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The effect of high methoxyl pectin and gellan including psyllium gel on Doogh stability

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Abstract

Influence of three stabilizers of psyllium seed hydrocolloid (PSH, 0.25%w/w), high methoxyl pectin (HMP, 0.25%w/w), and gellan gum (GG, 0.05%w/w) alone or binary mixture on the phase separation during 21d of storage, mean particle size and distribution, ξ-potential, rheology, microstructure and sensory characteristics of an Iranian fermented milk drink "Doogh" was investigated. Results revealed that the stability of Dooghs prepared with binary combinations of HMP, PSH and GG was highly more than samples manufactured with each studied hydrocolloids alone. Use of binary mixture of PSH and HMP with the lowest instability rate (6.08%) and the highest absolute negative ξ-potential (-7.10 mV) led to a desirable effect on the critical sensory attributes. The maximum consistency coefficient under power-law rheological model (R^2>0.97) was found for Dooghs formulated with GG alone. This study provides important data on the performance of different types of polysaccharides which can be used in formulating acidified milk drink systems.

Keywords: Acidified dairy drink, Physical stability, Rheology, Gellan gum, Psyllium mucilage, Storage time

1 Introduction

There are many types of fermented dairy drink under different names in different regions of the world. Some examples are yogurt drink in Europe, kefir and kumiss in the Middle East, ayran in Turkey and Doogh in Iran.[1] Doogh is made by mixing yogurt, water, and a few salt as well as some aqueous extracts of local herbs.[2-4] In recent years, consumption of this drink has become very common in Iran and other Asian countries.[5-7] It has many benefits due to the presence of probiotic and prebiotic microorganisms which can increase the nutraceutical value of the final product.[6] However, Doogh like other acidic dairy drinks has a serious problem because its low pH (≤ 4) causes phase separation leading the casein accumulation and gives a product with undesirable non-uniform appearance.[2,6,8] Furthermore, a reduction in salt and fat amounts can noticeably reduce physical stability of this dairy drink.[1,6,9] On the other hand, heat treatments such as pasteurization have a key importance in production of dairy products, since it is a common step in the processing of milk products. The heat treatment exposes reactive groups on the protein which were previously inaccessible; and this, in turn, affects the rheological properties of the products.[7]

It has been reported that polysaccharide hydrocolloids can considerably prevent phase separation in acidic dairy drinks by the changing functional properties of milk proteins especially kappa-caseins and viscosity enhancement.[2,9-16] Since hydrocolloids addition causes an undesirable taste, choosing the optimal concentration of applied hydrocolloids is considered an important factor in order to stabilize fermented dairy products.[9] Studies have also shown that various factors such as ionic power, protein-polysaccharide ratio, pH and final concentration of the product have great effects on the physico-chemical properties of protein-polysaccharide mixed systems.[1]

Psyllium seed is an excellent source of natural soluble fiber, and has almost 8% more soluble fiber than oat bran on a per weight basis.[17,18] The seed husk is containing high amounts of hydrocolloids obtainable by separating the outer layer of grains by grinding or scratching and forms approximately 25% of the whole grain.[19] Hydrocolloid of psyllium seed is a white fibrous substance that absorbs water and becomes a clear and colorless gel. Polysaccharide presence in this hydrocolloid is an anion from L-arabinose, D-xylose and D-galacturonic acid.[19-21] Hydrocolloid properties of psyllium seed include a high tendency to absorb water at about 20 times that of its original volume. Hydrocolloid is chemically inert and cannot be digested and absorbed in the body. Also, this seed as a medicinal material is an active polysaccharide used to treat unhealthy conditions such as diarrhea, irritable bowel syndrome (IBS), constipation, diabetes, colon cancer and high cholesterol.[22-24] Askari *et al.*[22] by extracting psyllium seed hydrocolloid (PSH) and evaluating its rheological properties demonstrated the rheological behavior is watery with shearing. Ladjeverdi *et al.*[25] have recently developed a stable low-fat yogurt gel using functionality of PSH for achieving the best rheological, textural and sensory characteristics using response surface methodology. Gharibzahedi *et al.*[21] reported that use of PSH in the presence of whey protein isolate can significantly improved physical stability of the ultrasonically prepared coconut oil-in-water emulsions containing canthaxanthin produced by *Dietzia natronolimnaea* HS-1 strain. Ahmadi *et al.*[26] earlier pointed out polysaccharide gel of psyllium had a good potential to be used in producing new biodegradable edible films with interesting specifications.

Study on the effect of different hydrocolloids such as tragacanth gum (TG), guar gum, high methoxyl pectin (HMP), and gellan gum (GG) on the Doogh stability showed that these polysaccharides as thickener agents can react with caseins having positive charge to create a strong three-dimensional network by trapping water and caseins.[1,7] Among these polysaccharides, pectin and TG were applied as a common stabilizer for this dairy drink. Pectin as a polysaccharide is composed of units of D-galacturonic acid with bounds of α-(1→4). It is a component of absorbent hydrocolloid that causes stability in a system via steric repulsion.[15] Some literatures have proposed formation of a weak gel network by pectin gel to prevent accumulation of casein deposits.[8,13,15] Also, the stabilizing mechanisms of TG, especially *Astragalus gossypinus* demonstrated that *tragacanthin* was responsible for an electrostatic reaction with casein in that *bassorin* also has a key role in sustainability by increasing viscosity of the constant phase.[6,9]

To the best of our knowledge, no study has been yet reported on the application of PSH in Doogh formulation and evaluation of its stability characteristics. Thus, this study has attempted to evaluate the ability of PSH used individually or in combination with GG, and HMP on Doogh stabilization over a 15 day storage period.

2 Materials and Methods

Materials and chemicals

Psyllium seeds used in this study were purchased from a local herbal store in Tehran (Iran). HMP and GG were provided from CP Kelco Co. (Lille Skensved, Denmark) and Heidelberg Co. (Colorado Springs, USA), respectively. All chemicals and culture media applied in this work were obtained from the Merck Chemical Co. (Darmstadt, Germany).

Preparation of psyllium seed hydrocolloid

For the extraction of PSH, 100 mL of hydrochloric acid (0.1 M) was indirectly heated to boiling temperature by a bottle containing water on a heater (Heidolph, Model R, Schwabach, Germany), and added 5 g of psyllium seeds and then continued heating and mixing (300 ×g) until the color change of seeds. The seeds were washed by 10 mL warm water and the resulted aqueous solution containing hydrocolloid was separated through a filter to completely collect the hydrocolloid. After this step, the filtered liquid containing hydrocolloid was mixed with 60 mL of 96% ethanol and maintained at 5°C for one day. Finally, PSH was isolated by centrifuging (Universal 320, Hettich, Manchester, UK) at 3500 ×g for 20 min. Hydrocolloid was obtained in a laboratory oven (Behdad Medical Equipment, Iran), dried at 40°C and prepared for further tests by grinding in a laboratory mill (IKA, Model A 11B, Germany).[20]

Doogh preparation

In order to produce Doogh samples, yogurt (40% w/w), NaCl (0.7% w/w), and each polysaccharides (PSH (0.25% w/w), HMP (0.25% w/w) and GG (0.05% w/w)) in a separate tank were added to 100 mL of distilled water. The polysaccharides amount in Doogh formulation was selected based on a preliminary literature review and experiment. The obtained dairy solution was heated at 60-65°C for 1 h after mixing yogurt, NaCl, hydrocolloid and water. In the next step, the mixture was homogenized (Oltratrax, Heidolph Persia, Germany) at the speed of 15,000 ×g until the achieving uniform and fine particles. Pasteurization was then done at 83°C for 1 min in a water bath (IKA-Werk, Germany). Doogh samples were put in to sterile containers and stored at 5°C. PH of the final product was 3.84 ± 0.09.

To study the combined effect of hydrocolloids, one hydrocolloid was added to water and yogurt based on the specified ratios, on completion of the determined duration, the second hydrocolloid was added and operations of homogenization, pasteurization and filling of sterile containers were performed. Doogh with a sour flavor is the most popular among consumers, thus sour yogurts with pH = 3.97 and acidity of 132-138°dornic were used. It is to be noted that the AOAC[27] standard method was used to measure acidity, dry matter and pH of the developed samples.

Physical instability measurement

Phase separation of the different formulations was carried out in glass tubes for 21 days. Amounts of serum phase (upper phase) were measured by a ruler and divided by the amount for Doogh content in each test tube and expressed as percentage.[3,4]

Viscosity measurement

The viscosity was measured at 23±1°C by a rotational viscometer (Brookfield DV-II+ programmable viscometer, Middleboro, MA, USA) equipped with a ULA spindle. This analysis was performed 24 h after the production at different rotational speeds dependent on torque amounts. The power law model was used to determine consistency coefficient and flow behavior index of the samples by fitting the experimentally measured shear stress-shear rate data.[28]

Determination of particle size

Particle size distribution of the samples was determined by dynamic light scattering using a Mastersizer 2000S (Malvern Instruments Ltd., UK). Measurements were made 1 day after the production according to the Fraunhofer principle.[4] Doogh samples were diluted before tests with deionized water at the ratio of 1:100 in order to avoid multiple scattering effects. 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 Eq. (1):

$$D_{43} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \qquad (1)$$

where, n_i is number of particles with diameter d_i.

ξ-potential measurement

Zeta-potential measurements of the 100-fold diluted particles were carried out using a Malvern Zetasizer IV (Malvern Instruments Ltd., UK). The zeta-potential measurements are reported as the averages of three separate injections, with three readings made per injection

Microscopic observation

Each Doogh sample (0.15 mL) was initially diluted with 20 mL of distilled water and added 1 mL of rhodamine B in order to color formation of casein particles. Observations were immediately visualized after the slides preparation of using a phase-contrast light microscopy (Leica Galen III, Germany) at 40× magnifications.

Microbiological analysis

Determination of coliforms, and mold and yeast population was according to the FDA-Bacteriological Analytical Manual.[29] Briefly, each sample was serially diluted (10-fold) in sterilized phosphate buffer solution (pH = 7.0). In the next step, 1 mL of Doogh and each diluent were transferred to two duplicated separate Petri-Dish and two of them poured plated with Violet Red Bile Agar (VRB) and other two with Potato Dextrose Agar (PDA) for enumeration of coliforms, and mold and yeast counts, respectively. After mixing completely, the VRB plates were incubated at 32ºC for 24 h and PDA plates at 20ºC for 5-7 days, respectively. After the incubation, all colonies grown on VRB and PDA were enumerated.

Sensory evaluation

Evaluation of sensory attributes of the produced Dooghs with various formulations during storage time in terms of taste, consistency, appearance, smell and overall acceptability was carried out by 20 trained panelists consisting of graduate students and staff members of *Tabriz University's Food Engineering Department* (Tabriz, Iran) who were familiar with the characteristic qualities of produced products.[4] In order to describe the sensory properties of the seven types of Doogh, the sensory profiling method was applied.[30] This procedure consisted of two phases, an initial phase to select, train and validate the assessors and a subsequent phase focusing on the evaluation of the samples. Trained panelists were thus selected on the basis of perceived verbal skills, motivation, regular use of dairy products and the ability to replicate their results. Panelists were between the ages of 25 and 45, 11 were female and 9 were male. Testers were seated in sensory booths with standard lighting. Each sample was presented twice, and the samples were presented in random order. Panelists used water to rinse between samples and unsalted crackers were available. A hedonic 9-point structured scale, in which 9 corresponded to most liked and 1 to most disliked. The median value or mode was used to represent the sensory data.

Statistical analysis

All experiments were repeated at least 3 times and the results presented as a mean of the obtained values with the standard deviation (SD). The results were subjected to analysis of variance (ANOVA), using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) software. Differences among mean values at a 5% level were examined by the least significant differences (LSD) test. Correlation analysis for studying the relationships between different traits was also performed employing Pearson's test.

3 Results and discussion

Phase separation

As shown in Fig. 1a, the Dooghs formulated with HMP and HMP-PSH respectively had the highest and lowest instability rate among other investigated samples ($p< 0.05$). In general, the stability of samples manufactured with each studied hydrocolloids alone was lower than those prepared with binary combinations of HMP, PSH and GG (Fig. 1a). Increase of serum separation in the produced Dooghs during storage (Fig. 1a) can be attributed to the sedimentation of large particles and aggregation of casein proteins at the bottom due to over acidification in low pHs (Fig. 1b).[31] Correlation analysis showed that the physical stability was strongly related with pH loss rate of the produced Dooghs during storage period (r^2 = - 0.929, p< 0.01). Table 1 shows the microbial analysis of different formulations of Doogh including the yeast, mold and coliforms count during the storage time. The mold and yeast (0.70-3.48 Log 10 CFU/mL) were dominant in this acidified drink during 21d storage period. However, growth of coliforms in the various formulations of Doogh was limited as only control sample and Dooghs containing HMP, GG and PSH had very low counts of coliform bacteria. Maybe, use of hydrocolloids alone provides suitable conditions to grow yeast/mold and subsequently reduces the medium pH. In contrast, combination between the used hydrocolloids can probably inhibit coliforms growth in the produced Dooghs. However, it is necessary to provide the hygiene status of materials and equipment applied for increasing microbial quality of the final product. For example, the quality of raw milk, adequacy of heat treatment, microbiological quality of edible salt and aromatic additives used in production, hygiene level of filling equipment and packaging containers and the air condition of the production hall are the most effective parameters on the microbial quality of the final Doogh.[11-13] Moreover, the storage temperature of Doogh should be controlled significant to extend shelf life of the product.[7]

It was demonstrated that the apparent viscosity of acidic milk drinks can increase by increasing pectin content and thus the low diffusivity under this condition can reduce the physical instability due to a decrease in the collisions between Doogh particles. [3,4,14,31-33] It seems that the use of a low concentration of HMP (0.25% w/w) provides high instability for the formulated Dooghs because more pectin at high levels of HMP to cover the casein particles and also to interact with water could be available for increasing the resistance of the fluid against flow.[11,34] Increase of the stability by combining HMP and PSH was probably because of the ability of these hydrocolloids to induce formation of a weak network structure in Doogh.[15] PSH enhances the consistency and stability of the natural systems because of the formation of a strong gel by hydrocolloid nature of the husk containing arabinoxylans with various structural features.[21] Arabinoxylans present in PSH structure are highly branched non-starch polysaccharides with a main chain of densely substituted β-(1,4)-linked xylopyranose residues. Single arabinofuranose and xylopyranose residues, or short side chains consisting of these monosaccharides, are attached at positions 2 and/or 3 of the main chain xylopyranose residues.[35-37] PSH thus is an anionic polysaccharide that bears a negative charge due to ionized carboxyl groups.[21] In acid medium, most of $-COO^-$ groups transform into –COOH groups and consequently a significant increase can be observed in the hydrogen bonding interaction among hydrophilic groups and the additional physical crosslinking degree.[38] Sila *et al.*[39] showed that the electrical charge on biopolymers may be also changed by their interactions with other ionic species in the environment. These interactions typically involve multivalent ions such as calcium that bind to oppositely charged groups on the biopolymer chain, altering overall charge characteristics. The presence of Ca^{2+} ions in Doogh medium can covalently form cross-links between free carboxyl and amino groups along neighboring polymer chains and can cause a decrease in negative charge. Therefore, adding Ca^{2+} ions resulted in an increase in the density of aggregates, and the network became stronger.[19,21] High stability of Dooghs formulated with PSH-HM can be due to polysaccharides molecules bonds either separately or in combination with proteins leading to the product stability through formation of a three-dimensional network or trapping the protein particles in the network.[40]

Rheology

Power law and Herschel-Bulkley models were applied to find the best shear stress-shear rate plots to describe the rheological behavior of Dooghs. Results showed that the power law was the better rheological model compared to the Herschel-Bulkley model (data not shown) because of due to the highest determination coefficient and lowest standard error (Table 2). Similar results were previously reported for Doogh[2], ayran[14,32], and other types of fermented milk beverages[41-43]. A rheological behavior change from Newtonian to pseudoplastic was found by the adding different hydrocolloids alone or in combination with each other (Table 2). The formation of inter- and intra-molecular interactions between chains is probably responsible for increase in the apparent viscosity in a constant shear stress and decrease in the flow behavior index.[6] Fig. 2 by presenting a shear-thinning behavior shows Dooghs formulated with 0.5% GG had the highest the consistency index (*m*). Table 2 shows the viscosity value of various Dooghs at a constant shear rate (0.416 s^{-1}). As considered in this figure, Dooghs formulated with GG and HMP respectively had the highest (0.0288 Pa.s) and lowest (0.0020 Pa.s) viscosity among the investigated samples. No significant difference ($p>0.05$) was observed for the values of viscosity (0.0048-0.0049 Pa.s) between control and Doogh containing GG-PSH. Also, the viscosity values of samples containing HMP in mutual combinations with GG and PSH and also Doogh containing PSH alone were in a similar range. High viscosity of Dooghs containing GG can be attributed to the formation of an electrostatic complex by increasing interaction between positively charged protein groups and anionic GG groups through the pasteurization process. [44,45] Indeed, association of acid-casein particles as control Doogh, existence of bridging between the casein particles via GG-casein associations, and fragile association of GG helices in term of weak gels provide a strong network against to flow.[34] Hasheminya *et al.*[7] also by studying effect of GG on rheological characteristics and stabilization of a fiber-enriched Doogh reported that the formation of such complex is possibly responsible for enhancement in the consistency coefficient and apparent viscosity in a constant shear stress and reduction in the flow behavior index. Low viscosity of Dooghs containing HMP alone was probably due to low concentration of this component in the formulation. It was demonstrated that HMP at high content has an interest ability to induce formation of a weak network structure in Doogh.[3] As can be seen in Fig. 3a, the favorable viscosity of PSH can be because of formation of a continuous cross-linked network due to its high molecular weight and the presence of an arabinoxylan with 1→4 linkages in the xylan backbone in alkaline extractable gelling (AEG) faction of PSH structure which was heavily substituted by short arabinose branches.[19,38] We believe that the formed complex structures by GG and PSH in combination with HMP can potentially modulate the system viscosity by providing a homogeneous and fine distribution among particles (Fig. 3b and c). Higher viscosity of Dooghs formulated with HMP-PSH than those formed by HMP-GG (Fig. 3) may be explained by the fact that the presence of many number of particles in their matrix (Fig. 3b and c) can increase resistance to flow. Poor viscosity of Dooghs formulated with combination of PSH and GG can be ascribed to extensive depletion flocculation (Fig. 3d) due to the high presence of non-adsorbed anionic polysaccharides of PSH and GG on casein micelles. Evaluation of zeta potential somewhat demonstrates this fact for Doogh formulated with PSH-GG (Table 2). Existence of large, irregular fragments of aggregated casein, separated from one another, and differing widely in size and shape in this formulation can often lead to “wheying off” – the formation of a clear separated layer or syneresis when polysaccharide stabilizers or thickeners are added to dairy-based beverages.[5]

Particle size and ξ-potential

Formation and growth of electrostatic complexes between polysaccharide and protein molecules can easily determine using particle size analysis. Table 2 indicates D_{32} values of the particles of produced Dooghs in this research. The lowest (5.39 μm) and highest (36.40 μm) D_{32} values respectively were for samples prepared with PSH alone and plain Doogh without any hydrocolloid ($p < 0.05$; Table 2). The microscopic image of Dooghs containing PSH alone (Fig. 3a) also confirms the results obtained using laser diffraction particle sizing. However, a high uniformity and low polydispersity index can be observed for Dooghs containing PSH according to the particle size distribution curve (Fig. 4). Casein particles in the absence of stabilizer formed large aggregates and had a wide particle size distribution from 0.1 to ~ 100 μm (Fig. 4). Particle size values of Dooghs stabilized with HMP, HMP-PSH and HMP-GG were in a similar range (6.26-8.39 μm) and had no significant difference with the control sample. This could be related to the efficient interactions between positive charged proteins and negative charged polysaccharides which resulted in formation of soluble complexes.[1] Nonetheless, the ξ-potential analysis demonstrated that HMP-PSH was the best biopolymer combination to improve the inter- and intra-molecular interactions in Doogh structure (Table 2). The surface charge of the casein stabilized particles is governed by the ionization degree of amino (NH_2) and carboxyl (COOH) groups of protein depending on the pH of the surrounding aqueous phase. At pHs lower than isoelectric point of casein protein, ξ-potential of the particles of control sample showed a relatively high positive surface charge (+11.91 mV), but when HMP-PSH was present in the system, charge reversal to -7.10 mV occurred, indicating the strong adsorption of this binary mixture on the surface of the Doogh particles. Dooghs composed of GG and PSH and also their binary mixture showed higher D_{32} values than the other samples. This fact showed that these components were not sufficient to completely cover the positively charged casein particles and caused bridging flocculation as well as casein aggregation.[46-48]

Sensory characteristics

Evaluation of consumer preference of an essential product as Doogh is one of the most important methods for determining its sensory quality. Results of the sensory evaluation during 21d storage in Fig. 5 showed that there was a difference between the Doogh samples containing hydrocolloids and the control sample in terms of taste, smell, consistency, appearance and overall acceptability. Moreover, a considerable decrease in sensory attributes of all the formulations of

Doogh by increasing storage time was observed (Fig. 5). The highest and lowest taste scores were respectively belonged to Dooghs containing PSH and PSH-HMP combination (Fig. 5a). The taste sensory attribute was strongly correlated with smell (r^2= 0.937) and overall acceptability (r^2= 0.860) values. Although fresh Dooghs formulated with GG had the highest consistency scores in comparison to the control, samples containing PSH-HMP maintained their high consistency up to the end of storage time (Fig. 5b). In general, the mean of consistency values presented by panelists was indicative of desirability of the samples containing hydrocolloids. Penna *et al.*[42] reported that a high consistency coefficient can positively increase the sensory acceptability of lactic beverages. Koksoy and Kilic[14] pointed out that a positive correlation with sensory acceptability of lactic drinks can be obtained with the providing high consistency coefficient and pseudo-plastic property. Joudaki *et al.*[3,4] showed that use of HMP in Doogh formulation can noticeably improve the consistency and consumer preference. A high correlation was also detected between consistency and appearance values (r^2 =0.909). The relatively stable appearance in samples containing HMP-PSH can thus be attributed to the least phase separation and the highest consistency during storage (Fig. 5c). Fig. 5 (d and e) confirms that the best smell and overall acceptability scores were for samples formulated with PSH hydrocolloid (PSH, GG-PSH and HMP-PSH). The correlation analysis showed that the smell values were positively associated with overall acceptability of Dooghs (r^2 = 0.967) formulated with the different formulations. Finally, it can be concluded that use of combination of PSH and HMP rather than their application alone had a desirable effect on the sensory characteristics of samples.

4 Conclusion

The present study showed that use of PSH, HMP and GG did not affect pH values of the developed Doogh samples during storage compared with the control sample. However, these hydrocolloids affected the attributes of consistency, sensory and instability rate. Addition of PSH separately and in combination with HMP increased the consistency, showed a dramatic reduction in the rate of phase separation and improved sensory properties of the produced Dooghs in comparison with other formulations prepared with the tested hydrocolloids. This effect was more happened in the samples containing PSH. In general, the use of PSH with HMP and GG can be recommended for improving the critical quality properties of Doogh and other acidified dairy drinks. This research provided a theoretical basis for Doogh processing; however, further studies is required due to the complex ingredients and reactions involved during Doogh processing and storage.

Acknowledgments

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Nomenclature

PSH	Psyllium seed hydrocolloid
HMP	High methoxyl pectin
GG	Gellan gum
TG	Tragacanth gum
IBS	Irritable bowel syndrome
D_{32}	Surface-weighted mean diameter
m	Consistency index
n	Flow behaviour index
S_{xy}	Standard error
R^2	Coefficient of determination

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Figures legend

Fig. 1. The instability rate (a) and pH value (b) of the different formulations of Doogh in this study

Fig. 2. Rheological behavior curves of Dooghs formulated with GG (∆), PSH (▲), HMP (○), HMP-PSH (■), HMP-GG (♦), and GG-PSH (●) in comparison to the control sample (◊)

Fig. 3. Light microscopy images of the Dooghs containing PSH (a), HMP-PSH (b), HMP-GG (c), and GG-PSH (d)

Fig. 4. Comparison of particles size distribution of Dooghs stabilized by HMP (□), PSH (○), GG (◊), HMP-PSH (▲), HMP-GG (■), and GG-PSH (●) with the control sample (∆)

Fig. 5. Evaluation of sensory attributes (a, taste; b, consistency; c, appearance; d, smell; e, overall acceptability) of the different formulations of Doogh during the storage time

Table 1. Microbiological analysis of the different formulations of Doogh during the storage time

Doogh type	Microbiological analysis (Mean±SD)					
	1st Day		7th Day		21th Day	
	Coliforms (Log 10 CFU/mL)	Mold and yeast (Log 10 CFU/mL)	Coliforms (Log 10 CFU/mL)	Mold and yeast (Log 10 CFU/mL)	Coliforms (Log 10 CFU/mL)	Mold and yeast (Log 10 CFU/mL)
HMP	-	0.21±0.08	0.75±0.41	1.25±0.10	1.30±0.41	2.06±0.23
GG	-	0.37±0.12	-	0.89±0.05	0.80±0.25	1.74±0.15
PSH	-	-	-	0.45±0.05	0.47±0.12	2.55±0.38
HMP-GG	-	-	-	0.37±0.13	-	3.48±0.42
HMP-PSH	-	-	-	0.22±0.01	-	0.70±0.19
GG-PSH	-	-	-	0.20±0.04	-	0.89±0.28
Control	-	0.62±0.02	1.90±0.37	0.40±0.11	2.83±0.37	1.09±0.04

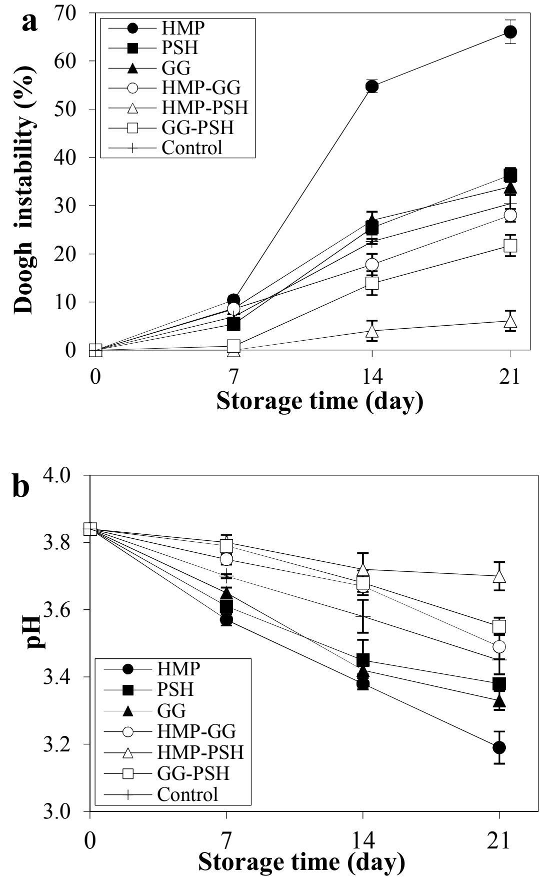
Table 2. Particle size, zeta potential and power law parameters for flow behavior curves of the developed Dooghs

Sample type[a]	Rheological parameters			$(R^2)^c$	$S_{xy}{}^d$	Particle size characteristics	
	m (Pa s^n)	*n*	Viscosity (Pa.s)[b]			D_{32} *(μm)*	*ξ-potential (mV)*
HMP	0.72[c]	0.81[b]	0.00204[d]	0.999	0.05	6.26±0.05[c]	-2.40±0.02[c]
PSH	0.83[c]	0.64[c]	0.00643[b]	0.989	0.02	36.4±0.31[a]	0.12±0.01[b]
GG	17.27[a]	0.65[c]	0.02880[a]	0.978	0.21	28.71±0.42[ab]	-0.32±0.01[b]
HMP-GG	0.80[c]	0.68[c]	0.00598[b]	0.984	0.16	8.39±0.79[c]	-0.21±0.01[b]
HMP-PSH	0.87[c]	0.98[a]	0.00690[b]	0.998	0.34	7.82±0.83[c]	-7.10±0.02[d]
GG-PSH	1.78[b]	0.73[c]	0.00480[c]	0.999	0.09	22.63±0.64[b]	0.45±0.02[b]
Control	1.70[b]	1.00[a]	0.00493[c]	1.000	0.07	5.39±0.26[c]	11.91±0.03[a]

[b] Values in the same columns followed by different letters (a-d) are significantly different ($p < 0.05$)

[b] Viscosity determined at constant shear rate of 0.416 s^{-1}

[c,d] R^2 and S_{xy} are determination coefficient and standard error, respectively.



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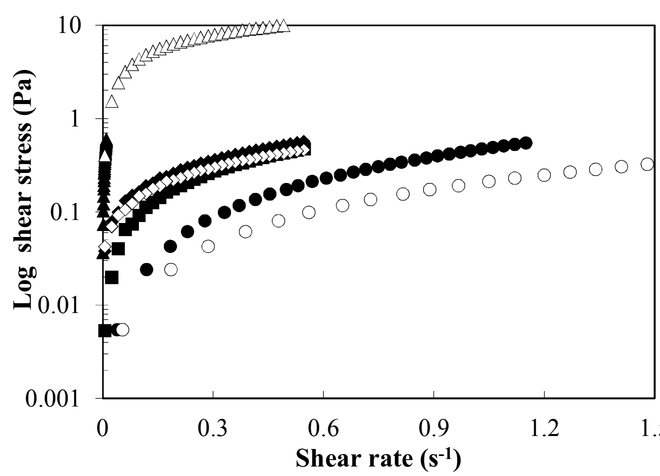

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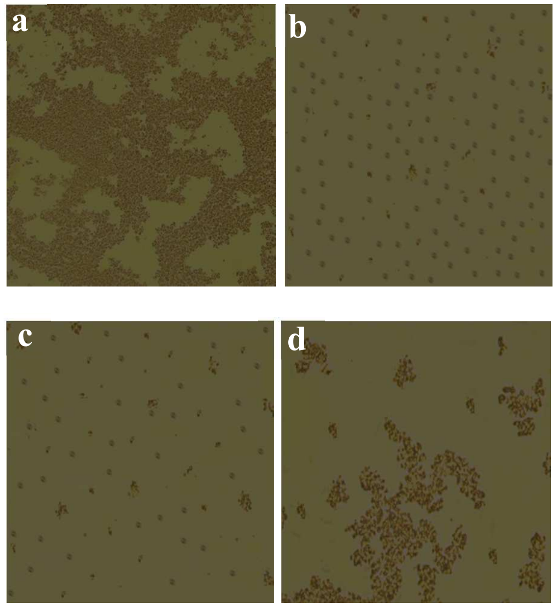

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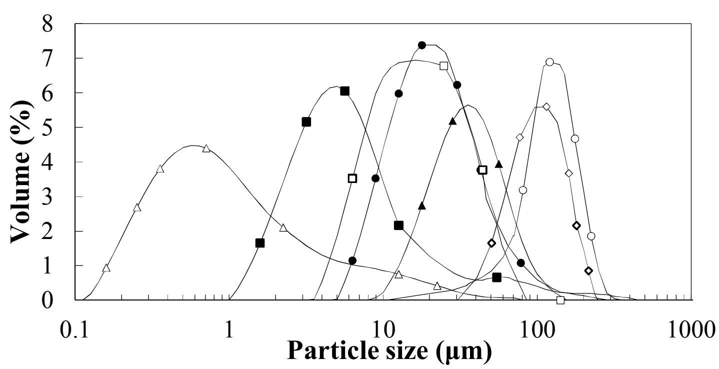

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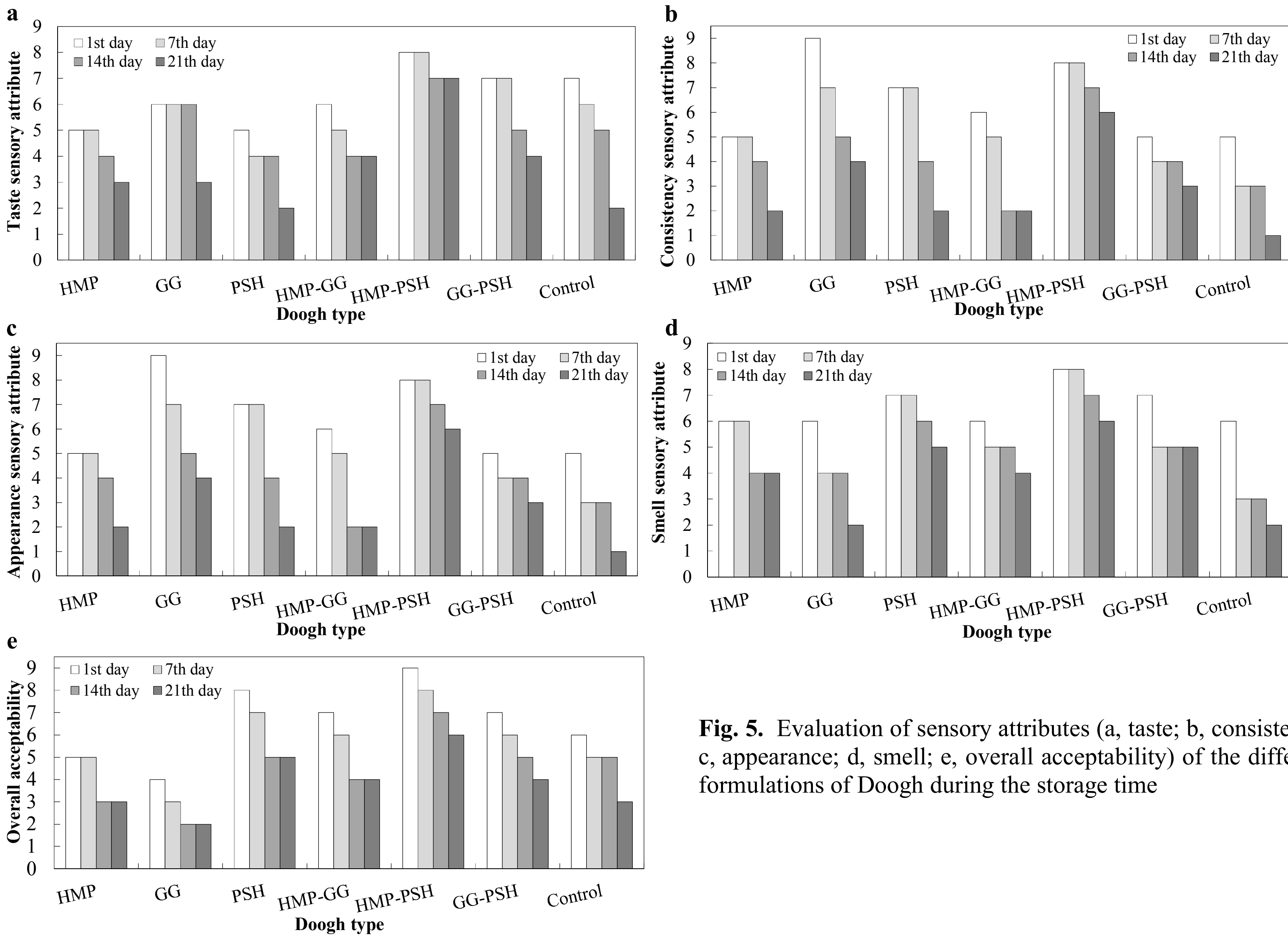

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