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Effect of drying techniques on physic-chemical & functional characteristics of gelatins from catla skins and its application

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Abstract
Carps are a major source of protein for millions of people in Asia. The wastes generated from such fishes during their processing are huge and can be effectively utilized for the preparation of gelatine, a protein. In present study, gelatin was prepared from catla skin and scales and different drying techniques like tray drying, freeze drying and drum drying were used during the drying processes. These gelatins were assessed for their physical, chemical, rheological properties and functional properties and compared with those of the commercially available gelatins. Tray drying of gelatin resulted in the highest yield 11.7%. Hydroxyproline content was highest for commercial gelatin 7.5%. while the commercial gelatins had greater L* (brightness) value (85).the tray and drum dried gelatins had greater and similar b* yellowness values (30-33). The odor profile by sensory evaluation of gelatins revealed least fishy odor for commercial and drum dried gelatins. FTIR (Fourier trans for infrared spectroscopy) analysis confirmed the conformational structure of gelatin while DSC (differential scanning calorimetry) results revealed the characteristics glass transition temperatures of the gelatins. SDS-PAGE pattern of the gelatin revealed major bands corresponding to chain and cross- linked component, chain. The gel strength of gelatine was measured and gelling ability were found more in drum dried gelatine (1256 gf). The emulsifying stability of the tray dried was the highest (73%) closely followed by drum dried gelatin. Drum dried gelatin had the least foaming capacity. While freeze dried gelatin had the highest water holding capacity (7.5 ml/g) drum dried gelatin had the highest fat binding capacity (17.5 ml/g). The drum dried gelatin showed the maximum solubility (92-93%) at Ph 3,7 and 11 indicating that the drum dried gelatins can be used in a wide range of food applications from acidic to alkaline without affecting the solubility.

Keywords: Drying techniques physic-chemical, characteristics

1. Introduction
The freshwater carps in Asia include several species and are the most widely used in aquaculture practices. Catla catla commonly called catla is one of the important freshwater carps cultured in many parts of South East Asia. The Asian carps contribute 15% to the total world fish production FAO, (2012). It is foreseen that in the years to come the consumption of processed carps will be on the rise leading to a large quantity of waste accumulation. The waste from processing industries of freshwater carps like Catla is large and normally discarded. The utilization of freshwater fish will have a new avenue for a better disposal of carps processing waste and good returns for aqua-culturist.

Drying plays a major role in food manufacturing or food processing activities. One of the last operations in the manufacture of gelatin, it controls to a large extent the quality of the final product. Drying is applied to a wide variety of food products, from cereals to finished goods, from raw materials to by-products. The processes used are numerous, according to the type and quantity of product to dry, the amount of water to eliminate, the final desired quality or functionality of the dried product. Different phenomena linked to water loss and temperature variation with time are observed during drying decrease in water activity, glass transition, crystallization, melting of fat, evaporation of volatile components, migration of component

2. Material and methods
Catla fish waste (skin and scales) were procured from the local market. Skins along with scales were cleaned by washing under tap water and then frozen at -20°C till further analyses. Extraction of gelatin was done by acid and alkaline treatment as discussed below.

3.1 Preparation of gelatin from Catla skin and scales

The extraction procedure of Yang *et al*., (2007) [33] and Niue *et al*., (2013) [23] was followed with a slight modification. The fish skins were thawed, rinsed in excess water to remove any superfluous material and added to a water bath containing 3200 L water, along with 5M hydrogen peroxide for 2 hours. Weightwas taken after the hydrogen peroxide treatment. Acid solution of 0.5N was prepared and the fish skins were treated in it for undergoing decalcification. They were then soaked in 0.5 M of sodium hydroxide solution (NaOH) for 1 hour and water washed further till neutralpH. This was further soaked in 0.05N sulfuric acid and washed with tap water till neutral pH. The final hot water extraction was carried out in tap water at 70^0 C for 5 hours.

Fig 3.1: Extraction of Gelatin from catla skin and scales

Fig 3.2: Gel formation

3.2 Drying of gelatins

After the extraction of gelatin in hot water, the resulting solution must be dried to get gelatin in solid form, either as sheets, granules, flakes or powder. This can be achieved by using different methods like tray drying, solar drying, freeze drying, drum drying, vacuum drying, spray drying. The details are discussed below.

3.2.1 Single effect evaporator

It removes all the water content present during the extraction of gelatin and the remaining liquors become more concentrated and because of this the boiling temperature rises.

3.2.2 Determination of gel strength

Gel strength of gelatin was determined by taking the 6.67% of dried samples were dissolved in 40 ml of distilled water and dissolve it hot water bath at 60^0 C. sample was transferred into standard bloom (2.5 cm in height). The sample was stored in refrigerator for 5 hours and cooled until a gel was formed. The gel strength was measured by (LLOYD instrument LR SK) using 10 mm plunger. The speed of the plunger was 50.00 mm/min and it stop at deflection of 6.00 mm. the penetration (gel strength) and compression was measured.

3.4 Proximate estimation

The proximate composition of Catla skin and scale was determined. The moisture, protein, fat and ash contents were 59.43±0.17,17.59±0.97, 8.5±0.50, 1.24±0.02 respectively. According to Gomez-Guillen *et al.,* (2002) the proximate composition for bigeye snapper (*Priacanthus hamrur)* protein content was 25.19%, moisture content 52.79% fat content of fish skin was less than 2%. Fish skin contains a large amount of collagen. (Nagai and Suzuki, 2000) [21] reported that the collagen contents in the fish skin waste of Japanese sea-bass, chub mackerel, and bullhead shark were 51.4%, 49.8%, and 50.1% (dry basis), respectively. Catfish skin had 50.2% moisture, 10.3% fat, 1.0% ash and 37.4% protein, which were comparatively lesser than catla skin and scales. According to Mourad Jridi *et al.,* (2003) skin of cuttlefish (*Sepia officinalis)* the extracted dried gelatins had high protein (89.47%), with low fat (0.21%) and ash (0.03%) contents. There latively low ash content suggests that the extracted gelatins are of good quality the desalted jellyfish (dried) contained protein as the major component (69.85±0.79%), moisture (9.88±0.07%), fat (8.76±0.37%) and ash content (1%), respectively (Sinthusamran *et al.,* (2014) [31] which was less than calta raw material.

4.1.2 Biochemical composition of gelatins from different drying techniques

Proximate composition of tray dried, freeze dried and drum dried and commercial gelatins were analyzed. For moisture analysis the obtained result was found to be less in drum dried (1.5±0.07) gelatin and higher in commercial gelatin (7.83±0.28), the protein content was found more in drum dried (97±0.70) gelatin than other dried gelatin. Benjakul *et al.*, (2009) reported that the moisture content was 8.73±0.15, protein 89.76±0.59, fat 0.38±0.03 and ash 0.35± 0.01 for squid skin gelatin. In this protein content is similar to freeze dried sample.

4.1.3 Yield of catla gelatin

Yield of gelatin was calculated for the different drying process employed *viz.*tray drying, drum drying, and freeze drying with 11.75%, 5.6%,6.2% respectively. 1.82% weight of the fish "Kerisi" was found to produce the least gelatin extract as compared to catla fish dried sample Gomez-Guellen *et al.*, (2001). The highest percentage of gelatin was obtained from "kerapu" 68.47% of its fish skin or 3.68% of total the fish weight. This was followed by "kembung" with a recovery of 67.82% of its skin or 2.04% of the total fish weight difference in the gelatin recovery could be due to the difference in the characteristics of the skin and scale of the fish. For example, "kerapu" and "kembung" apparently have harder skin and scale. Gomez-Guellen *et al.,* (2001). Gelatin yield from "jenahak" was 55.21% over the weight of the skin.

Table 4.3: Yield of gelatin using different drying process

Types of gelatin	weight of raw material(kg)	Yield (kg)	Yield %
Tray dried	5.00	0.58	11.75
Drum dried	5.00	0.28	5.60
Freeze dried	5.00	0.30	6.20

4.1.4 Hydroxyproline

Hydroxyproline is an important amino acid involved in formation of structure of gelatin gel and also a gelatin film. Hydroxyproline content of from tray dried, freeze dried and drum dried gelatins were 4.86%, 4.11%, 6.97% respectively whereas commercial gelatin had 7.55%. Commercial gelatin had higher hydroxyproline content than the others. Gelatin from bigeye snapper (*Priacanthus tayenus*) Binsi *et al*., (2009) [6] showed that protein content and hydroxyproline (Hyp) yield of skin gelatin increased with increasing extraction temperature and time (p<0.05.According Chandra *et al*., (2015) [7] by catla fish gelatin hyp is 8.50%.Hydroxyproline is believed to play a role in stabilizing triple helical structures due to its hydrogen bonding ability through its hydroxyl group (Mizuno, Hayashi, & Bachinger, 2003). Shyni *et al*., (2013) had reported 5.29% for rohu skin gelatin which is lower than catla waste (drum dried gelatin). The lower hydroxyproline content is due to the low hydrogen bond forming capacity of gelatin with water when kept for gelling in 4^0 C. The Hydroxyproline content of gelatin was found to be 8.73±0.25% Venkateshwarlu *et al.*, (2012).

4.1.6 Antioxidant assays

Antioxidant activities studied included reducing power, ferrous ion chelation, DPPH (1, 1- diphenyl-2- picrylhydrazyl) radical scavenging, ABTS and Nitric oxide scavenging.

Antioxidant assays were performed for the different dried gelatin. Due to many oxidation processes and reactions, the use of a one method to confirm the antioxidant activity of gelatin cannot provide a clear idea of the antioxidant potential. The results have been displayed in [table 4.5]. From the results obtained, commercial gelatin showed higher scavenging activity in DPPH 75.50% and drum showed less DPPH 8.28%. The freeze-dried gelatin found to be higher in NO 18.93% and less in tray dried gelatin. Freeze dried gelatin showed higher % in ABTS 76.97% and less in commercial gelatin (27.00%). All gelatin samples showed higher metal chelation activity in the radical scavenging assays. Katari *et al., (*2012) [17] the cobia skin was reported to have high result (77.60%) when DPPH redical was inhibited. The presence can be the reason for the high antioxidant concentration.

4.2. Physical properties

4.2.1 Gel strength

Gel strength is an important function of gelatin and is determined by amino acid composition. The quality of gelatin is determined by gel strength (penetration and compression). Gel strength values of gelatins were Tray drying 360.21g, freeze drying 1309.50g and drum drying had 1256.41g. The gel strength was found more in drum dried gelatin as compared to commercial, tray dried and freeze dried gelatin. The gel strength of commercial gelatin is 471.25 g which is higher than sea bass fish 369g Sinthusamran *et al.,* (2014) [31]. According to Gomez Guillen *et al.,* (2002) the gel strength of shark skin gelatin is 111.9 gm. and he also reported that gel strength of cod skin is 80 g which is having less gel strength as compared to tray dried, freeze dried and drum dried gelatin of catla waste. Cho *et al*., (2005) has reported 426g for yellow tuna fish skin. Muyonga *et al*., (2004) [20] reported the gel strength of tuna skin gelatin is 230g. Gel strength of yellow tuna fish skin and tuna skin gelatin has less gel strength compared to gelatin from catla.

4.2.2 Texture profile analysis

The texture profile analysis was performed. The gelatin solution with the same concentration 6.67% was prepared. Freeze dried gelatin have a higher hardness value than the commercial gelatin. The hardness related to gel strength peak force and compression during first compression. The hardness value will be a customizable value which can be preferred for imparting more or less hardness in food product. Chandra *et al.,* (2015) [7] reported that amongst the gelatin of rohu, catla, mrigal and tilapia, catla gelatin showed highest chewiness value of 12.85 N followed by rohu which was 6.7N and Tilapia at 5.1 N. By observation it showed freeze dried gelatin had 2401.31 and more chewiness.

4.2.3 pH

pH values are dependent on the gelatin extraction processes. pH values observed for tray drying, freeze drying drum drying and commercial were 7.35, 6.55, 8.16, 5.40 respectively. Jamilah, Harvinder, (2002) [13] reported that pH values for gelatin extracted from the skin of black tilapia 3.81 and red tilapia 3.05 which more acidic than those of catla gelatin. Chandra *et al*., (2015) [7] reported that catla fish had pH 6.7 ± 0.1 which is more acidic in nature as comparison to present study. The pH of gelatin from Blackspotted Croaker (*Protonibea diacanthus*) was found to be 5.5 the acidic pH of the gelatin solution obtained was affected by the washing treatment.

4.2.4 Colour

The tilapia skin gelatin had bright and whitish-yellow color, similar to the color of commercial gelatins, which usually varies from pale yellow to dark amber Jongjareonrak *et al.*, (2010). The values for L*, a*, and b* of the extracted gelatin were 89.25 ± 11.79, - 0.44 ± 0.07, and 2.48 ± 0.14, respectively gelatin from tilapia skin is similar to commercial gelatin. Babji *et al.*, (2013) had reported that for catla and red tilapia gelatin. The lightness was observed to be less as compared to catla gelatin. A lighter colour (L*-values) was observed for gelatin gels extracted at 45^0C, compared with those of gelatin extracted at 55^0C. At an extraction temperature of 45^0C, the L*-values of gelatin gels slightly decreased with seabass skin could be used as a replacer for bovine or porcine gelatin**.** Sittichoke Sinthusamran *et al.,* (2015).

4.2.5 Odour

According to Jamilah *et al*., (2002) [13] Fishy odour in the red tilapia gelatin was barely detectable but this was not for the gelatin from black tilapia. This may be due to the stronger muddy odour and flavour associated with black tilapia. Muyonga *et al.,* (2004) [20] reported that the Nile perch skin gelatin had considerable fishy odour and he had used activated carbon for the extraction the second time and did not have much odour. According to Benjakul. S *et al*., (2015) [5] fishy odour intensity of gelatin increased when seabass skin stored in ice was used for longer time, with corresponding increase in the content of volatile aldehydes and alcohols.

4.2.6 Particle size analysis

Particle size was analyzed by catla fish gelatin for the different dried gelatins in which Drum dried material measurements were analyzed using Vernier calipers due to the larger size of the gelatin flakes. By comparing with the commercial gelatin, the diameter was found more in drum dried gelatin and less in tray dried gelatin and the width of drum dried gelatin was more as compared to freeze dried, tray dried and commercial gelatin. Binsi *et al.,* 2017 reported that the fish gelatin solution had 12.7 nm diameter with volume 90.3% as compared to catla fish gelatin diameter of drum dried gelatin found more (2.77 mm).

4.2.7 DSC analysis

The thermal property of the gelatins extracted from catla waste was determined by this analysis. Their corresponding transition temperatures are shown when they undergo heating scan from 30 to 140°C. The results were 106.27^0C for tray dried, 111.01^0C for freeze dried and 96.27^0C for drum dried which were higher than that for bovine gelatin Tg (57.9^0C).Weng *et al*., (2014) [32] reported that Tg for tilapia skin gelatin at pH 7 was 115^0C which is higher than freeze dried, tray dried and drum dried gelatin. Kajornsak Chuaynukul *et al.*, (2014) [8] reported that the glass-transition temperature (Tg) of Gelatin molding compound resin which is made up of fish gelatin or bovine gelatin is 52.6 – 57.9 °C and degradation temperature (Td) of 248.83 – 249.35 °C, depending on gelatin type

4.2.8 FTIR (Fourier transform- infrared spectroscopy)

Hashim *et al.*, (2010) reported that FTIR spectrum for extracted fish gelatin at 4^0C using 0.01 N acetic acid solution. It shows three major peak regions marked as 1 (3600-2700 cm^{-1}), 2 (1900-900 cm^{-1}), and 3 (400-900 cm-1). The regions are assigned to the bonds: Amide A and B; Amide I, II and III; and Amide IV, V and VI Similar results were also observed by, Muyonga *et al.* (2004) [20].

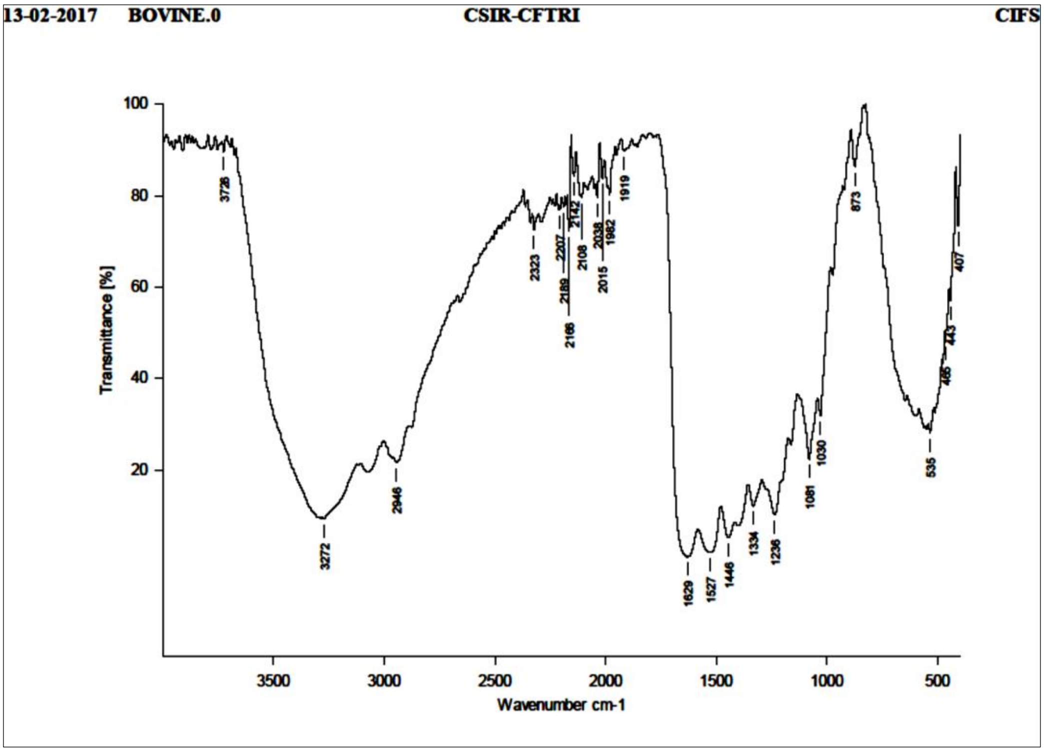

Fig 4.14: FTIR of commercial gelatin

4.3. Rheological properties

4.3.1 Viscosity

Analysis commercial gelatin and freeze dried have similar viscosity and as compared to tray dried and drum dried material commercial and freeze dried have high viscosity. Ward and Courts, (1997) [14] viscosity of gelatin extracted from skins of Catla, Stripped cat fish, Black tilapia and Black king fish at 45^0C were found to be 9.5 cP, 8.21 cP, 6.9 cP and 13.15 cP which is higher than commercial gelatin as well as freeze dried, tray dried and drum dried gelatin. Gudmundsson and Hafsteinsson, (1997) reported 7.5 cP as the highest viscosity in cod skin gelatin.

4.3.2 Dynamic viscosity

According to Chandra *et al*., (2015) [7] the gelling and melting point of temperature of gelatin were 13.7^0 C and 23.3^0 C as determined by stress rheometer. Binsi *et al.*, (2009) [6] reported that the DVB of Bigeye snapper *(Priacanthus hamrur)* fish gelatin was in the temperature range of 20–5^0C and 5–20^0C.The storage modulus (G') values were higher than loss modulus (G") values during both gelling and melting. The relatively higher rate of increase of storage modulus values at less than 12.2^0C indicates gelation had started at around 12.2 ^{0}C

4.4 Functional properties

4.4.1 Emulsifying properties

Commercial gelatin was found to ES more than freeze dried gelatin but less than drum dried gelatin. Zakira *et al.*, (2011) had reported that cuttlefish bone and skin gelatin showed 48.70% and 56.34% of emulsion capacity respectively. At 1% gelatin concentration EA and ES were 25.97± 1.05 and 24.32

± 0.94 respectively whereas at 3% concentration EA and ES were 37.26±1.21 and 17.51± 1.41 it was observed that emulsifying properties at 3% concentration is higher. EAI of all gelatin samples increased as the concentration of gelatin increased. Jridi *et al.*, (2013) by comparison with catla waste (dried gelatin) cuttlefish skin showed less value it had less emulsifying capacity and stability. Killekar *et al.* (2012) [15] reported emulsifying capacity and stability at 55.66% and 32.5% respectively.

4.4.2 Foaming capacity

Gelatin can form and stabilize foams and is widely used in confectionary product such as marshmallows Lassoued *et al.*, (2014) [18]. Foam formation is generally controlled by transportation, penetration and reorganization of protein molecules at the air-water interface f *et al.*, (2012). The values for foaming capacity were94%(TD) 60% (FD), 20% (DD) and 92%commercial.

4.4.3 Fat binding

The values were3.7 TD, 3.6 FD, 5.0 DD and 2.8 for commercial gelatin. By observation drum dried gelatin has higher fat binding capacity when compared to other dried gelatins and commercial gelatin had the least fat binding capacity. Cho *et al.*, (2004) reported that Tyrosine which is hydrophobic amino acid in shark cartilage was observed to be 1.17% which was higher than that of porcine gelatin. Kaewruanga, Benjakul, & prodpran, (2014) [8] reported that the fat binding capacity of squid skin was 9.2 g oil/g protein which is higher than present study and commercial gelatin.

Fig 4.26: Fat binding capacity in Tray dried and drum dried Gelatins

4.4.5 Turbidity

According to Alexandre da Trindade alfaro, (2013) [2] Wami Tilapia skin gelatin solution had turbidity of 67 ± 4.3 which is higher than present study. Turbidity values are widely dependent on the efficiency of the clarification process. According to Muyonga *et al., (*2004) [20] Turbidity values of Nile perch (*Lates niloticus)* skin and bone gelatin are largely dependent on efficiency of the clarification (filtration) process. Nile perch skin gelatin had turbidity of 20.5– 158 NTU while bone gelatins had turbidity of 109–517 NTU.

4.4.6 Solubility

At neutral pH, the maximum solubility (93%) was shown by drum dried gelatin followed by commercial gelatin (79%). Tray and freeze-dried gelatins showed similar solubility at pH 7 (78-79%) and pH 9 (82-83%). Jinhan Shon *et al.*, (2011) Reported that the solubility of skate skin collagen was 82.7±1.87% at neutral pH which is similar to freeze dried gelatin

4.5 Dairy spread

4.5.1 Sensory evaluation

Dairy spread Sample A had slightly lower buff color (5.5) compared to sample B (6.5). Spread had good set of 7.8 for sample A and 8.8 for sample B. Spread ability was good (7.1-7.5) which was a desirable attribute. Both the samples A and B had sticky texture and highly adhesive. Smoothness attribute for sample A was 8.9 and for sample B was 7.5. Greasiness was of higher intensity for sample B compared to sample A Sample A had beany note of higher intensity than sample B, while sample B had higher intensity of fishy note compared to sample A. Slight buttery note was perceived for both the samples. Salty taste was high (6.5) for sample B. However, both the dairy spread samples are acceptable with overall quality score of 10.5 and 10.1 for sample A and sample B respectively.

4.5.2 Texture profile analysis

According to L.H Cheng *et al., (*2008) texture attributes of low-fat spread sample was analyzed and Firmness, adhesiveness, compressibility properties were found to be significantly increase with higher substitution of fish.

Table 4.12: Texture profile analysis of dairy spread

Sample	Hardness (gf)	Cohesiveness	Springiness	Gumminess (N)	Chewiness (J)	Adhesiveness (gs)
Spread A	1.194	0.596	0.804	0.726	0.612	0.172
Spread B	2.193	0.642	1.117	1.143	1.389	0.328

Conclusion

In this study the extraction of gelatin was carried out from catla skin and scales using different drying process i.e Tray drying, freeze drying and drum drying during the final step of the extraction protocol. The physiochemical and functional properties of the dried gelatins were compared with commercial gelatin. The extracted gelatin showed higher protein content in drum dried (97±0.70) as compared to commercial and other dried gelatin. The hydroxyproline (7.55%) and fat content (0.94) were higher in commercial gelatin as compared to the others. With regards to functional properties tray dried gelatin showed higher emulsifying stability. Drum dried gelatin had the least foaming capacity. While freeze dried gelatin had the highest water holding capacity (7.5 ml/g) drum dried gelatin had the highest fat binding capacity (17.5 ml/g). Analysis of turbidity and solubility at various pH revealed that, freeze dried gelatin showed greater turbidity at pH 5 and pH 7 and as compared to commercial gelatin and drum dried gelatin showed the maximum solubility (92-93%) at pH 3, 7 and 11. As in the study of rheological properties commercial gelatin (4.75cp) and freeze dried (4.25cp) gelatin is more viscous than tray dried (2.00cp) and drum dried (2.75cp) and dynamic visco-elastic studies revealed that drum dried gelatins had the least melting temperature upon sol-gel transition. Based on the above studies drum dried gelatins were used in the preparation of a dairy spread and its sensory and textural properties were compared with another dairy spread made with commercial gelatin. The Spread ability was good in both (7.1-7.5) which was a desirable attribute. Both the samples A and B had sticky texture and highly adhesive. However, both the spreads were acceptable.

Recommendations

Such studies on storage can be continued to the dairy spreads prepared in the above investigation and the possibility of preparing better, nutritious and healthier emulsion spreads with reduced fat content can be explored by varying the concentration of the protein with the fat.

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