



## Scrutinizing the different pectin types on stability of an Iranian traditional drink “Doogh”



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### ABSTRACT

Doogh is a fermented dairy drink which is highly consumed by Iranian people. Stability of this healthy drink was investigated in terms of sedimentation rate, viscosity, density and particle size characteristics including surface-weighted mean diameter ( $D_{32}$ ), Span and particle uniformity. Eight treatments were performed as randomized complete block design (RCBD) with three replications. Three types of pectin (high methoxyl pectin (HMP), grapefruit-seed extract pectin (GSEP) and amidated pectin (Ceamsa pectin (CSP)) at a constant concentration (0.35%w/w), three levels of salt (0.50, 0.75 and 1.00%w/w) and two dry matter contents (DMCs, 4 and 5% w/w) were used to produce the Dooghs. The results showed that the maximum stability and viscosity, and the minimum  $D_{32}$  were obtained by application of CSP, GSEP and HMP, respectively ( $p < 0.05$ ). Pectin type had no significant difference on the density values of Dooghs. The lowest sedimentation rate, viscosity, density and  $D_{32}$  were achieved in the minimum concentrations of salt and dry matter. The ANOVA analysis also revealed that the interaction of pectin type, salt concentration and DMC had a significant effect on the Span and particle uniformity. A maximum physical stability was found for the prepared samples with 0.35%w/w CSP, 0.5%w/w salt and 4%w/w dry matter. Evaluation of sensory attributes also confirmed that this formulation had the highest overall acceptability value.

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### 1. Introduction

The structural and textural characteristics of colloidal food systems depend not only on the functional attributes of the individual ingredients, but also on the nature and strength of the interactions between protein and polysaccharide [1]. Doogh as an acidified milk drink is produced by adding salt (maximum 1%) and water (50–60%) into yoghurt (40–50%) and it is characterized by a pH < 4.5 and non-fat solid more than 3.2 wt% [2]. Casein (CSN) micelles present in the milk dispersions aggregate at pH < 4.6, thus a stabilizer agent needs to be added to them to prevent phase separation and wheying off [3]. In this condition, the protein–polysaccharide interactions can enhance the stability depended on the type of the polymer, concentration of the polysaccharide present and the environmental conditions of the solution (i.e. temperature and ionic strength) [4].

Pectin among carbohydrate biopolymers is one of the most usually applied hydrocolloids and gelling polysaccharides in the food industry. This anionic polysaccharide consists of a backbone of galacturonic acid partly methyl-esterified and branches of

arabinose, galactose and xylose. This hydrocolloid is usually classified by the degree of esterification (DE) in high-methoxyl pectin (HMP), if half or more of the carboxyl groups are esterified, and low-methoxyl pectin (LMP), if less than half of the carboxyl groups are esterified [5]. The DE has a decisive effect on pectin solubility and its gelation properties. Marozienne and de Kruif [6] reported that pectin at pH < 5.0 can prevent aggregation and sedimentation of the CSN particles through electrostatic and steric stabilization. High-methoxyl pectin (HMP) has been extensively used as an ideal stabilizer in acid milk drinks as it aids in preventing flocculation of milk CSNs and thus improving and maintaining the desirable properties of dairy products.

We applied two commercial pectins namely Ceamsa pectin (CSP, an amidated pectin) and grapefruit seed extract pectin (GSEP) to produce stable Dooghs with suitable physical and sensory attributes. Only a few scientific reports of their stability effects in colloidal solutions could be found in the literature. The data were compared to the results obtained from Dooghs formulated with HMP. Thus, the aim of our research was to better understand the influence of different pectin types including the HMP, CSP and GSEP for the preparation of stable Dooghs and investigation of their sedimentation rate, density, particle size, rheological and sensorial characteristics.

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## 2. Materials and methods

### 2.1. Materials

Fresh skim milk was provided from the Pegah Dairy Co. (Iran, Tehran). It contained  $2.0 \pm 0.1\%$  fat,  $2.8 \pm 0.2\%$  protein and  $4.7 \pm 0.2\%$  lactose. A commercial reference culture (Yo-Mix™ 502) for traditional acidic yogurt was obtained from Gemak-Danisco Co. (Ankara, Turkey). Citrus HMP was provided from Sigma Chemical Co. (St. Louis, Mo, USA). CSP as an amidated pectin was purchased from CEAMSA Co. (CEAMSA, CEAMGEL 9623, Spain). GSEP is a commercial product derived from the seeds and pulp of grapefruit (*Citrus paradisi* Macf., Rutaceae). GSEP was obtained from ABC Techno Inc. (Tokyo, Japan). Sodium chloride (99.5% purity) and lactic acid were provided from Merck Chemical Co. (Darmstadt, Germany).

### 2.2. Doogh preparation

For the Doogh preparation, the method of Kiani et al. [7] was adopted with slight modifications. The used milk was pasteurized in a water bath at  $80 \pm 1^\circ\text{C}$  for 30 min. After cooling to  $40^\circ\text{C}$ , it was inoculated by the commercial reference culture and transferred to plastic cups, incubated at  $43^\circ\text{C}$  until the pH reached  $4.3 \pm 0.1$ . The pH values of Dooghs were measured using a glass pH electrode (Mettler Toledo, Schwerzenbach, Switzerland). In order to measure the dry matter content (DMC) of the produced yogurt, about 1.0 g sample on a boiling bath was evaporated and then oven-dried at  $100^\circ\text{C}$  to a constant weight. In the next step, the resulting yoghurt was diluted and blended using a classic blender (PBI International, Milan, Italy). Three types of used pectin (0.35% wt) in the separate treatments gradually added to deionized water. The pectin-salt mixture by adding salt (0.50, 0.75% and 1.00% wt) was produced, heated ( $80^\circ\text{C}$  for 15 min), and cooled down to ambient temperature. To achieve full hydration, the mixture was kept at room temperature overnight. In this step, the obtained solutions were added to the partial diluted yoghurt kept at room temperature. The DMC standardized at 4 and 5% w/w for the different formulations, including solids originated from yoghurt, typical pectin and salt. Afterwards, the mixture passed through a high-pressure homogenizer (APV 2000, Denmark) at  $185 \pm 5$  bar. At least two separate samples were prepared for each treatment.

### 2.3. Stability analysis

In order to determine serum separation, freshly prepared samples (15 ml) were transferred to cylindrical tubes (internal diameter 15 mm, height 120 mm), and stored for 30 days at  $4.0 \pm 0.1^\circ\text{C}$ . Each phase has a clear appearance that could be easily detected visually. The amount of sedimentation was measured and expressed as a percentage of the total sample volume.

### 2.4. Viscosity measurement

Viscosity of the prepared samples was measured using a steady stress rheometer (Brookfield DV-II, LV Viscometer, USA) equipped with the ULA spindle, as previously recommended by Gharibzadeh et al. [8]. The experiments were carried out at  $25 \pm 2^\circ\text{C}$  and different rotational speeds depending on their torque values. After primary experiments, the appreciate torque value found between 10 and 100% of the measuring range to obtain reliable results. Tests were performed in triplicate immediately after production of the samples. The power law model was applied to determine the consistency coefficient and the flow behavior index of the samples

using the shear stress data obtained from increasing shear rate measurements as follows (Eq. (1)):

$$\sigma = K\dot{\gamma}^n \quad (1)$$

where  $\sigma$  is shear stress,  $K$  is the consistency coefficient,  $\dot{\gamma}$  is shear rate, and  $n$  is the flow behavior index.

### 2.5. Density determination

The density was measured at room temperature ( $25 \pm 2^\circ\text{C}$ ) using a hydrometer (KEM, Kyoto, Japan) in appropriate range. The measurement was carried out in triplicate.

### 2.6. Analysis of particle size characteristics

Particle size of the samples 1 day after preparation was measured by light scattering, using a Mastersizer 2000S (Malvern Instruments Ltd., UK). Sample analysis was carried out after sample preparation and during storage time, based on the principle of Fraunhofer [9]. Size distribution was characterized by volumetric percentage and mean particle diameter obtained by surface-weighted mean diameter ( $D_{32}$ ) of the Doogh particles, based on the following equation [10,11]:

$$D_{32} = \left( \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \right) \quad (2)$$

where  $n_i$  is the number of droplets of radius  $d_i$ .

The Span is the distribution width of the particles in the dispersion, which was calculated using the following equation (Eq. (3)) [4]:

$$\text{Span} = \left( \frac{d(v, 90) - d(v, 10)}{d(v, 50)} \right) \quad (3)$$

where  $d(v, 10)$ ,  $d(v, 50)$  and  $d(v, 90)$  are diameters at 10%, 50%, and 90% cumulative volume, respectively. In other words,  $[d(v, 90) - d(v, 10)]$  is the range of the data, and  $d(v, 50)$  is the median diameter.

Moreover, deviation from the median which is an indicative of polydispersity was reported as uniformity (Eq. (4)) [4]:

$$\text{Uniformity} = \frac{1}{d(0.5)} \left( \frac{\sum V_i |d(0.5) - d_i|}{\sum V_i} \right) \quad (4)$$

where  $V_i$  is the volume of the number of particles existing between the two consecutive diameters.

### 2.7. Sensory analysis

Sensory characteristics of the produced samples with different formulations including taste, mouthfeel and overall acceptability were evaluated by 30 testers including graduate students and staff members of Tehran University's Food Engineering Department who were familiar with the characteristic qualities of the commercial Dooghs. Panelists were seated in sensory booths with standard lighting. A hedonic 9-point structured scale, in which 9 corresponded to "most liked" and 1 to "most disliked". Each sample was presented twice, and the samples were presented in random order.

### 2.8. Statistical analysis

Results were expressed as mean  $\pm$  standard deviation of the mean using all available data. The obtained results were subjected to analysis of variance (ANOVA), applying randomized complete block design (RCBD), using SPSS 13 software (SPSS Inc., Chicago, IL, USA). The  $F$ -test was used to determine significant effects of pectin

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