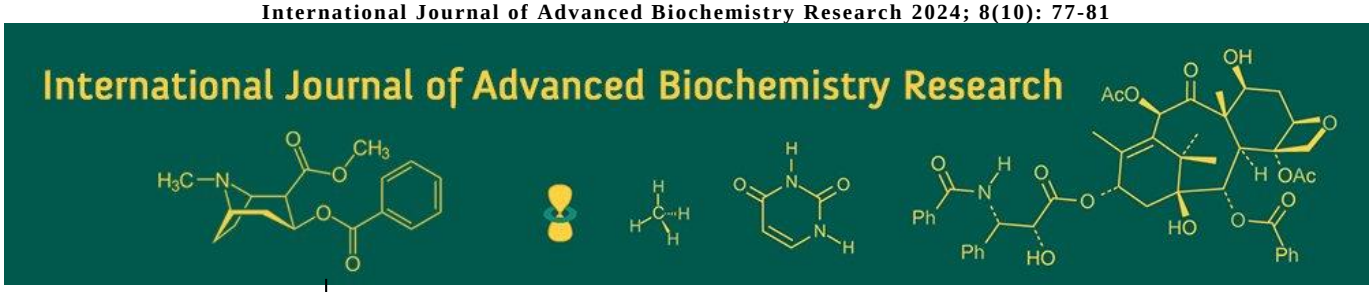

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Effect of temperature and gelling agents on stability of whey protein isolate based gels

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Abstract
Whey proteins from bovine milk are of high quality and their incorporation into the diet can improve the health status of individuals. Chewy gels with a variety of colours and sour or sweet flavours appeal to all age groups. Hence gels can be used as a delivery system of proteins and other nutrients in the diet provided their stability is maintained. Apart from attracting customers, whey protein-based gels would be an excellent means to provide high-quality proteins with all essential amino acids. Hydrocolloids like gelatine, carrageenan, and agar agar were used as gelling agents in the study. The effect of four different temperatures on hydrocolloid gels was analysed (20 °C, 25 °C, 30°C and 35°C). A ten member sensory panel evaluated the samples based on a nine point hedonic scale. Among the different gelling agents gelatine gel was found to have the lowest melting point of approximately 30 °C. Carrageenan and agar agar gel were stable even at 35 °C. They measured higher melting points than gelatine and were stable at room temperature. The highest acceptability sensory score was for the agar agar-based gels. Gelatine being sensitive to moderate temperature and being of animal origin affects consumer acceptability. Hence, agar agar was found to be the best hydrocolloid for formulating whey protein isolate gels based on sensory score and gel stability at room temperature.

Keywords: Whey protein isolate, gel, gelling agent, hydrocolloids

Introduction
Whey is an important by-product of the dairy industry owing to the continuous market growth for products like cheese and paneer. Due to the superior nutritional and functional qualities of whey solids, a significant amount of whey is converted into whey powders. Its utilization has paved new dimensions and opportunities for dairy technologists all over the globe. Whey is usually used as the raw material to isolate lactose and to prepare sweet whey powder, demineralized whey, delactosed whey, whey protein isolate (WPI), and whey protein concentrate (WPC), (Kilara. (2015)) [1]. Commercial dried whey protein products can be classified into two categories based on their protein content as whey protein isolate (WPI) with 90% protein and whey protein concentrate (WPC), with 50–85% protein. From a nutritional perspective, whey protein has several advantages, including high purity, high bioavailability, high utilization rate, and a balanced essential amino acid profile (Sharma, R. (2019) [2]. Whey protein-derived bioactive peptides have exhibited a wide range of functional, biological and therapeutic properties varying from anticancer, antihypertensive, and antimicrobial effects. In addition, their functional properties involve gelling, emulsifying, and foaming abilities (Saadi *et al*., 2024) [3]. Whey proteins are relatively small amphiphilic molecules that have good water solubility over a wide pH range. The ability of whey protein to assemble into different kinds of carriers is mainly based on its surface properties, such as hydrophobicity, electrical charge, and chemical reactivity. (Jiang *et al.* (2024) [4].
Food gels are becoming popular nowadays due to their aesthetic appeal. However, there isn't much nutritional value for these gels. Gel-forming hydrocolloids used in the study are agar, carrageenan, and gelatine. According to Zandona *et al.* (2021) [5], whey-derived ingredients like WPC and WPI are added to beverages with high protein content, mainly sports drinks and drinks for the malnourished. Whey protein can therefore be added to food items in our regular diets to provide them with protein. Gels being semisolid systems with good acceptability can be used to address protein deficiencies by incorporating whey proteins.

Mahajan (2015) [6] conducted a consumer survey on the amount of protein consumed by adult Indians in their diet and found that nine out of ten Indians consumed low protein diets. According to IPSOS (France), a leading global market research company study conducted in 2018, 84% of Indian vegetarian diets and 65% of non-vegetarian diets are low in protein (Jagmeet (2020)) [7]. In this study, gels are identified as an appropriate delivery system of whey proteins owing to abate protein deficiency.

Materials and Methods

The experiment was conducted in the laboratory of the Department of Dairy Chemistry, Verghese Kurien Institute of Dairy and Food Technology, Mannuthy, Kerala, India, during the year 2023-2024. The major ingredients for the preparation of whey protein isolate-based gels were whey protein isolate (WPI 90), sugar, gelling agents like gelatine, carrageenan and agar agar. Whey protein isolate was procured from Medizen Labs Pvt Ltd, Bengaluru, Karnataka, India. The gelling agent's agar agar and kappa carrageenan were procured from Marine Hydrocolloids, Kochi, Kerala, India and gelatine from Brew Lab, New Delhi, India. Colour and flavour were procured from Bake King, Thrissur, Kerala and the chemicals were purchased from Chemind Chemicals, Thrissur Kerala, India.

Preparation of Whey protein solution

Accurately weighed whey protein isolate was dissolved in preboiled water at 70 ℃ by continuous stirring. The whey protein solution was prepared in this way to yield a final protein content of seven per cent in the gel after standardisation based on gel consistency.

Table 1: Nutritional information of whey protein isolate as per manufacturer

SL No	Parameter	Value
1	Total fat (%)	1.06
2	Protein (%)	90
3	Energy (Kcal /30g)	113
4	Carbohydrates (%)	1.6

Pre-treatment of gelling agents

A different procedure was followed for each gelling agent for the preparation of hydrocolloid-based gels. In each case, 100 ml gel was prepared and standardised based on preliminary trials considering the stability of gels. Each hydrocolloid gel is considered as a treatment as follows.

T_1-Gelatine based gels
T_2-Agar agar based gels
T_3-Carrageenan gels

Pre-treatment of the gelling agents were done for preparation of 100 ml gel as given below.
Gelatine (6% of gel) was soaked in 20 ml of distilled water for a minimum period of five minutes. Then the gelatine solution was added to distilled water (about 60 ml) at 50 ℃.
Carrageenan (1% of gel) was ground in a mixer along with the definite quantity of sugar (3-4%) and then transferred to 30ml of distilled water at 60 ℃ or above.
Agar agar (1.2% of gel) was dispersed in 60 ml of distilled water at room temperature to form a colloidal solution.

Gel preparation

Carrageenan gels

To the pre-treated carrageenan sugar mixture (30 ml), the remaining sugar (96-97%) which was made into a sugar syrup of 50 ml was added. Then, both sugar syrup and carrageenan sugar solution were blended uniformly and heated to obtain a final concentration of 50% sugar in the gel. Afterwards, the mixture was cooled to 70 ℃. 30 ml of whey protein solution, flavour and colour were blended uniformly to the carrageenan sugar syrup mixture (70 ml) at 70 ℃. Gel is formed upon cooling to room temperature (30 ℃) and is stored under refrigerated condition at 4℃.

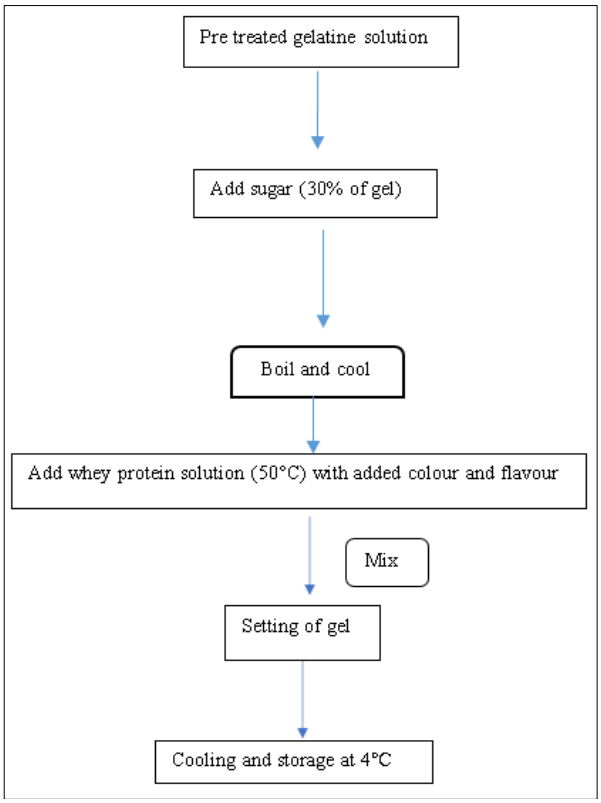

Fig 1: Preparation of gelatine based gel

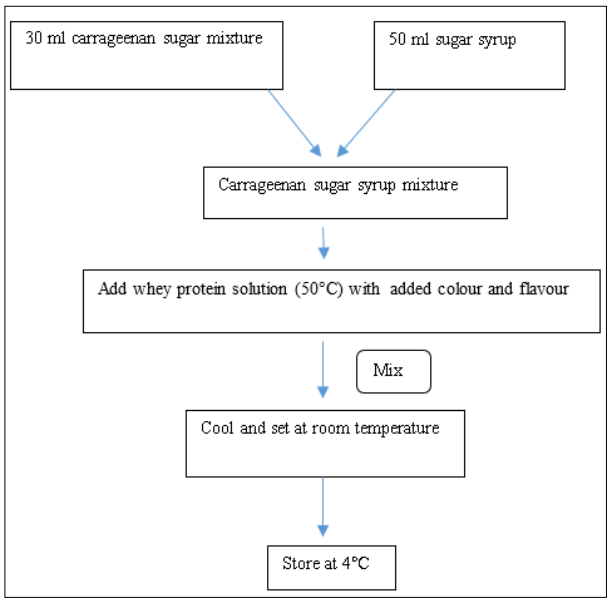

Fig 2: Preparation of carrageenan based gel

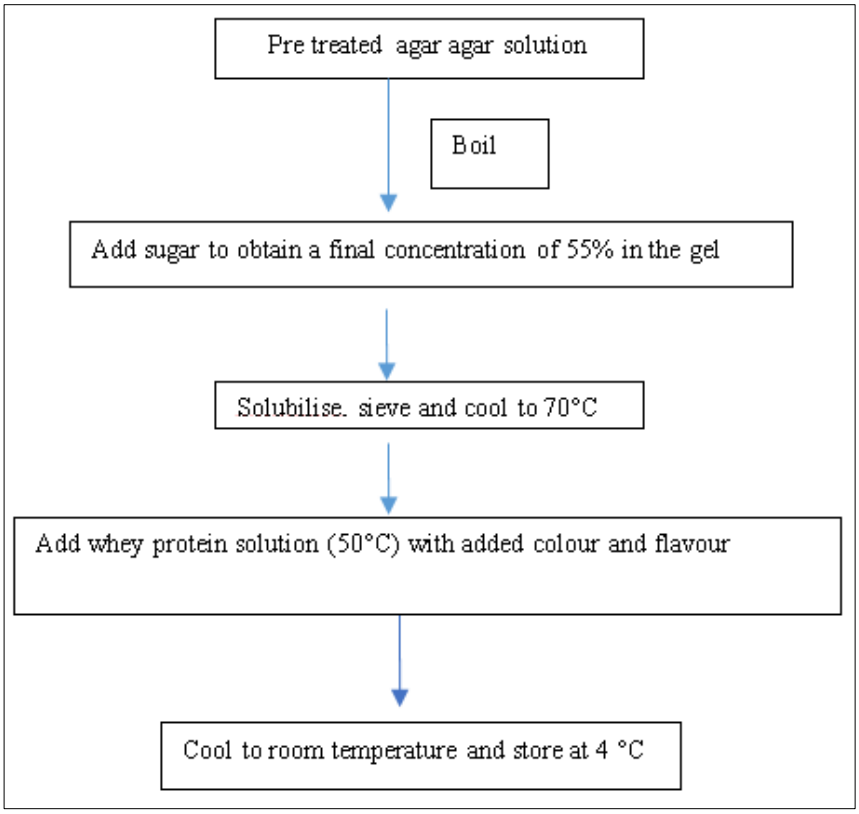

Fig 3: Preparation of agar based gels

Gelatine gels

Pre weighed quantity of sugar (30% of the gel) was added to the boiling pre-treated gelatine solution. After complete solubilisation of sugar, stopped boiling and cooled to 50 ℃. 20 ml of whey protein solution (50 ℃) with added flavour and colour were blended with the gelatine-sugar mixture (80ml) at 50 ℃ to form a uniform product. For setting, the mixture was kept under refrigerated condition at 4 ℃.

Agar agar gels

Pre-treated agar agar solution was boiled to obtain a transparent solution. To the agar agar solution sugar was added to obtain a final concentration of 55% with respect to the gel. After complete solubilisation of sugar, the mixture was sieved and cooled to 70 ℃. Whey protein solution blended with flavour and colour (20 ml) at 70 °C was added to the agar agar-sugar solution of 80ml at the same temperature. The mixture was cooled at room temperature to induce gelation and kept under refrigerated condition at 4 ℃ for storage.

Sensory analysis and storage study

Sensory analysis was conducted by a trained panel of 10 members based on a nine-point hedonic scale. The parameters taken for the sensory analysis were flavour, colour, body and texture, appearance and overall acceptability. Blueberry, strawberry, pineapple and watermelon flavours were used in the study during the sensory analysis. The effect of temperature on storage conditions of whey protein isolate gels was also studied. Storage study was conducted at 20 °C, 25 °C, 30 °C and 35 °C immediately after preparation. Statistical analysis was done using the Kruskal Wallis test in SPSS software version 27.0.

Results and Discussion

Three different hydrocolloid based whey protein gels were formulated using selected gelling agents gelatine, carrageenan and agar agar. The prepared gels were kept at four different temperatures 20 ℃, 25 ℃, 30 ℃ and 35 ℃. Figure 4, 5 and 6 represents agar agar gels, carrageenan gels and gelatine gels respectively. These gels kept at 20 ℃, 25 ℃, 30 ℃ and 35 ℃ are represented as Fig 4 (A, B, C and D respectively.

Fig: 4(B), 4(C) and 4(D) represents agar agar gels kept at 20 ℃, 25 ℃, 30 ℃ and 35 ℃. They were stable even at a higher temperature of 35 ℃.

Fig 5: 5 (A), Fig 5(B), Fig 5(C) and Fig 5(D) represents carrageenan gels kept at 20 ℃, 25 ℃, 30 ℃ and 35 ℃. From the figure, it is clear that gels were stable even at a higher temperature of 35 ℃.

Fig 6: 6(A) and 6(B) show that gelatine gels remained stable at 20 ℃ and 25 ℃ whereas Figure 6(C) and 6(D) reveals that gelatine gels melted at temperatures 30 °C and 35 °C respectively

Sensory analysis was done on freshly prepared treatments. The analysis revealed that the gels based on gelatine were unstable in tropical environments. The result of the experiment was found to be in accordance with Johnston-Banks (1990) [8] who found that gelatine has a lower melting point than polysaccharides. The utility of gelatine is therefore confined to ready-to-eat food items that are kept in the refrigerator or cooling cabinet. While the other polysaccharides agar and carrageenan did not melt at room temperature and exhibited a stable configuration even at 35 °C. Shimada *et al* (1993) [9] who measured the melting temperature of different gels by the upside-down test tube method stated that melting temperature of carrageenan gels and gelatine gels were about 50 °C and 30 °C respectively. Armisen and Gaiatas (2009) [10] in their study on gelation and melting properties of agar found that agar is capable of forming gel that does not melt below 85 °C. They also concluded that gelation and melting of agar gels are based only on the formation of hydrogen bridges. Therefore it can be inferred that even after incorporating protein in the gel, heat stability is not significantly reduced.

Table 2: Sensory evaluation of whey protein based gels using different gelling agents

Sensory attributes	Hydrocolloids			$\chi 2$ value
	T_1	T_2	T_3	
Colour	7.8± 0.632	8.15±0.579	7.55±0.685	4.725^{ns}
Flavour	7.85±0.807	7.9±0.421	7.69±0.718	0.228^{ns}
Body and texture	7.2±1.032	8.05±0.598	7.5±0.471	5.556^{ns}
Appearance	7.65±0.818	8.1±0.737	7.6±0.459	2.995^{ns}
Overall acceptability	7.4±0.966	8.2±0.707	7.45±0.437	3.844^{ns}

The values are the mean of ten values.
The means with same superscripts in a row does not differ significantly ($p \leq 0.05$).
a, b, c depicts significance at 5% level

All the three treatments when considered for the sensory analysis, the statistical approach as per the Kruskal Wallis test do not infer any statistical difference between sensory analysis of different hydrocolloid gels. But the sensory analysis scored the highest overall acceptability for agar agar-based protein gels with an average score of 8.25. Recently, extensive research works have been conducted on fortification or supplementation of nutrients using jellies. In a study conducted by Younas *et al.* (2021) [11], jellies were formulated with food-grade calcium salts and chicken eggshell powder to cope with calcium deficiency. Despite naturally available calcium-rich sources, calcium-fortified jellies can be consumed by individuals who are incapable of taking sufficient calcium from their diet. Food-grade calcium salts like calcium carbonate, and calcium gluconate were used for the fortification of jellies. Junsara *et al.* (2023) [12] optimized jelly using different levels of crude protein extracts from Sung Yod and Hom Rajinee varieties of rice brans. The vegetarian equivalent of gelatine is speculated to be agar (Mahamud *et al.* (2023)) [13].

Conclusion

Whey protein isolate-based edible gels were formulated using different hydrocolloids. Three gelling agents namely carrageenan, agar and gelatine were used for the study. Sensory panelists gave the highest preference to the whey protein gels made using agar. The gelatine gels showed the least stability at room temperature melting almost instantly at 30°C. So it was confirmed that gelatine gels should be stored at 25°C or below. At refrigerated condition, gelling ability of gelatine gels were good. But as gelatine is of animal origin, it may not be acceptable to all consumers. Being derived from

plants, agar would be accepted by more people. Carrageenan and agar based gels are possible options and both remained stable even at a higher storage temperature of 35°C. Based on sensory score agar was found to be the best gelling agent for formulating protein gels based on whey protein isolate. Most of the protein supplements are available in powder or dry form. Recent research on protein supplements has revealed several adverse consequences such as dehydration brought on by protein breakdown. Whey protein gels are superior to regular supplements as it is a water-bound media for whey protein isolate and hence hydrogels can be the perfect solution to this scenario.

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