoshdouni farahani reports was provided by Islamic Azad University Science and Research Branch. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

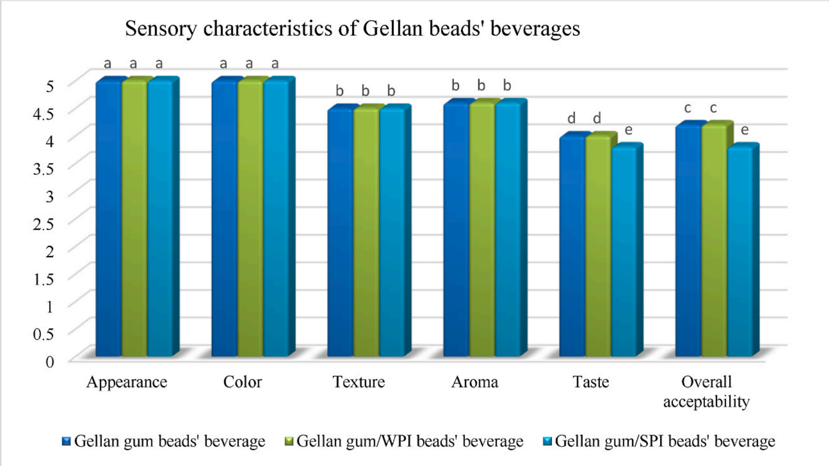

Fig. 1. Sensory characteristics of Gellan beads' beverages. Values are mean ± SD from triplicate determinations. Different letters show the statistical significant differences ($P < 0.05$).

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