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Research Article
Functional Properties of Bovine Bone Gelatin and Impact on Physicochemical, Microbiological and Organoleptic Quality of Set Yogurt

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Abstract

Background and Objective: Improvements in the shelf life of yogurt can be brought about by addition of gelatin not only increase its nutrient content but also improve its properties. The objective of this study was to extract the gelatin from bovine bone, characterize and understand their functional properties and to study the effect of its incorporation on the quality of yogurt. **Materials and Methods:** Gelatin was extracted from bovine bones after their characteristics and functional properties were analyzed in comparison with commercial gelatin (CG). The effects of bovine gelatin (BG) addition on properties of yogurt added with bovine gelatin (YABG) were studied. **Results:** The yield of BG was 6.32±0.20% and the pH of BG was 9.63±0.01. It was observed that BG and CG had higher solubility at low pH with a maximum value observed at pH 4. A significant effect ($p<0.01$) of ionic strength was observed. Increasing the NaCl concentration to more than 2% resulted in a significant decrease of the solubility. BG showed higher foaming expansion (FE) and higher foaming stability (FS) than CG. Increasing the concentration of BG and CG decreased the emulsifying activity index (EAI) but increased the stability index (ESI). Significant effects of BG rate on acidity and pH of YABG were observed. Viscosity of YABG was increased significantly ($p<0.01$) with increasing the BG rate. YABG added with 1.5% of BG recorded the highest viscosity. In addition, there was a significant effect of BG addition on *Streptococcus thermophilus* counts. According to sensory properties, addition of BG had significant effect on adhesiveness, cohesiveness and taste of the YABG. Sensory results indicated a preference for YABG with 1.5% of BG. There was no significant effect of BG on the odour and aftertaste of YABG. **Conclusion:** The bovine bone could serve as raw material for the extraction of gelatin with desired functional. The addition of 1.5% of this gelatin had a considerable effect on the physiochemical properties and the texture of YABG.

Key words: Gelatin, yogurt quality, functional properties, conservation, extraction, fermentation, post-acidification

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Yogurt is fermented milk made by the protocooperative action of two homofermentative bacteria *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*[1-3]. It is a popular product throughout the world [4] and it is accepted by consumers because of its flavour and aroma, mainly acetaldehyde and texture[1] especially among health conscious consumers, since it has nutritional benefits. Texture is a prime characteristic of yogurt quality and the addition of a stabilizer, functioning as a gelling agent or thickener, such as gelatin or other hydrocolloids has been shown to provide good stability and desirable texture. Among these additives the gelatin, widely used in food, is a soluble protein compound obtained by partial hydrolysis of collagen, the main fibrous protein constituent in bones, cartilages and skins[5,6]. It is one of the most popular biopolymers, it is used in food because of its functional and technological properties[7]. Gelatin can be used as a foaming, emulsifying and wetting agent in food, pharmaceutical, medical and technical applications due to its surface-active properties[8,9]. Gelatin improves the texture of yogurt. As a result, it gives a firmer product with less tendencies to syneresis. This effect has been attributed to the interaction of gelatin with the casein matrix of yogurt to develop a stronger three-dimensional network[10]. Generally, the traditional sources of gelatin are bovine and porcine skins and bones. With pig's skin, the derived gelatin was accounted for the highest (46%) output, followed by bovine hides (29.4%), bones (23.1%) and other sources (1.5%)[11]. A number of studies have addressed properties of fish gelatin[6,8,9,12,13]. Gelatins from land animal sources are preferred over marine sources due to their superior gel strength, melting point and viscosity[14].

In slaughterhouses and meat processing plants, not only fresh meat but also fresh bone material is obtained. A small portion of the bone material obtained is supplied, like the meat, to butchers for sale as soup bones. However, the major part of this valuable source of collagen goes as wastes. Production of gelatin from ossein is the better way of utilisation of the processing wastes from the meat industry. With this background, the present study was undertaken to extract the gelatin from bovine bone, characterise and understand their functional properties in comparison with commercial gelatin. The effects of bovine gelatin on the physicochemical and sensory properties of set yogurt were evaluated.

MATERIALS AND METHODS

Preparation of bovine bone: Bovine bone was obtained from different butchers of Mostaganem, Algeria (April, 2014-March, 2016). The bone material was cut into small pieces and degreased by washing it for 30 min with hot water (85-90°C). This process completely removed any residual fat. Prepared bone was then placed in polyethylene bags and stored at -20°C until use.

Gelatin extraction: Before gelatin extraction, the prepared bone was treated with dilute (2%) hydrochloric acid HCl (analytic grade) for 48 h at room temperature to remove non-collagenous proteins. Acidic-treated bone was washed with tap water until the wash water was neutral. The bone was then soaked in 1 N sodium hydroxide NaOH (analytic grade) for 48 h with gentle stirring at 4°C. The alkaline solution was changed every 12 h to swell the collagenous material in the bone matrix. Alkaline-pretreated bone was washed thoroughly with tap water until wash water became neutral. During these periods, the calcium phosphate and calcium carbonate, which are bound to the bone material and provide it with its firmness, are converted into their soluble forms. On completion of the demineralization, all that is left is the proteinaceous structural framework of the bone: The actual raw material or ''ossein'' that is used for the extraction of gelatin[15]. The final extraction was carried out in 3 volumes of distilled water at 100°C for 90 min. The clear extract obtained was filtered with Whatman filter paper (No. 1). The filtrate was then kept in a tray and dried in oven at 40°C for 24 h. The thin film of dried matter was powdered, weighed, packed and stored at ambient temperature (25±2°C) for further study.

Gelatin analyses

Yield of gelatin: The yield of the gelatin obtained was calculated according to Jridi *et al.*[8]:

$$\text{Yield (\%)} = \frac{\text{Weight of dry gelatin (g)}}{\text{Weight of initial dry bone (g)}} \times 100$$

Ash and moisture content: The moisture content was determined by drying in an oven at 100°C. for 4 h. The ash's contents were determined by incinerating the dry matter in a muffle furnace at 600°C, according to the AOAC method[16].

Determination of pH: The pH value of BG and CG was measured using the British Standard Institution method (BSI)[17]. For the gelatin solution, a 1.0% (w/v) gelatin solution was prepared by adding 1 g of gelatin in 99 mL of distilled water. The mixture was heated to 45°C for 5 min, for dissolving gelatin. The solution was allowed cool down to room temperature before pH measurement. The pH was measured using a pH meter.

Solubility: The solubility of BG and CG was determined by the method of Singh *et al.*[9]. The gelatin was dissolved in 0.5 M acetic acid (analytic grade) to obtain a final concentration of 3 mg mL^{-1} and the mixture was stirred at 4°C for 24 h. Thereafter, the mixture was centrifuged at 5000 rpm for 15 min at 4°C. The supernatant was used for solubility study.

Effect of pH on solubility: The pH of gelatin solution (3 mg mL^{-1}, 8 mL) was adjusted with either 6 N NaOH (analytic grade) or 6 N HCl (analytic grade) to obtain the final pH ranging from 1-10. The volume of solution was made up to 10 mL by deionised water previously adjusted to the same pH as the gelatin solution. The solution was centrifuged at 5000 rpm for 30 min at 4°C. Protein content in the supernatant was determined by the Biuret method[18] using bovine serum albumin as a standard. Relative solubility was calculated in comparison with that obtained at the pH giving the highest solubility.

Effect of NaCl on solubility: Gelatin solution (3 mg mL^{-1}, 5 mL) was mixed with 5 mL of NaCl (analytic grade) in 0.5 M acetic acid at various concentrations to give the final concentrations of 0, 1, 2, 3, 4, 5 and 6%. The mixture was stirred continuously at 4°C for 30 min, followed by centrifuging at 5000 rpm for 30 min. Protein content in the supernatant was measured[18] and the relative solubility was calculated as previously described.

Foaming properties: Foam expansion (FE) and foam stability (FS) of BG and CG are determined as described by Nagarajan *et al.*[13] with a slight modifications. Fifty milliliters of gelatin solution (2% w/v) was transferred into 250 mL cylinders and homogenized with a Robert BoscHHausgerate GMBH mixer (type CNHR8, FD8905, Slovenia) at room temperature (28-32°C). The sample was allowed to stand for 0 and 30 min. The FE and FS were then calculated determined by Nagarajan *et al.*[13] using the following equations :

$$FE\ (\%) = \left(\frac{V_T}{V_0}\right)\times 100$$

$$FS\ (\%) = \left(\frac{V_t}{V_0}\right)\times 100$$

Where:

V_T = Total volume after whipping
V_0 = Original volume before whipping
V_t = Total volume after standing at room temperature for 30 min

Emulsifying properties: The emulsion activity index (EAI) and the emulsion stability index (ESI) of gelatins were determined according to the method of Lassoued *et al.*[19], with a slight modification. The gelatin solutions were prepared by dissolving dry gelatin in distilled water at 60°C for 30 min. Aliquot of 30 mL of gelatin solutions at different concentrations (0.5, 1, 1.5 and 2 g/100 mL) were homogenized with 10 mL of corn oil for 1 min at room temperature (25°C) using Robert BoscHHausgerate GMBH mixer (CNHR8 type, FD8905, Slovenia). Aliquots of the emulsion (50 mL) were taken from the bottom of the container at 0 and 10 min after homogenization and diluted 100-fold with 0.1 g/100 mL SDS solution (analytic grade). The mixtures were mixed thoroughly for 10s using a vortex mixer. The absorbance of the diluted solutions was measured at 500 nm using a spectrophotometer (Optizen 2120UV, 249244-141028-00, Korea). The absorbance's were measured immediately (A0) and 10 min (A10) after emulsion formations were used to calculate the emulsifying activity index (EAI) and the emulsion stability index (ESI)[20]. All determinations are means of at least three measurements according to Pearce and Kinsella[20]:

$$EAI\ (m^2\ g^{-1})\ (\%) = \frac{2\times 2.303\times A\times N}{\phi\times C\times 10000}\times 100$$

Where:

N = Dilution factor
C = Weight of protein per unit volume (g mL^{-1})
ϕ = Oil volumetric fraction (0.25)
A = A_{500}

The ESI represents the difference of the EAI at 0 and 10 min at 500 nm and was calculated by the following equation (Pearce and Kinsella[20]):

$$ESI\ (min) = \frac{(A_0\times \Delta T)}{\Delta A}$$

Where:

A_0 = A_{500} at time of 0 min
ΔT = 10 min
ΔA = A_{10}-A_0; A_{10}: A_{500} at time of 10 min

Yogurt manufacture: The reconstituted milk used was prepared at a rate of 140 g L^{-1} of milk powder (26% fat). The milk was homogenized and heated to 90°C for 3 min for pasteurization. Once cooled to 45°C, the gelatin was respectively incorporated into the milk samples at levels of 0, 0.1, 0.3 and 0.6%. Inoculation of specific lactic strains of yogurt (CHR HANSEN Denmark) was carried out a leaven

of 3% (3 mL of leaven in 300 mL of reconstituted milk) and report of strains *Streptococcus thermophilus* (YC-X16) and *Lactobacillus bulgaricus* (CHN-11) of 2S/1L. Each experimental parameter was represented in triplicate tests, with three pots of 100 mL. After incubation of the samples at a temperature of 45°C for 4 h of the fermentation phase, yogurt added with pectin were cooled and preserved at 4°C for 21 days post acidification period.

Measurement and control: The physicochemical and microbiological analyzes were carried out at 2 and 4 h during the period of fermentation, while during the period of post acidification, they were made weekly for a period of 21 days of storage at 4°C.

Physicochemical analysis: The physicochemical analyses were carried out according to AOAC methods[21].

pH and acidity: The pH measurement was carried out by a pH meter calibrated with two solutions: One basic and one acidic and at a temperature of 25°C. The Dornic acidity was determined by titration of 10 mL of yogurt with 0.1 N NaOH (analytic grade) using phenolphthalein (analytic grade) as an indicator colour. Results were expressed as degree Dornic[22].

Viscosity: Viscosity was measured using a falling ball viscometer using a glass tube and a normalized ball equipped with a chronometer at 25°C. The viscosity was expressed in Pascal sec (Pas).

Microbiological analysis: The enumeration of *Streptococcus thermophilus* was carried out according to the method described by the International Dairy Federation (IDF Standard 117)[23]. The M17 agar was used for the enumeration of *Streptococcus thermophilus*. Microbiological count data were expressed as colony-forming units (CFU) per mL of yogurt.

Organoleptic test: Throughout the period of post acidification (7th, 14th and 21st days of storage at 4°C), the organoleptic quality of experimental yogurt will be evaluated by a jury of panelists with 10-point scale. The organoleptic test consists in appreciating the experimental yogurt according to five parameters: Taste, cohesiveness, adhesiveness, aftertaste and odour.

Statistical analysis: The results of the physicochemical and microbiological analyses were statistically treated by a factorial bivariance analysis in total randomisation, followed by comparison of means according to the test of Newman and Keuls (Stat Box 6.4). However, those relating to the organoleptic test were treated according to the nonparametric test of Friedman (Stat Box 6.4).

RESULTS AND DISCUSSION

Characteristics of gelatin: The gelatin extraction yield was 6.32±0.20%. The ash content of bovine bone gelatin was similar to that of commercial gelatin. The lowest moisture content was recorded with bovine bone gelatin, while commercial gelatin had high contents (Table 1). The pH of bovine bone gelatin was 9.63 as shown in Fig. 1, indicating their category as type A (acid gelatin).

The solubility at different pH values of BG and CG were depicted in Fig. 2. The BG and CG revealed minimum solubility of 47.44 and 27.77% at pH 7, respectively. On either side of this pH, solubility increased and maximum solubility was recorded at pH 4 ($p < 0.01$). In general, both gelatins were solubilised in the acidic pH range (1-6). The solubility of BG was generally higher than that of CG in the alkaline pH range, while at below pH 4.0, the solubility of BG was lower than that of CG. The relative solubility of BG and CG were maintained in the presence of NaCl up to 2%. A marked decrease in solubility was observed with an increasing NaCl concentration. A drastic decrease in gelatins solubility was observed with 3% NaCl or above ($p<0.01$). The relative solubility of BG is higher than that of CG over the 0-6% concentration range (Fig. 3).

Foam expansion (FE) and foam stability (FS) of bovine gelatin were 150.66±22.03 and 114.66±8.08%, respectively, while those of commercial gelatin were lower (Table 2). The emulsifying activity index (EAI) and emulsion stability index (ESI) of BG at different concentrations, in comparison with CG

Table 1: Gelatins ash and moisture contents of BG and CG

Sample	BG	CG
Ash (%)	13.33±0.57	13.66±0.57
Moisture (%)	7.87±0.62	10.12±2.79

The results were expressed as mean followed by standard error, BG: Bovine gelatin, CG: Commercial gelatin

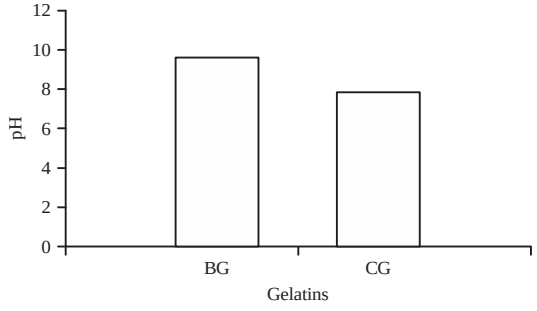

Fig. 1: pH of bovine gelatin (BG) and commercial gelatin (CG)

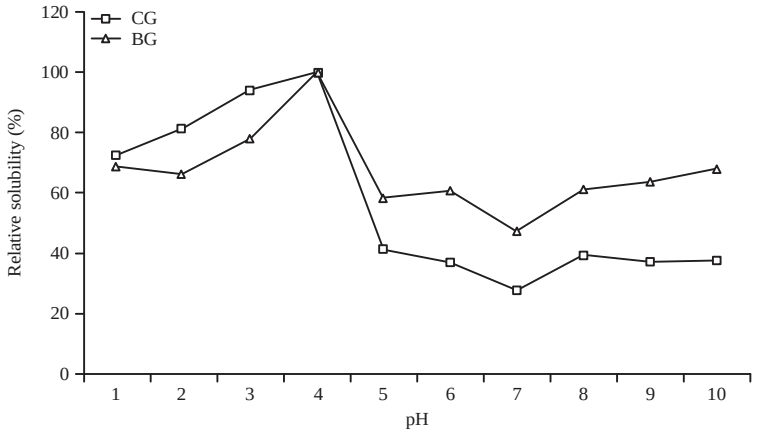

Fig. 2: Relative solubility (%) of bovine gelatin (BG) and commercial gelatin (CG) influenced by pH

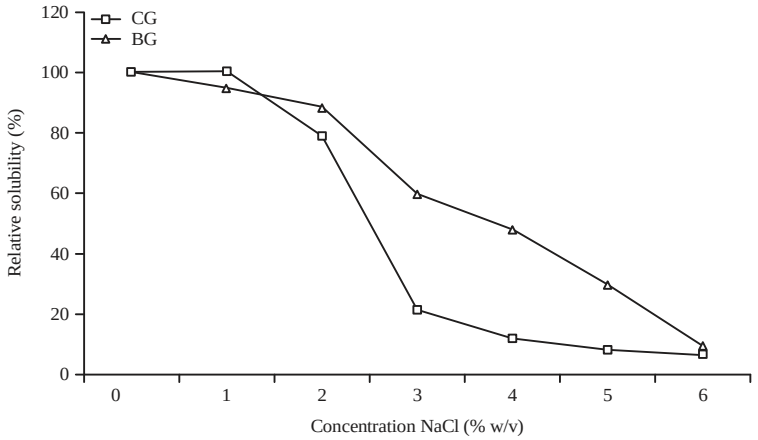

Fig. 3: Relative solubility (%) of bovine gelatin (BG) and commercial gelatin (CG) as affected by NaCl concentration

Table 2: Foaming properties of BG and CG

Sample	FE (%)	FS (%)
BG	150.66±22.03	114.66±8.08
CG	127.33±5.03	113.55±2.69

The results were expressed as mean followed by standard error, FE: Foam expansion, FS: Foam stability, BG: Bovine gelatin, CG: Commercial gelatin

Table 3: Emulsion activity index (EAI) ($m^2 g^{-1}$) and emulsion stability index (ESI) (min) of BG and CG at different concentrations

Sample	Gelatin concentration (g/100 mL)	EAI ($m^2 g^{-1}$)	ESI (min)
BG	0.5	21.51±1.59^a	24.33±1.73^c
	1.0	10.19±0.64^b	47.51±1.32^b
	1.5	10.58±1.59^b	60.37±2.49^a
	2.0	8.41±0.46^b	66.04±2.72^a
CG	0.5	20.53±0.34^a	20.62±0.84^c
	1.0	7.14±0.92^b	13.38±1.54^c
	1.5	3.83±0.11^c	43.91±1.59^b
	2.0	2.57±0.26^d	56.81±1.04^a

The results were expressed as mean followed by standard error, Means in the same column with different small letter superscripts are significantly different ($p<0.05$), EAI: Emulsion activity index, ESI: Emulsion stability index, BG: Bovine gelatin, CG: Commercial gelatin

were shown in Table 3. The EAI and ESI of BG were higher than CG. The EAI values of BG and CG decreased with the increase of gelatin concentration ($p<0.01$). The ESI of the both gelatins (BG and CG) increased with increasing concentration ($p<0.01$). The higher ESI was found when a concentration of 2% was used. For this concentration, the ESI of BG and CG was 66.04±2.72 and 56.81±1.04 min, respectively.

Quality of yogurt added with bovine gelatin (YABG): The pH of YABG is presented in Table 4. After 2 h of fermentation, the pH values varied 4.90, 4.88, 4.86 and 4.75 on average for the BG levels of 0, 0.5, 1 and 1.5%, respectively. Also noted during the 21 day storage period, the mean pH values of the YABG decreased from 4.50±0.01 to 4.35±0.04. The pH values of YABG significantly ($p<0.05$) decreased during fermentation and post acidification periods (Table 4). Adding BG (0.5-1.5%) into the yogurt samples significantly ($p<0.05$) increased the values of titratable acidity during the

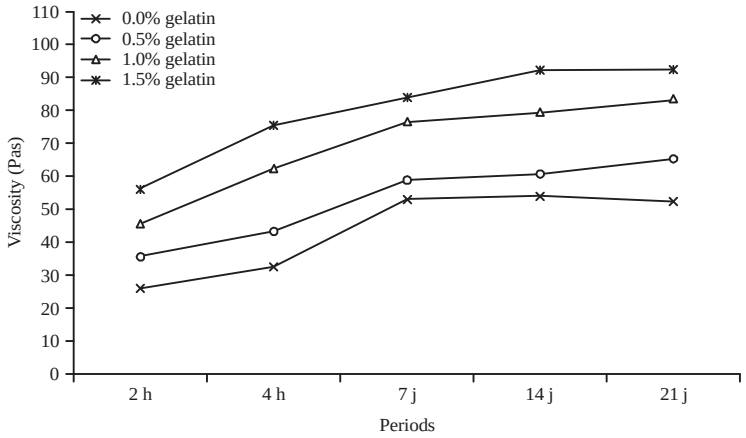

Fig. 4: Evolution of viscosity (Pas) of yogurt added with bovine gelatin

Table 4: Evolution of pH of yogurt added with bovine gelatin

	Bovine gelatin added doses (%)							
Periods	0	0.5	1	1.5	Period F_2	Effect F_1	Effect F_2	Effect $F_1 \times F_2$
Fermentation								
2 h	4.90 ± 0.01^{a}	4.88 ± 1.16^{a}	$4.86^{ab} \pm 0.01$	$4.75^{ac} \pm 0.01$	4.86 ± 0.49^{a}	*	*	*
4h	4.63 ± 0.01^{a}	4.59 ± 0.01^{a}	4.57 ± 0.09^{ab}	4.56 ± 0.01^{ac}	4.59 ± 0.04^{a}			
Post-acidification								
7j	4.42 ± 0.01^{a}	4.47 ± 0.02^{ac}	4.51 ± 0.01^{acd}	4.58 ± 0.01^{ac}	4.50 ± 0.01^{b}			
14j	4.38 ± 0.04^{a}	4.39 ± 0.01^{ac}	4.40 ± 0.01^{acd}	4.49 ± 0.01^{abcd}	4.42 ± 0.01^{c}			
21j	4.24 ± 0.06^{a}	4.34 ± 0.04^{ac}	4.35 ± 0.04^{bcd}	4.45 ± 0.06^{ace}	4.35 ± 0.04^{c}			
Gelatin dose (F_1)	4.48 ± 0.02^{c}	4.53 ± 0.44^{b}	4.54 ± 0.04^{b}	4.57 ± 0.02^{a}				

The results were expressed as mean followed by standard error, *Significant effect ($p<0.05$) of bovine gelatin addition, Means in the same column with different small letter superscripts are significantly different ($p<0.05$), F_2: Factor studied experimental periods, F_1: Factor studied dose of gelatin added, $F_1 \times F_2$: Interaction of the two factors studied

Table 5: Evolution of acidity (°D) of yogurt added with bovine gelatin

	Bovine gelatin added doses (%)							
Periods	0	0.5	1	1.5	Period F_2	Effect F_1	Effect F_2	Effect $F_1 \times F_2$
Fermentation								
2h	70.00 ± 2.01^{a}	72.33 ± 2.52^{ad}	74.00 ± 2.65^{bcd}	76.67 ± 1.53^{ae}	73.25 ± 0.49^{d}	*	*	*
4h	79.67 ± 6.51^{a}	81.00 ± 3.61^{b}	86.33 ± 4.16^{ad}	88.33 ± 5.29^{ae}	83.83 ± 4.28^{c}			
Post-acidification								
7j	95.00 ± 2.01^{a}	90.00 ± 3.21^{b}	91.00 ± 2.65^{ad}	78.00 ± 7.55^{ae}	91.25 ± 0.01^{c}			
14j	105.00 ± 3.21^{a}	102.67 ± 2.00^{b}	95.00 ± 4.01^{ad}	93.33 ± 1.53^{ae}	100.89 ± 2.44^{b}			
21j	115.00 ± 3.00^{a}	111.00 ± 2.52^{b}	98.67 ± 3.05^{ad}	95.17 ± 5.51^{bcde}	104.96 ± 3.16^{a}			
Gelatin dose (F_1)	92.93 ± 3.15^{a}	91.40 ± 2.39^{a}	89.00 ± 2.84^{b}	88.90 ± 4.14^{b}				

The results was expressed as mean followed by standard error, *Significant effect ($p<0.05$) of bovine gelatin addition, Means in the same column with different small letter superscripts are significantly different ($p<0.05$), F_2: Factor studied experimental periods, F_1: Factor studied dose of gelatin added, $F_1 \times F_2$: Interaction of the two factors studied

fermentation period or acidity values varying from 79.67 ± 6.51 to 88.33 ± 5.29 °D for BG rates of 0, 0.5, 1 and 1.5%, respectively. Furthermore, the values of titratable acidity for all the samples studied were increased when stored at 4°C for 21 days. In addition, during this period, it appeared that the acidity was inversely proportional to the incorporation rate of the gelatin in the YABG ($p<0.05$) (Table 5). During the fermentation and post-acidification periods, the viscosity showed a remarkable increase with the increase in the rate of BG incorporate in the YABG ($p<0.05$). Thus, during these two experimental periods, it appeared that the tests containing 1 and 1.5% of gelatin had the highest viscosity values (Fig. 4). During the period of fermentation, the number of *Streptococcus thermophilus* was all the more important that the rate of BG was high in the YABG ($p<0.01$) or mean values of 27×10^7, 34×10^7, 35×10^7 and 43×10^7 CFU mL^{-1} at 2 h and 29×10^7, 36×10^7, 54×10^7 and 66×10^7 CFU mL^{-1} after 4 h of fermentation for the respective BG incorporation rates

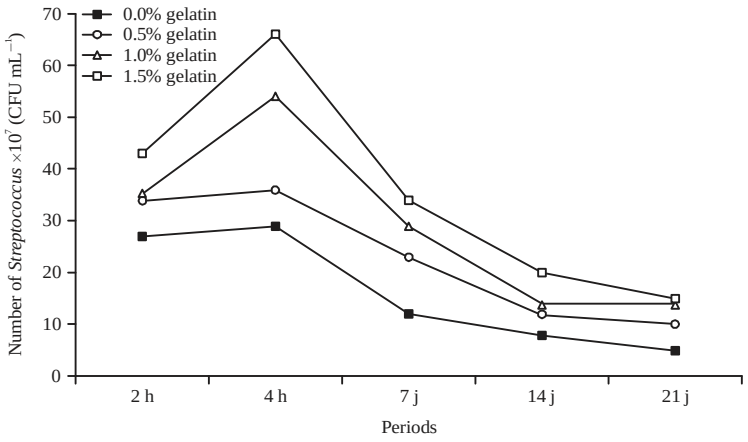

Fig. 5: Evolution of the number of *Streptococcus thermophilus* (CFU mL^{-1}) of yogurt added with bovine gelatin

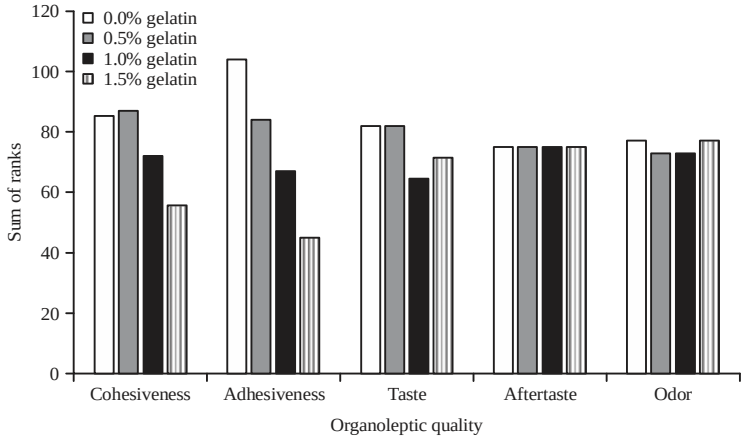

Fig. 6: Evaluation of the organoleptic quality of yogurt added with bovine gelatin during 21 days of cold storage at 4°C

of 0, 0.5, 1 and 1.5%. Moreover, for the post-acidification period, all the YABG showed a significant drop in the evolution of averages of these germs in function of the time in the 21st day of conservation of samples to the positive cold of 4°C ($p<0.01$) (Fig. 5).

Organoleptic quality of yogurts added with gelatin: During the post-acidification period, the tasting jury rated the taste for acidity of the YABG as acceptable with more preference given to samples prepared at 1 and 1.5% of BG (Fig. 6). During the post-acidification period, panelists estimated that the adhesiveness of YABG containing gelatin was better in comparison with the control. This adhesiveness was improved ($p<0.05$) with the increasing of the BG incorporation rate in the YABG or values ranging from 104, 84, 67 and 45 sums of ranks for BG levels of 0, 0.5, 1 and 1.5%, respectively. The best adhesiveness values were recorded in the tests prepared at a severe rate of 1.5% gelatin (Fig. 6). Cohesiveness was increased with increasing the BG dose incorporated in the YABG ($p<0.05$), or values of sums of ranks of the order of 85.5, 87, 72 and 55.5 for the respective rates of 0, 0.5, 1 and 1.5% of BG incorporated (Fig. 6).

Furthermore, the tasting panel did not detect any aftertaste in all the YABG so the gelatin concentration of the various experimental tests did not have a significant effect on the odour of the YABG. Moreover, the quantity of exuded whey was negligible in the YABG containing 1 and 1.5% of BG as compared with the control samples and those supplemented with 0.5% of BG.

Characteristics of gelatin: The extraction yield of the gelatin depends on the raw material used and on the suitable extraction process. Ktari *et al.*[24] found that the extraction yield of gelatin from zebra blenny (*Salaria basilisca*) skin was 14.8%. The addition of pepsin during swelling process was noted to increase the yield to 18%. These findings are in agreement

with the results reported by Lassoued *et al.*[19] and Jridi *et al.*[25]. Increasing yield of gelatin from splendid squid (*Loligo formosana*) skin was obtained when extraction temperatures increased[13]. Moisture content of BG and CG were well below the limit prescribed for edible gelatin (15%)[26]. Ash contents of gelatin, both BG (13.33%) than CG (13.66%), were quite high when compared with the recommended value (2.6%)[27] and the limit given for edible gelatin (2%)[26]. The same results were found by Ahmad and Benjakul[28]. The high ash content in the gelatin indicated the presence of inorganic salt, which might be generated during the pretreatment with either alkali or acid[28]. Shakila *et al.*[29] reported that the moisture contents of gelatins extracted from the bones of grouper (*Epinephelus chlorostigma*) and red snapper (*Lutjanus campechanus*) were in the range of 4.10 and 6.24%, respectively. Such higher ash contents were also observed in grouper and red snapper bone gelatins (6.58 and 10.32 %)[29]. There are two types of gelatin with different characteristics including type-A, acid-treated collagen (isoelectric point at pH 7-9) and type-B, an alkaline treated (isoelectric point at pH 4-5)[30,31]. According to Shyni *et al.*[32], the pH of the gelatin extracted from the skins of skipjack tuna (*Katsuwonus pelamis*), dog shark (*Scoliodon sorrakowah*) and rohu (*Labeo rohita*) was 4.29, 4.34 and 4.17, respectively. The pH reported for gelatin from cod skin was ranged from 2.7-3.9[33]. Cheow *et al.*[34] and Shakila *et al.*[29] reported that the pH of the bovine gelatin was 5.48 and 6.18, respectively. In the solubility analysis, the BG had higher solubility in acidic pH from 1-4 and the lesser in pH 7. Kittiphattanabawon *et al.*[35] and Singh *et al.*[9] obtained the same results. Lassoued *et al.*[19] revealed minimum solubility of gelatin from thornback ray skin (*Raja clavata*) at pH 5. Lee *et al.*[36] showed that the solubility of gelatin was higher at pH 3 than at neutral pH. When the pH is lower or higher than pI, the net charge of protein molecules are greater and the solubility is increased by the repulsion forces between chains[37]. In contrast, when the total net charges of protein molecules are zero, the hydrophobic-hydrophobic interaction increases, thereby leading to the precipitation and aggregation at the pH. The decrease in solubility of BG with an increasing NaCl concentration could be described as being due to a 'salting out' effect, which occurred at relatively high NaCl concentrations[35,37]. Similar behaviours were also found by Kittiphattanabawon *et al.*[35], Lee *et al.*[36] and Veeruraj *et al.*[38]. The FE and FS of BG were similar to those obtained by Ahmad and Benjakul[28]. According to Hafidz *et al.*[39], the FE of bovine and porcine gelatin was 99 and 90%, the FS was 91.67 and 87.67%, respectively. Foam formation is generally controlled by the transportation, penetration and reorganization of the protein molecule at the air-water interface. A protein must be capable of migrating rapidly, to the air-water interface, unfolding and rearranging at the interface to show good foaming ability[40]. Foam stability depends on the nature of the film and indicates the extent of protein interaction with the matrix. Foams with higher concentration of proteins were denser and more stable because of an increase in the thickness of interfacial films[15]. Present study results showed that EAI of BG and CG decreased as the concentration of gelatin increased. Similar results were reported Binsi *et al.*[41], Ahmad and Benjakul[28] and Khiari *et al.*[7]. This possible resulted that at high concentration, gelatin with higher hydrophilicity in nature might interact with each other, thus lesser amount of gelatin were available to be localized at the oil-water interface. Therefore, a thinner film stabilizing the oil droplet was postulated. At low protein concentrations, protein adsorption at the interface is diffusion-controlled[42]. It was noted that EAI and ESI of bovine bone gelatin were frequently higher than that of fish gelatin[31,43]. Early studies by Kim *et al.*[44] reported that cod bone gelatin had emulsifying properties similar to those of a commercial emulsifier, such as Tween-80. A positive correlation between the BG concentration and the ESI was found (increasing the concentration of gelatin solutions increased the ESI). Similar results were previously observed by Kaewruang *et al.*[45] and Khiari *et al.*[7]. The stabilisation of emulsion against coalescence/flocculation is greatly dependent on the force of electrostatic repulsions between the adsorbed proteins on the interfacial protein film[28].

Quality of yogurt added with bovine gelatin: The increase in the titrable acidity and the decrease in pH of YABG was the result of lactic acid production from the fermentation of milk lactose by the two specific strains of yogurt: *Streptococcus thermophilus* and *Lactobacillus bulgaricus*[3,46]. Similar results were obtained by Cais-Sokolinska *et al.*[2], who found that the pH of yogurt decreased during fermentation from 6.7-4.11. The results showed that an increase in the concentration of BG, significantly increased product acidity and decreased the pH of YABG ($p<0.05$).These results can probably be explained by the fact that gelatin is rich in certain growth factors. These results agree with those of Kumar and Mishra[1] and Supavititpatana *et al.*[10] and Mehmood *et al.*[47]. Viscosity of YABG increases with concentration of BG and storage time. Increasing viscosity during storage could be due to the protein rearrangement and protein–protein contact[4,48]. Furthermore, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria lead to the increase in yoghurt viscosity by producing

special mucous substances of polysaccharide nature. These substances form, primarily with casein, permanent aggregates of high resistance to mechanical action and, hence, of high resistance to syneresis[49-53]. Zhang *et al.*[54] reported that exopolysaccharide producing by *Streptococcus thermophilus* ST1 strains improved the viscosity of cow yogurt. The addition of gelatin to the milk during preparation of the yogurt changed the microstructure of the product by the formation of flat sheets or surfaces, which interacted with the casein matrix, enclosing granules of casein in several zones. The gelatin seemed to connect the granules and chains of milk proteins and consequently improved yogurt viscosity[55,56]. Gelatin would take the network of casein formed as a basis for the creation of its network during cooling, enclosing the granules of milk proteins and putting out interconnecting bridges. During cooling the final gelatin network is constructed in close interaction with themilk protein network[57]. During the fermentation period, an increase in the concentration of BG, significantly increased the number of *Streptococcus thermophilus*. These results could be explained that *Lactobacillus bulgaricus* produces short peptides and amino acids that stimulate the growth of *Streptococcus thermophilus*[58]. After about 4 h post incubation, *Streptococcus thermophilus* was inhibited at pH values between 4.4-4.5, while *Lactobacillus bulgaricus* could tolerate acidic pH[59]. On the other hand, the gel of gelatin formed increased the ability of the protein to immobilize water resulting in a drastic decrease of microbial activity[60].

Lactobacillus bulgaricus and *Streptococcus thermophilus* contributed to the taste, aroma and avour of YABG. They acidify the yogurt, resulting in a tangy lactic acid taste and produce aromatic compounds[59]. These two microorganisms by their mutually growth change the milk compounds. They produced aroma substances that in cooperation with other metabolites produce substances defining the original aroma and flavour of the yogurt[61]. The addition of gelatin increased adhesiveness and cohesiveness of YABG. Similar results were reported by Kumer and Mishra[1]. Moreover, gelatins modify the texture of YABG. These results agree with those of Fiszman *et al.*[56], who investigated the effect of gelatin addition on the texture of acidic milk gels and yogurt. They found that the smooth bridge of gelatin with a double network structure seemed to be located inside the casein micelles, which could retain the aqueous phase more efficiently, thus reducing syneresis. In addition, the assessment of rheological yoghurt (adhesiveness and cohesiveness) was linked to exopolysaccharides produced by specific strains of yoghurt *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.

CONCLUSION

Gelatins could be successfully extracted from the bone of bovine. The functional properties, including solubility, foam expansion, foam stability, emulsion capacity and emulsion stability of BG were in general higher than those of CG. The addition of gelatin to yogurt significantly affected sensory, texture parameters and had a significant effect on the pH and acidity of YABG. Furthermore, the addition of 1.5% of BG to yogurt resulted in a significant increase in viscosity, cohesiveness and adhesiveness. Results indicate that the addition of 1.5% of BG to the yogurt can yield yogurts with characteristics that are associated with good eating quality.

SIGNIFICANCE STATEMENT

This study discovers the effect of bovine gelatin addition on the quality of yogurt. This study will help the researchers to uncover the critical area of gelatin extraction and conservation of yoghurt that many researchers were not able to explore. Thus, a new theory on this natural additive, can be used as an alternative to chemical additives for structuring yogurt and others foods.

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