Comparative study on effect of pectin, gelatin and modified starch replacement with fish gelatin in textural properties and graininess of Non-fat yogurt

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*Abstract_* **This study aimed to evaluate the quality of set style yogurt with replacing skim milk powder with high bloom gelatin (HBG), low bloom gelatin (LBG), cold water fish skin gelatin (FSG), low methoxyl pectin (LMP) and modified tapioca starch (MS), and effect of these thickeners on textural and physicochemical properties of samples. Firmness is one of the major sensory attributed important to customer preference. Because of low total dry material (9%) of raw milk lower concentration of thickeners didn't have suitable effect on textural properties of samples, higher value of textural parameter related to firmness and fracturability was found in samples contained 1% HBG. Viscosity of samples increases with addition of thickeners and have a straight relationship with stabilizer concentration and viscosity, in HBG contend samples slope of viscosity vs. concentration curve was highest and maximum viscosity obtained with 1% HBG addition. Except pectin all samples pH decreased over time, but MS significantly increased acidification process. All kinds of, gelatins and modified starch decrease synersis, but a reverse correlation between pectin concentration and synersis. Also all of types of gelatin decreased number of grains and grain perimeter but starch significantly increased graininess in all concentrations (p<0.05). Graininess trend in samples contained LMP decreased up to 0.05% but, higher concentration of LMP lead to more graininess. However FSG showed the best effect on decreasing of graininess**.

Keywords-component; textural properties, Non-fat yogurts, replacement, Gelatin, pectin, modified starch

INTRODUCTION

In recent years health concerns have led development of customers interests to reduce consumption of high fat contain foods, which cause open way to growing markets of healthier foods, with good mouth feel, natural ingredients and lower in fat (Lobato-Calleros et al., 2004), also because of Nutritional, remedial and also low caloric characteristics of low-fat and non-fat yogurt , its consumption significantly increased. Yogurt defines as composite gel that constitute three-dimensional casein network aggregated through isoelectric precipitation that brought with acid bacteria action and act as basic structure of yogurt, and filled with denatured serum proteins and fat globules. It can say fat globules acting as structure promoters of protein network in yogurt. The development of elastic gel with solid-like behavior causing changes in micelle structure due to solubilization of calcium phosphate during fermentation (Aguirre-Mandujano et al., 2009; Purwandari et al., 2007). Therefore, particular composition and structural arrangement of raw milk are responsible for palatability of yogurt. Texture is one of the most important properties that determine yogurt quality and customer satisfying (Crion et al., 2012). Reduction or elimination of fat from raw milk hardly affects physical and textural properties. Texture and stability of yogurt significantly affects by starter culture and manufacture condition of yogurt too (LAl et al., 2006). Lobato-Calleros et al., 2004 reported that yogurt produced by varying levels of protein, fat and hydrocolloids provides a wide range of consistency and brittleness (Lobato-Calleros et al., 2004). Sandoval-castilla et al., (2004) reported fat reduction in yogurt significantly decrease tension and firmness of product (Sandoval-castilla et al., 2004). Low fat desirable yogurt can achieve by change in formulation e.g., use of fat replacers, dairy ingredients and carbohydrates or controlling production conditions such as heating temperature (Torres et al., 2012).

To improve texture in low-fat yogurt one must seek the reinforcement of protein network to build up the structure. Therefore, large numbers of investigators have studied making change on formulation of low fat yogurt to improve its acceptability with different hydrocolloids (Aguirre-Mandujano et al., 2009; Sahan et al., 2008; Decourcelle et al., 2003; Amiri aghdai et al., 2010; Razmkhah sharabiani et al., 2010) and various dairy based protein concentrations (Lobato-Calleros et al., 2004; Sandoval-castilla et al., 2004; Matumoto-Pintro et al., 2011; Amatayakul et al., 2006; Guzmán-González et al., 2000; Marafon et al., 2011). Hydrocolloids are widely used in food industry as thickeners, stabilizer and gelling agent to improve textural properties of products. Particularly stabilizer in yogurt use to improve consistency and reduce synersis. The common stabilizers in yogurt manufacture include gelatin, pectin, starch, alginate, carrageenan, Arabic gum, locust beam gum, karaya, Xanthan and like them. Gelatin is one of the natural, multifunctional and popular hydrocolloids with wide range of applications and no limitation in use; the main sources of gelatin include cattle and bovine slaughterhouse by-products like skins, tails, hides and bones. Although gelatin has unique applications pessimism and concerns still persist among some of customers, this pessimism is mainly due to religious constraints (such as Jewish and Muslims limitations to use pig and non-religious slaughtered animals, Hindus prohibition in cow related products) and increasing vegetarian/vegan movements, in addition after outbreak of mad cow disease (bovine spongiform encephalopathy, BES) in 1980s and increasing concern about animal tissue-derived like collagen and gelatin capability for transmitting pathogenic vectors, increasing interest has been paid to find and use a suitable alternative substitutes. Skins and bones form fish processing plants are the best sources introduced for gelatin extraction. Two major advantages of marine sourced gelatins are these they don't have risk of BES out break and also acceptable for both Islam and

Judaism (Karim et al., 2008 and 2009; Kittaphattanabawon et al., 2010). Gelatin, corn starch, modified starch, and pectin are common stabilizers in dairy industries and there is large numbers of researches that studied on effects of these thickeners on microstructural, rheological, textural and physicochemical properties of yogurt. They find out these thickeners significantly increase firmness and deformability and reduce wheying- off (Fisezman et al., 1999; Gonçalvez et al., 2003; Kim et al., 2009; Supavititpatana et al., 2008). But little works have been done to date on effect of marine sources gelatin as a new source of gelatin on yogurt properties. Therefore, this study was aimed to develop of marine gelatin as thickener to improve low caloric yogurt.

MATERIALS AND METHODS

Commercial skim milk powder (fat contain lower than 0.5%) (Pegah dairy Inc. Tehran, Iran), Low bloom (bloom 170) and high bloom (bloom 390)gelatins (Merck, Germany), cold water fish skin gelatin (FSG)(bloom 140) (Sigma Chemical company, St. Louise, Missouri, USA), LMP (CP Kelco, Denmark) and modified starch (esterified cross link tapioca starch)(Prokar, Turkey) , were purchased and used without future purification. All chemical used were of analytical grade. .

Yogurt manufacture

Skim milk powder prepared from reconstituted at 35$\pm$1°C with moderate mixing. Gelatins (LBG, HBG, and FSG), LMP and MS were hydrated separately. Different samples contained a:0.25%, b:0.5%, c:0.75% and d:1% various gelatin and modified starch and a:0.05%, b:0.15%, c:0.25% and d:0.35% LMP and blank sample were prepared and total solid of milk with thickener adjust at 9%. Thus 21 batch of yogurt were made in total. The mixtures were separately homogenized using an Ultra Turrax blender (T25, IKA, Merck, Germany) at 24,000rmp for 5min. Then homogenates were heated at 95°C for 5min. This heat treatment causes firmer texture and increases storage modulus in yogurt than unheated (3), samples subsequently cooled to fermentation temperature (43°C) in cold water bath. Formulated milk mixed with direct vat set culture[1] (YX-11, Chr. Hansen, Hamilton, New Zealand) and dispersed into plastic cups, ca. 100 g, and incubated at 42.5$\pm$1 °C until pH 4.5-4.6. Following incubation all samples moved to 4 °C for 17 hours. After this time experiments was repeat three times.

Physicochemical properties measurements

PH and acidity measurement

The pH was measured 17 hours after incubation using previously calibrated digital PHmeter (Jenway, 3505, Uk). Titratable acidity was measured according to AOAC method (AOAC International, 1997), using NaOH 0.1 N, approximately 9gr of sample which diluted with approximately the same volume of distilled water and use phenolphthalein solution as an indicator. Titrable acidity was expressed as percentage of lactic acid using the following Eq. (1).

$$\text{Lactic acid}(\%) = \frac{0.1\text{M NAOH(mL)} \times 0.009}{\text{sample (g)}} \times 100 \quad (1)$$

Water Holding Capacity and Synersis

Rearrangement and drainage of acid inducted casein network in yogurt occurs during storage. For measuring synersis, whey which separated cooling without any change in yogurt structure removed. The relative amount of whey drained off was calculated as synersis index per 100 gr of initial sample.

Water holding capacity (WHC) measured according method described by Sahan et al.,2008, 5 g of yogurt was centrifuged at 4500rmp for 30min, after centrifuging the supernatant was removed and the peller was collected and weighted, the WHC was calculated according followed Eq. (2):

$$\text{WHC} = [1 - \frac{w_t}{w_i}] \times 100 \quad (2)$$

Where w_t is weight (g) of pallet and w_i is initial weight (g) of sample. (sahan et al., 2008)

Penetration test

Tests were performed according method described by Fisezman (Fisezman et al., 1998) with a TA-XT2 texture analyzer (model CT3, Brookfield Engineering Laboratories. Middleboro, MA, USA). About 100gr of samples were incubated in cylindrical jars and without remove samples from their jars at 4°C test was performed. A cylindrical flat probe with 12.7 mm diameter was used at a speed of 1 mms^{-1} for 25mm penetration. Following parameters was records:

- Fracturability (g): the first significant discontinuity in the curve as the plunger penetrated 25 mm.
- Firmness: maximum force (g) occurring at the end of penetration (12).

Viscosity

Viscosity is defined as resistibility of material against deformation and especially in yogurt indicates slimy fluid of samples (LAl et al., 2006). Viscosity was measured using oscillatory viscometer (Myr, V2L, Viscotech, Spain) that equipped with Brookfield circulator (Brookfield Engineering Laboratories. Middleboro, MA, USA) and a concentric cylindrical double skin device to control temperature at 4°C with L4 spindle rotation of 30rmp. Small sample adaptor has been applied and about 18ml of samples were transfers into cup and for relative comparison between treatments viscosity reading was taken at the point of 20th minute of experiment.

Graininess

Graininess was defined as the number and mean perimeter of grains those measured according method described by Küçükçetin et al., 2009. A glass plate (92×150×6 mm) was surrounded with two metal bars with height of 0.6 mm fixed aside the glass as frame. The yogurt was poured onto glass plate and spread by hand using a metal bar to form continues yogurt layer with 0.6 mm thickness through the frame. The glass plate was moved to dark chamber and placed on an illuminated plate. Image of transmitted sample was taken with a digital camera (PC1620, Canon, Japan) with resolution of 4000× 3000 and 256 gray scale color depth. The image analysis was performed with Adobe Photoshop CS2, version 9 (adobe systems incorporated, USA) and Image J 10.40g softwares (Wayne Rasband, National Institutes of Health, USA). The number of samples indicating a perimeter greater than 0.5mm and mean perimeter of grains were evaluated. All measurements were performed in duplicate.

[1] *Streptococcus thermophilus and Lactobacillus bulgaricus*

Statistical analysis
Results were evaluated statically using SPSS package program version 19. Also differences between means were determined by Duncan's multiple range tests at a level of 0.05.

RESULTS AND DISCUSSION

pH and titratable acidity
As shown in table 1, pH value for most of samples reduced during cooling and ranged from 4.29 to 4.65 (table 1) with 0 to 0.26 decrease in pH, in samples contain various type of gelatin there is no significant different were noted, but according the table 1, pectin prevented pH reduction during storage. Pectin supposes prohibiting lactic acid Bactria action and also significantly increases coagulation time. Thus additions of pectin may cause reduction in sensory properties of yogurt. Decourcelle et al., 2003 and Pang born et al., 1974 found a decrease on aroma perception by increase pectin concentration. Modified starch absolutely increase pH reduction and decrease coagulation time, it can be because of starch composition that influence lactic acid bacteria growing rate.

Synersis and water holding capacity
Whey separation is an important defect in yogurt qualification. That occurs due to shrinkage of the gel and appeares as whey on the gel surface of set type yogurt that is defined as synersis (Sahan et al., 2006). All kind of used stabilizers significantly decreased synersis except for LMP. LMP significantly increased synersis. Evertt et al., 2005 reported that in high levels of LMP the flow units of network are increasingly covered by pectin and the aggregates are partially statically stabilized, therefore casein network begins to lose structural integrity and expels serum phase. This lead to increased synersis and decreased WHC. Other stabilizers connect the granules and chains of milk proteins and provide continues and homogeneous doubled network structure with minimum free ends, this more interconnected network would binding aqueous phase more efficiently. These results agreed with those of Fiszman et al., 1997; Amir aghdai et al., 2010; Razmkhah sharabiani 2010; Sahan et al 2006; Gonçalvez et al., 2003 who reported the effects of thickeners on synersis reduction.

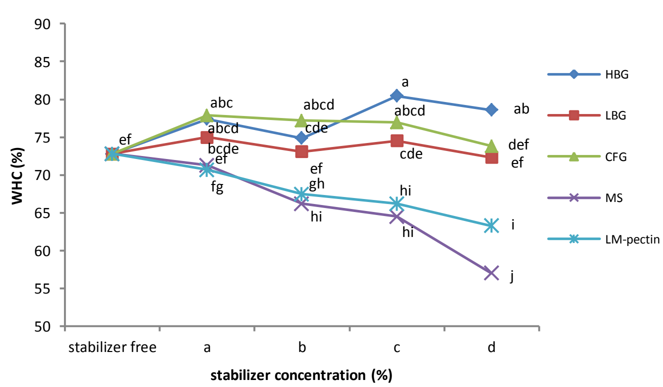

Figure 1. Effect of different stabilizers in various concentrations (a: 0.25% , 0.5%. 0.75% and 1% for LBG, HBG, FSG and MS- a:0.05, b:0.15, c:0.25 and d:0.35% for LMP) on synersis.

WHC is defined as drainage occurs during apply stress and indicates protein network resistance against shear stress. WHC ranged from 57% to 80%, all types of gelatin had positive effect on WHC. Yogurts contained HBG demonstrated maximum resistance to shear, and lowest WHC was in MS contained samples. It indicated that modifies starch constituted lean links with casein network those easily broken with stress.

Texture analysis
In lower concentration of all stabilizers reduced fracturability and firmness in samples, due to in absent of fat as a structure promoter of protein network, reduction of casein contain and replace it with thickeners in low concentrations make a swoon network and hand low value of thickeners couldn't sustain casein network (Sandoval-castilla et al., 2004). In low values of thickeners formed smaller number of junction points in protein network, and much more open structure in the samples would be contribute to lower firmness(Fisezman et al., 1997). Maximum loss in firmness was occur in 0.25% FSG contained yogurt, in this samples the WHC was reduced with addition of thickener, thus bloom of FSG is not a suitable stabilizer to improve texture of non-fat yogurt, and network that processed with replacement of dry material of milk with FSG had lower density. It supposes that FSG as a thickener is unable to binding free water in protein network, therefore increase WHC and significantly decreases firmness and fracturability of the samples. Fiszman et al., 1997 reported addition of gelatin caused increase firmness of yogurt in 12.5% and 14% day mater content.

Apparent viscosity
Viscosity is the strength of the gel resistant to breaking. Because of shear thickening properties of Yogurt as a thixotropic gel, its viscosity often decreases during mixing, and also recovers a part of the original structure and increase after cessation of shearing. The trend of apparent viscosity of samples contained various amounts of stabilizers, is shown in Fig 1. The graphs indicate that thickeners increase gel resistant and increase breaking strength and viscosity. Apparent Viscosity had a direct correlation with stabilizer concentration. Its thickeners assumed that binding with free water and trap it in casein network thus increase viscosity of sample (Sahan et al., 2006). Therefore after 20min stress lowest the viscosity (5430 pa.s) obtained in thickener free sample, and the highest viscosity (12500 Pa.s) obtained in samples containing 1% high bloom gelatin. Fish skin gelatin had lower effect on apparent viscosity (table 3). (Sahan et al., 2006; Fiszman et al., 1997 and Amir aghdai et al., 2010 obtained same result.

Graininess
The number of grains, total area and mean perimeter of grains of yogurt one day after production were calculated and varied from 20 to 48514, 50 to 174602 mm^2 and 2 to 8.67 mm per 6 gr sample, respectively (fig 4, and table 3). The number and the total area of grains determined for modified starch containing yoghurt was significantly ($p<0.05$) higher than other samples, therefore because of high amount and total area of grains in MS added yogurts, statistical software didn't indicate significant different between other samples grains values, but regardless of MS contained samples and in comparison of other samples Fish skin gelatin significantly decrease number of grains and their total area, but highest perimeter of grains was in this samples. All types of gelatin decreased graininess, but only in low concentration LMP reduced the graininess. Also increasing of LMP amount graininess

increased too. The highest mean perimeter of grains obtained in FSG added samples, but this different wasn't significant ($p<0.05$).It seems that in fat absent various gelatins fill the gaps formed by β-lactoglubolin filament, resulting in a reduction of surface hydrophobicity and reduce granulation, also gelatin as an emulsifier with increase hydrophilic surface in network increase the water absorbance in network thus reduce synersis. Pectin and modified starch had reverse effect. Increasing in hydrophobic surface caused increasing in grain formation and synersis. Graininess results confirm synersis test results.

Conclusions

Various strategies exist to improve the gel stability of set-yogurt, such as increase total solids of milk, etc., stabilizers are common added to control textural defects and increase consistency in yogurt. This study indicated that addition of some of stabilizers without increase in total solid (9%total solid) of improved properties of yogurt, but the other have undesirable or insignificant effects. As the level of HBG increased improved the firmer network and in addition made a smoother texture, but other stabilizers replacement cause undesirable changes on textural properties or doesn't have significant effect on them, it can be explained that these samples total solid contained of yogurt (9%) was lower than total solid contain of common milk (12.5%). Therefore, it wasn't product strong casein network. Also, viscosity trend during time for all kinds of stabilizers was increasing, and except pectin other kinds of additives decrease synersis and all types of gelatin make a smother texture. In this study, cold water fish skin gelatin was a new additive for yogurt, this kind of gelatin significantly reduces graininess and synersis of samples, but because of low gel strength of this has bad effects on textural properties of yogurt. Generally reduction of total solid causes some defects in texture of yogurt, but stabilizers can surmount some of these defects.

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Table 1.pH and acidity variation during cooling

	Initial pH				**pH reduction**				**Acidity (%)**				**Coagulation time**
	a	**b**	**c**	**d**	**a**	**b**	**c**	**d**	**a**	**B**	**c**	**d**	
HBG	4.45	4.5	4.58	4.54	0.15^{abcd}	0.093^{bcd}	0.063^{cd}	0.06^{cd}	0.825	0.78	0.755	0.76	4:32
LBG	4.62	4.55	4.49	4.55	0.07^{cd}	0.093^{bcd}	0.103^{bcd}	0.09^{bcd}	0.755	0.885	0.825	0.745	3:50
FSG	4.49	4.6	4.58	4.48	0.103^{bcd}	0.033^{d}	0.063^{cd}	0.106^{bcd}	0.885	0.76	0.85	0.895	3:45
MS	4.43	4.42	4.35	3.28	0.236^{ab}	0.25^{a}	0.226^{ab}	0.213^{abc}	0.81	0.74	0.86	0.9	3:28
LM-P	4.64	4.61	4.59	4.6	0.01^{d}	0^{d}	0.003^{d}	0.006^{d}	0.84	0.86	0.84	0.74	5:22
Stabilizer free	4.6				0.023^{bcd}				0.76				4:30

Table 2. Effect of stabilizers on textural properties of samples (a: 0.25% , 0.5%. 0.75% and 1% for LBG, HBG, FSG and MS- (a:0.05, b:0.15, c:0.25 and d:0.35% for pectin).

	fracturability				**firmness**			
	A	**b**	**c**	**d**	**a**	**b**	**c**	**d**
HBG	14^{ijk}	22^{ef}	34.33^{b}	59.66^{a}	22^{efg}	26.33^{ef}	40.33^{c}	55^{b}
LBG	21.33^{efg}	15.66^{hi}	15.33^{hij}	27.33^{c}	25.33^{ef}	25.66^{ef}	23^{efg}	32.33^{d}
FSG	10.33^{l}	12.66^{ijkl}	10.5^{l}	12^{kl}	16.33^{h}	18^{gh}	16^{h}	18.33^{gh}
MS	20.33^{fg}	18^{gh}	12.33^{jkl}	25.33^{cd}	20.22^{fgh}	25.33^{ef}	22.66^{efg}	25.33^{ef}
LMP	19.33^{fg}	10.66^{l}	19.33^{fg}	23.66^{de}	27.33^{de}	21.66^{efgh}	83.66^{a}	32.33^{d}
Stabilizer free	20.25^{fg}				32.5^{d}			

Table 3. Effect of stabilizers total area and mean perimeter of grain of samples (a: 0.25% , 0.5%. 0.75% and 1% for LBG, HBG, FSG and MS- (a:0.05, b:0.15, c:0.25 and d:0.35% for pectin).

	Total area				**Mean Perimeter**			
	A	**b**	**c**	**d**	**a**	**b**	**c**	**d**
HBG	354.5 ^{c}	389 ^{c}	463.5 ^{c}	342 ^{c}	2.983 ^{a}	2.909 ^{a}	2.635 ^{a}	2.936 ^{a}
LBG	561 ^{c}	503.5 ^{c}	496 ^{c}	254.5 ^{c}	2.813 ^{a}	2.496 ^{a}	2.474 ^{a}	3.014 ^{a}
FSG	132 ^{c}	247.5 ^{c}	267 ^{c}	232.5 ^{c}	2.735 ^{a}	2.813 ^{a}	2.46 ^{a}	3.955 ^{a}
MS	785 ^{c}	74069 ^{b}	130823 ^{a}	6455 ^{b}	5.34 ^{a}	4.222 ^{a}	2.813 ^{a}	2.18 ^{a}
LMP	1033.5 ^{c}	1387.5 ^{c}	1117 ^{c}	2170.5 ^{c}	3.344 ^{a}	2.959 ^{a}	2.769 ^{a}	2.813 ^{a}
Stabilizer free	1909.5^{c}				2.813^{a}			

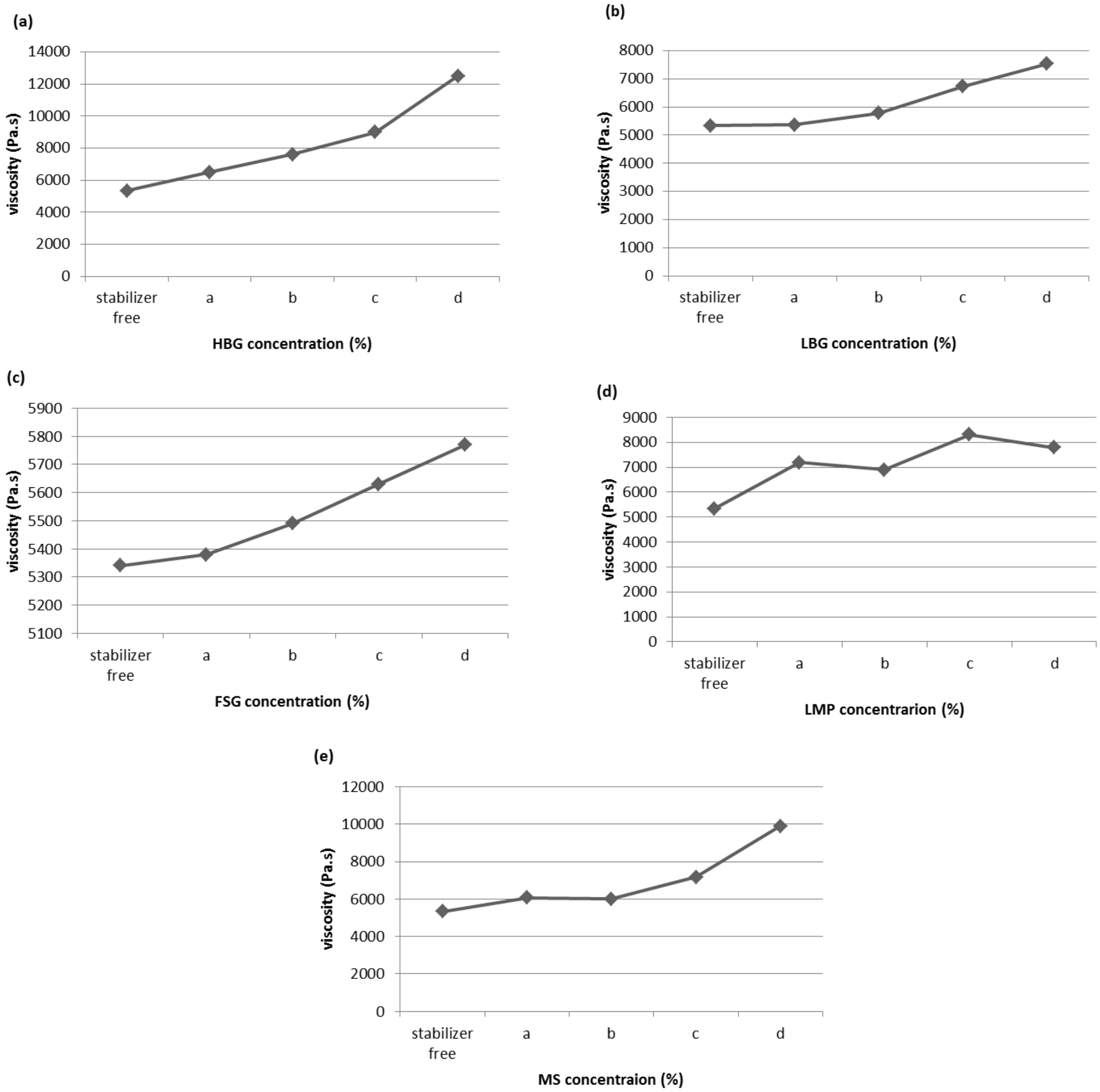

Figure 3. Effect of stabilizers on viscosity trend of samples (a: 0.25% , 0.5%. 0.75% and 1% for LBG, HBG, FSG and MS- (a:0.05, b:0.15, c:0.25 and d:0.35% for pectin), (a)high bloom gelatin, (b)low bloom gelatin, (c) fish skin gelatin, (d)low methoxyl pectin and (d) modified starch.

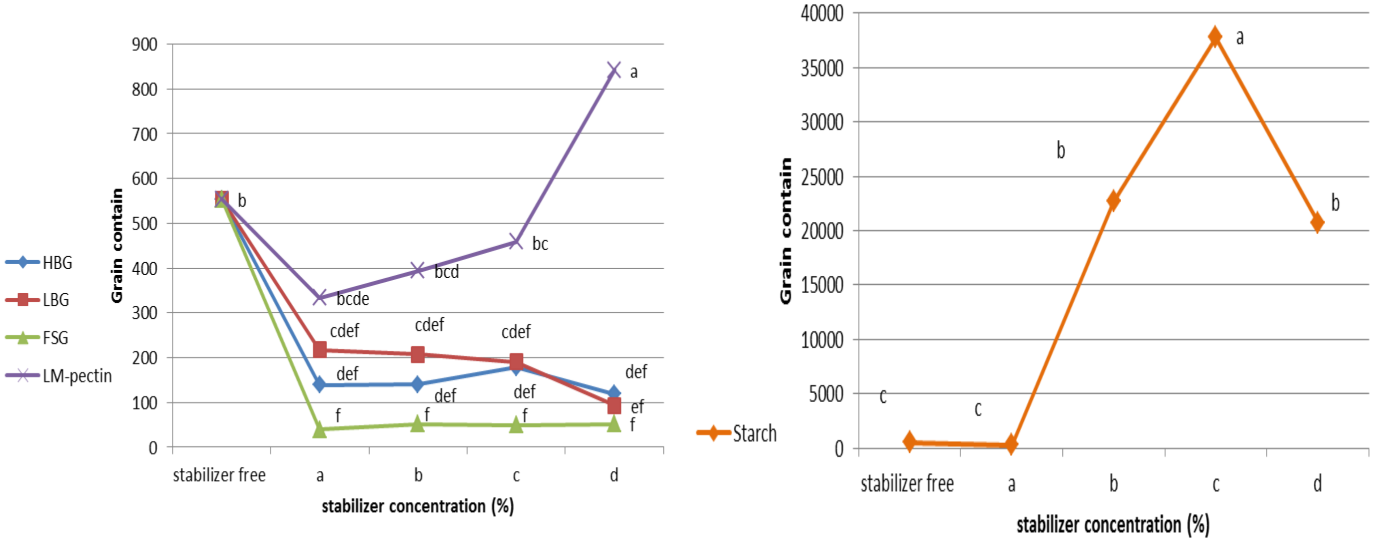

Figure 4. Stabilizers effect on graininess (a: 0.25% , 0.5%. 0.75% and 1% for LBG, HBG, FSG and MS , a: 0.05%, b: 0.15%, c: 0.25% and d :0.35% for pectin).