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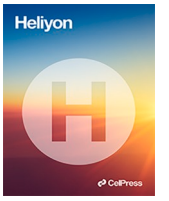

Research article

Plasticizer–gelatin mixed solutions as skin protection materials with flexible-film-forming capability

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ABSTRACT

To demonstrate the feasibility of plasticizer–gelatin solutions as novel skin protection materials from a physical aspect, we evaluated the rheological properties of the solutions and the mechanical properties and textures of their dried sheets and films. Three types of sugars and polyols were employed as organic plasticizers and mixed with gelatin in solutions at plasticizer/gelatin weight ratios of 0.13–1.67. The plasticizers minimally affected the viscosities and gelation temperatures of the gelatin solutions, but they remarkably softened dried gelatin sheets, except for propylene glycol. Glycerol exhibited the best plasticizing effects, but the sheets obtained using glycerol showed tacky textures. Preliminary investigations on the film-forming properties of the solutions on the human skin showed that the fructose–gelatin solution at a weight ratio of 1.0 formed a flexible thin film with a texture and mechanical properties similar to those of a commercially available polyurethane-based flexible film dressing. In terms of physical properties, we conclude that the fructose–gelatin solution has potential as a skin protection material that transforms from a solution to a film on the skin.

1. Introduction

Gelatin is a polypeptide derived from the hydrolytic degradation of collagen. The Unites States Pharmacopeia/National Formulary defines it as a product of the partial hydrolysis of collagen derived from the skin, white connective tissues, and bones of animals. Thus, the denatured collagen α-chain is the main component of gelatin with minor portions of dimer, trimer, and degraded molecules [1,2]. The physicochemical properties of gelatin differ from those of original collagen because the rod-like triple-helix structure of collagen is lost upon thermal denaturation and degradation. Therefore, the quality of gelatin is contingent upon the method of preparation employed [3]. Gelatin has characteristic gelling properties [4] and offers processability to produce soft capsules [5,6] and edible films [7,8], making it valuable in the food and pharmaceutical industries.

To process gelatin capsules and edible films, plasticizers are usually added to avoid gelatin vitrification [6,9]. Water acts as a

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plasticizer [10,11] as well as the major solvent of gelatin, but the volatility is not preferable for the thermal processing of gelatin. Various nonvolatile organic compounds such as polyols [12–14], polyethylene glycols with various molecular weights [15,16], amines [15], fatty acids [17], and alditols [18,19] have been used as plasticizers for gelatin. Of the plasticizers that have been examined, glycerol (GLY) is the most effective one for gelatin [20,21] owing to its excellent plasticizing efficacy and hygroscopicity [6]. Using these plasticizers alone or in combination, physicochemical, mechanical, and pharmaceutical properties can be adjusted for food and pharmaceutical applications.

Besides, gelatin has been one of the most useful biopolymers in the biomedical field. Gelatin has been used as a bioink [22], supportive material for 3D bioprinting [23], tissue engineering scaffold [24], and carrier for drug delivery [25]. It offers thermoresponsive gelling properties, processability to various shapes (e.g., microparticles), cell affinity, nontoxicity, and bioabsorbability, making it promising for biomedical applications. Plasticizers have rarely been used in those applications because they can be leached from gelatin-based materials used in aqueous solutions or in the body. Consequently, the flexibility of plasticized gelatin has not been thoroughly investigated.

Here, we propose a gelatin-based skin protection material conceptualized as follows: A gelatin solution containing a biologically safe plasticizer can be topically applied to the human skin. The solution can be air-dried to form a thin film that adheres to the skin. The plasticizing effects make the film flexible similar to a synthetic polymer-based film dressing, but the gelatin film can be noninvasively removed with warm water without stripping the stratum corneum of the skin. This concept can be realized owing to the characteristic properties of plasticizer–gelatin mixtures. Although sugars are biologically safe, cost-effective, and have been investigated as plasticizers for polysaccharides [26] and other proteins in food applications [27], their plasticizing effects on gelatin have not been extensively investigated. The idea of plasticizing gelatin membranes with sugars is well known; however, the plasticizing effects on plain gelatin are very limited. Sucrose (SUC) is the only sugar whose plasticizing effects on plain gelatin films [28] and gelatin–starch blended films [29] have been evaluated.

Therefore, the objective of this study was to prove the viability of our concept in terms of physical characteristics by investigating not only the rheological properties of sugar–gelatin solutions but also the mechanical properties and textures of sugar–gelatin sheets and films, with the aim of creating a unique gelatin-based skin protection material. These properties were compared to those obtained using polyols as well-investigated plasticizers. Moreover, the water vapor barrier (WVB) properties of the films were evaluated. Our study has the potential to facilitate the creation of novel, noninvasive skin protective films as well as groundbreaking wound-healing gels and sheets, which can be attributed to the scaffolding properties of gelatin [30].

2. Materials and methods

2.1. Materials

Gelatin (Type B from bovine bone; Nitta Gelatin Inc., Osaka, Japan), fructose (FRU; FUJIFILM Wako Pure Chemical Corporation (abbreviated FUJIFILM Wako), Osaka, Japan), glucose (GLU; FUJIFILM Wako, Osaka, Japan), SUC (FUJIFILM Wako, Osaka, Japan), GLY (FUJIFILM Wako, Osaka, Japan), propylene glycol (PPG; FUJIFILM Wako, Osaka, Japan), 1,3-buthylene glycol (BG; FUJIFILM Wako, Osaka, Japan), and a commercial polyurethane-based stretchable wound dressing Multi Fix® (MF; ALCARE Co, Ltd, Tokyo, Japan) were used. The chemical reagents have been used without further purification.

2.2. Preparation of gelatin solutions and sheets

2.2.1. Gelatin solutions

Plasticizers (FRU, GLU, SUC, GLY, PPG, and BG), gelatin, and water were mixed to achieve various gelatin/water and plasticizer/gelatin weight ratios in solutions and plasticizer/gelatin weight ratios in dried sheets (Table 1). First, pure water containing plasticizers was poured into a container with gelatin granules to achieve predetermined gelatin/water and plasticizer/gelatin weight ratios. In

Table 1
Compositions of plasticizer–gelatin mixed solutions.

Compositions in solution			Gelatin/water	Plasticizer/Gelatin
Gelatin (%)	Plasticizer (%)	Water (%)	(w/w)	(w/w)
15	25	60	0.25	1.67
15	20	65	0.23	1.33
15	15	70	0.21	1.00
15	10	75	0.20	0.67
15	5	80	0.19	0.33
15	2	83	0.18	0.13
13.2	20.8	66	0.20	1.58
14.3	14.5	71.2	0.20	1.01
15.8	5.5	78.7	0.20	0.35

The upper six types of solutions were used to prepare gelatin sheets and films and perform rheological measurements. The lower three types of solutions were used only for rheological measurements.

addition, a gelatin solution containing no plasticizers was prepared (CONT). The mixtures were stood overnight in a refrigerator and then warmed at 60 °C for 15 min to dissolve gelatin completely. The mixed solutions were transferred to 15-mL and 50-mL tubes and stored at 4 °C to form the gels. Each gel was melted only once upon warming at 60 °C for 12 min before use for the experiments described below. We designated the plasticizer–gelatin mixed solutions as abbreviated name of plasticizer_plasticizer to gelatin ratio. For example, a fructose–gelatin mixed solution at a fructose/gelatin ratio of 1.67 (w/w) was designated as FRU_1.67 solution. The odd-numbered quantities of the plasticizer/gelatin ratios were derived from the integral numbers of the solution compositions (Table 1).

2.2.2. Gelatin sheets

The gelatin sheets were prepared from the solutions containing plasticizers upon air-drying. The gelatin solutions were poured into silicone rubber containers (10 × 10 × 0.5 cm or 10 × 15 × 0.5 cm) and left in a room with controlled temperature and relative humidity (22°C-23 °C and 50 %–52 %, respectively) for 2 d. Air-drying under heating is typically used to create gelatin films and sheets from solutions, but we opted for air-drying at room temperature in order to prevent the formation of distorted and uneven gelatin sheets. The dried sheets formed in the containers were peeled off and cut into small pieces for testing as described below. The designations of the plasticizer–gelatin blended sheets corresponded to those of the mixed solutions: for example, a fructose–gelatin blended sheet at a fructose/gelatin ratio of 1.67 (w/w) was designated as FRU_1.67 sheet. The corresponding thin films formed on the human skin were designated as FRU_1.67 films.

2.3. Rheological properties of gelatin solutions

2.3.1. Rheometer

An MCR502 and MCR302e rheometer (Anton Paar, Graz, Austria) with an upper parallel sensor (diameter 30 mm) was employed to conduct the steady flow viscosity (η) and gelation temperature (T_{gel}) measurements of the gelatin solutions. The rheometer was equipped with a Peltier-controlled bottom to control the sample temperature. The upper parallel sensor and the horizontal bottom were covered with the originally equipped Peltier-controlled hood [31] to minimize water evaporation from the gelatin solutions.

2.3.2. Measurements

The η and T_{gel} of the gelatin solutions were determined by conducting sequential rotation–oscillation measurements. A portion of the gelatin solution (60 °C) was set in the sensor of the apparatus at 40 °C. After 2 min, rotation at a shear rate of 20 s^{-1} was initiated. The shear stress obtained in 12 s were employed to calculate η. Consequently, the measurement mode was shifted from rotation to oscillation under controlled deformation (shear deformation: 0.1 %; frequency: 1 Hz) to evaluate dynamic viscoelastic properties. The set temperature was decreased from 40 °C to 23 °C at a rate of 1 °C/min during oscillational measurements. Changes in storage (G') and loss modulus (G'') were recorded. The temperature at which G' intersected G'' was defined as T_{gel}.

2.4. Textures of gelatin sheets and films

2.4.1. Gelatin sheets

All tests were conducted in a room with controlled temperature and relative humidity (22°C-23 °C and 50 %–52 %, respectively). Tactile sensation evaluation was conducted to evaluate the textures of the gelatin sheets. The textures were classified into three groups: slippery like a hard plastic film (with no frictional resistance), smooth like a plastic wrap film used for food (with frictional resistance), and tacky or too soft.

2.4.2. Gelatin films formed on human skin

The tactile sensation evaluation of the gelatin sheets on human skin (female, age 24) was approved by the Business Ethics Review Committee of Tokyo Metropolitan Industrial Technology Research Institute (ME2021-5). Before starting examinations, we obtained the explicit consent concerning the examination from the subject. FRU–gelatin films were formed on the skin upon air-drying. A portion of the FRU_1.00 and FRU_1.33 solutions at 40 °C was put on the skin of antebrachium, spread with a finger, and then stood for 10 min to form thin films upon air-drying. Moreover, a thin film was formed from the CONT solution using the same procedure. Further, MF was cut in a small piece, put on the skin according to the product instructions. The adhesion of the films was ensured by pushing the films on the skin with fingers. Permission was obtained from the subject to include her photographs in the paper.

2.5. Mechanical properties of gelatin sheets

Uniaxial tensile tests were conducted on the gelatin sheets, which were cut into a dumbbell-shape with a parallel part (20 mm × 4 mm). The thicknesses of the specimens at plasticizer/gelatin ratios of 1.67, 1.33, 1.0, 0.67, 0.33, and 0.13 (w/w) were 0.227 ± 0.068 mm, 0.208 ± 0.097 mm, 0.201 ± 0.096 mm, 0.129 ± 0.048 mm, 0.089 ± 0.018 mm, 0.081 ± 0.017 mm (mean ± SD; n = 16–24), respectively. The specimens (n = 4) were mounted in the grips (gauge length of 30 mm) of a mechanical tester (TA.XTplus; Stable Micro Systems, Godalming, UK) equipped with a 50-N load cell. Uniaxial tensile loads were applied to the specimens at a deformation rate of 1 mm s^{-1} up to a deformation of 20 mm, and nominal stress–strain curves were calculated assuming that initial specimen length of 20 mm and constant cross-sectional areas of the specimens. Young's modulus was determined from the linear regions of the curves at an early stage (strain: 0.01–0.04). Breaking strain and stress were determined from the breaking points when the specimens broke until the maximum deformation.

2.6. Water vapor barrier properties of gelatin sheets

The WVB properties of gelatin sheet were evaluated using Tewameter® TM300 attached to Cutometer 580 MPA® (Courage + Khazaka, Köln, Germany) along with custom-made water-vapor-generating containers. Tewameter® is a specially developed probe used to measure the transepidermal water loss (TEWL) of the human skin. A hole (diameter 10 mm) was made in the center of the polypropylene lid of a centrifugation tube (50 mL), covered with the circular test pieces (diameter 25 mm) of the FRU_1.00 sheets and

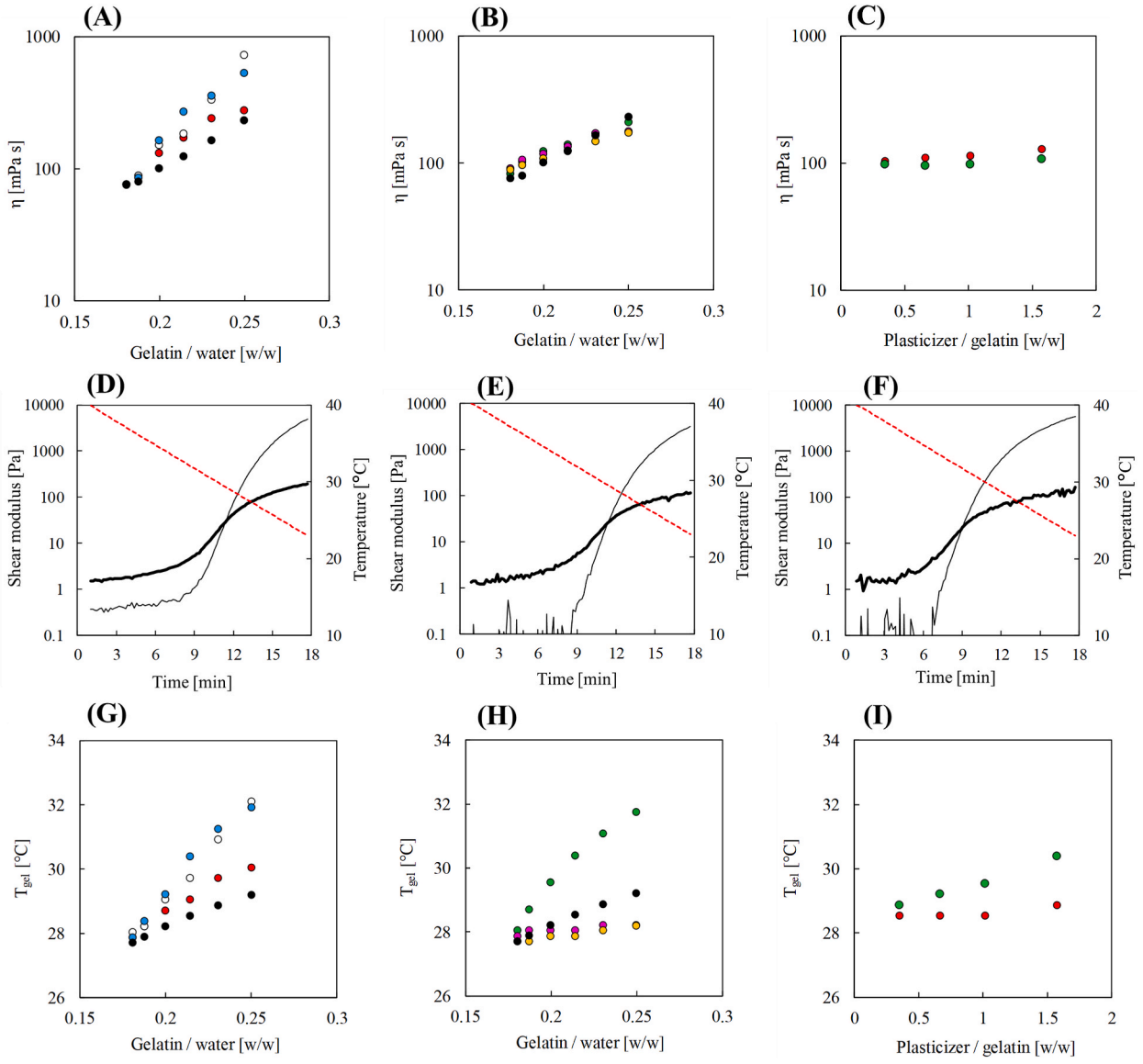


Fig. 1. Rheology data of plasticizer–gelatin mixed solutions.
(A–C) Steady flow viscosities (η). In Figure A, fructose (FRU, red circle), glucose (GLU, white circle), sucrose (SUC, blue circle), no sugars (CONT, black circle). In Figure B, glycerol (GLY, green circle), propylene glycol (PPG, pink circle), 1,3-buthylene glycol (BG, orange circle), no sugars (CONT, black circle). In Figure C, the η of gelatin solutions containing FRU (red circle) and GLY (green circle) were obtained at a constant gelatin/water ratio of 0.2 (w/w).
(D–F) Representative rheology graphs of plasticizer–gelatin mixed solutions. The narrow solid line, broad solid line, and broken red line indicate shear storage modulus, shear loss modulus, and temperature, respectively. D, CONT solution (gelatin/water = 0.25 (w/w)); E, FRU–gelatin solution (FRU/gelatin = 1.67 (w/w)); F, GLY–gelatin solution (GLY/gelatin = 1.67 (w/w)).
(G–I) Gelation temperatures (T_{gel}). In Figure G, fructose (FRU, red circle), glucose (GLU, white circle), sucrose (SUC, blue circle), no sugars (CONT, black circle). In Figure H, glycerol (GLY, green circle), propylene glycol (PPG, pink circle), 1,3-buthylene glycol (BG, orange circle), no sugars (CONT, black circle). In Fig. I, the T_{gel} of gelatin solutions containing FRU (red circle) and GLY (greens circle) were obtained at a constant gelatin/water ratio of 0.2 (w/w).

MF using a thick double-sided adhesive tape. The FRU_1.00 sheets with different thicknesses were produced. The test pieces of MF were prepared via laminating two, three, and four layers. Water (30 mL) was added into the centrifugation tube; consequently, the lid was closed. This tube was stood in a room at constant temperature and humidity for over 1 h. The probe of the apparatus was tightly attached to the center of the sheet. The apparatus body was recorded as TEWL in g/m^2/h, the amount of water vapor per unit time passing through the probe. After measuring for 60 s, the data obtained in the last 10 s was averaged and used as the WVB of each specimen (designated as WVBexp). Additionally, blank tests without sheets were performed (designated as WVBblank).

2.7. Thermal analyses of gelatin sheets

The glass transition temperature (T_g) of the FRU–gelatin and CONT sheets were determined using differential scanning calorimetry (DSC; Model DSC-60, Shimadzu, Tokyo, Japan). The cut pieces of the sheets (approximately 10 mg) were precisely weighed and sealed in aluminum pans using a crimping tool. Each pan was set on the sample stage of the DSC apparatus and heated from room temperature to 110°C-160 °C at a rate of 10 °C/min under constant N_2 flow. The peak temperature of the DSC graph obtained by the first scan was defined as T_g.

2.8. Statistics

The mechanical data of gelatin sheets and MF were statistically analyzed. Comparison between the two groups was performed using Student's t-test to identify statistical significance ($p < 0.05$), and the data from three groups were compared using one-way analysis of variance. Significant differences among the three groups were identified using Tukey's test and considered significant when $p < 0.05$.

3. Results and discussion

3.1. Rheological properties of gelatin solutions

The rheological measurements of the gelatin solutions were conducted to evaluate their fluidic and gelation properties. From a physical point of view, these two physical properties are related to ease of application to the skin. Fig. 1A and B shows the η values of the sugar–gelatin and polyol–gelatin solutions, respectively, as a function of gelatin/water weight ratios, where water was replaced with the plasticizers (Table 1). Similar to the CONT solutions, the η values of the sugar–gelatin solutions increased as the gelatin/water weight ratios increased, although higher η values were observed for GLU and SUC (Fig. 1A). The η plots of the polyol–gelatin solutions almost overlapped with those of the CONT solutions (Fig. 1B). To separate the effects of the plasticizer/gelatin and gelatin/water ratios, the gelatin solutions with FRU/gelatin and GLY/gelatin ratios of 0.35–1.58 (w/w) were prepared at a constant gelatin/water ratio of 0.2 (w/w). The η values of the solutions were almost constant and independent of the plasticizer/gelatin weight ratios (Fig. 1C), suggesting that the fluidity of plasticizer–gelatin solutions is predominantly dependent on gelatin concentration and not on plasticizer concentration. The minor effects of sugars and polyols on η are due to the absence of intermolecular networks between those compounds and gelatin formed via electrostatic and hydrophobic interactions in solutions.

Fig. 1D–F shows the representative rheology graphs of the gelatin solutions obtained using dynamic viscoelastic measurements conducted at linearly decreasing temperature from 40 °C to 23 °C. The CONT solution with a gelatin/water ratio of 0.25 (w/w) exhibited a typical gelation curve of a temperature-responsive sol-to-gel transition (Fig. 1D). The solution kept fluidity ($G' < G''$) at 40°C-35 °C; consequently, G' sharply increased and intersected G'' at temperatures near 30 °C. When the measurements were terminated at 23 °C, the solution completely changed to a gel ($G' \gg G''$). Similar rheology graphs were obtained from the FRU_1.67 and GLY_1.67 solutions, respectively (Fig. 1E and F), as the representatives of sugar–gelatin and polyol–gelatin solutions. All plasticizer–gelatin mixed solutions showed intersections of G' and G'' similar to those of CONT solutions, but the temperatures at which G' and G'' intersected (T_{gel}) varied among the solutions.

The T_{gel} values of sugar–gelatin and polyol–gelatin solutions, as well as CONT solutions, increased as the gelatin/water weight ratio increased (Fig. 1G and H), similar to the η values of the solutions. In a series of FRU–gelatin solutions in which water was replaced with FRU, the T_{gel} value of the FRU_1.67 solution was only 1.8 °C, higher than that of FRU_0.33 (Fig. 1G). However, the FRU/gelatin weight ratio (0.35–1.58 w/w) did not affect T_{gel} (Fig. 1I). The T_{gel} value of the GLY_1.67 solution was 3.0 °C higher than that of GLY_0.33 (Fig. 1H). However, the T_{gel} values of the GLY–gelatin solutions increased by only 1.5 °C when the GLY/gelatin weight ratio increased from 0.35 to 1.58 (w/w) at constant gelatin/water weight ratio. The minor effects of the plasticizers on T_{gel} are due to the structural transition of gelatin molecules, which occurs independently in gelatin/plasticizer mixed solutions. In solutions, gelatin chains are in the coil conformation [32]. When gelatin solutions are cooled, a reverse coil-to-helix transition occurs to form transparent and rigid gels [33]. This physical crosslinking occurs between "junction zones"; a partial reversion of gelatin molecules to triple helical collagen-like sequences makes junction zones with pyrrolidine-rich sequences [34] and connected each other by hydrogen bonds [35]. The presence of organic compounds in gelatin solutions seems to minimally affect the self-assembly of gelatin molecules.

Considering the topical application of gelatin solutions on the human skin from a physical aspect, η and T_{gel} are important characteristics as they relate to applicability and ease of use. The rheological investigations demonstrated that these properties were predominantly affected by the gelatin/water weight ratio, not by the presence of sugars and polyols (Fig. 1), suggesting that the flexibility of gelatin sheets and films can be adjusted irrespective of the usability of gelatin solutions.

3.2. Appearances and textures of gelatin sheets

Furthermore, the ideal physical properties of skin protection materials include transparency and surface properties, in addition to their tensile mechanical properties. Transparent and yellowish sheets were prepared from the plasticizer–gelatin solutions upon air-drying (Fig. 2). Although the appearances of the sheets were almost identical, their textures were different, greatly depending on the plasticizer type and content (Table 2). Overall, the slippery surface of gelatin sheets changed to a smooth surface on which frictional resistance was felt, and some sheets exhibited tackiness as the content of the plasticizer increased. Among the plasticizers, GLY increased the sheet tackiness the most, followed by BG. In contrast, all SUC–gelatin and PPG–gelatin sheets exhibited slippery surfaces, such as hard plastics and plain gelatin. FRU and GLU showed moderate effects on tacky feelings.

Assuming that the tacky feeling of plasticizer–gelatin sheets is due to the plasticization of gelatin, the order of tacky feeling (GLY $>$ $>$ BG $>$ GLU $>$ FRU $>$ SUC $\approx$ PPG) should be the same as the order of softness of the sheets (GLY $>$ FRU $\approx$ GLU $>$ SUC $>$ BG $>$ PPG), as shown in Fig. 3. The order of tacky feeling could be explained by the softness of the sheet except for BG and FRU. BG is a fluidic material at ambient temperature, resulting in bleeding from the sheet (Table 2). Thus, the tacky feeling of the BG–gelatin sheets could not be attributed to the plasticization of gelatin. In contrast, FRU was a plasticizer with low tackiness, despite its high plasticizing effect. Although we do not have data to explain the reason for this favorable property, the stickiness of the plasticizer itself may have been involved.

3.3. Mechanical properties of gelatin sheets

The most crucial physical characteristics for designing skin protection materials are their mechanical properties, particularly their tensile properties. Mechanical tests were conducted on gelatin sheets to evaluate the plasticizing effects of sugars and polyols. Vitrified CONT sheets were frequently cracked by punching with a blade. Only two specimens showed stress–strain behaviors with low reproducibility, resulting in averaged Young's modulus of 34.6 GPa. Fig. 3A and B presents the typical stress–strain curves obtained from a series of FRU–gelatin and BG–gelatin sheets. The data from FRU_1.67 were not included because the sheets were too soft. Both sheets at a plasticizer/gelatin ratio of 0.13 (w/w) showed sharp increases in stress just after the start of the pulling of the specimens and then broke at strain $<$0.1. The slope of the stress–strain curves became low-pitch, and the stretchability increased with the plasticizer content. Finally, the sheets showed no breakage until strain $\leq$1.0. Fig. 3C and D shows the Young's moduli of the gelatin sheets as a

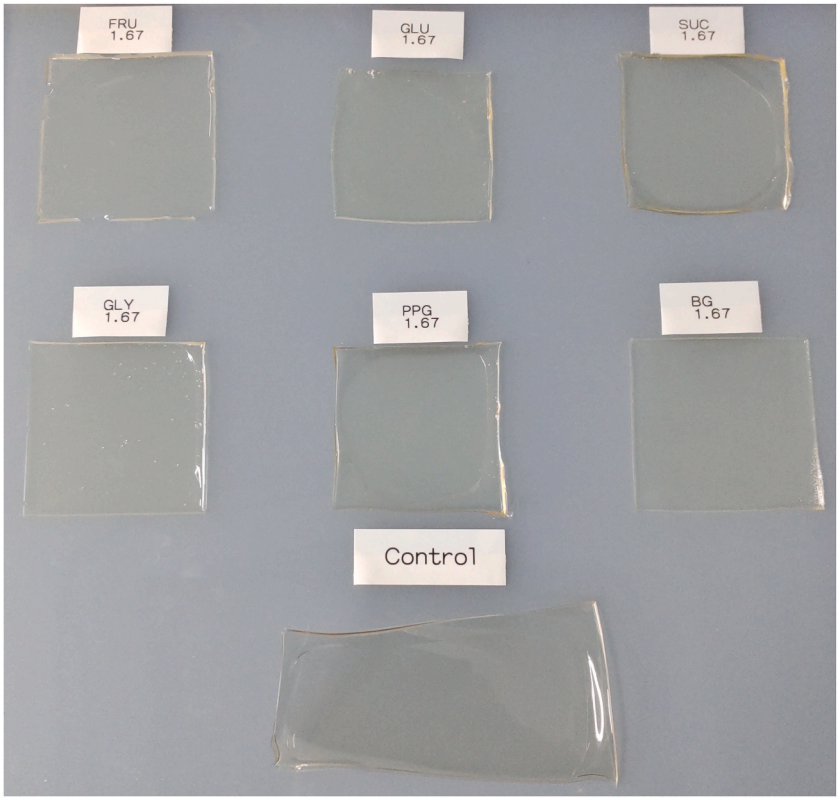


Fig. 2. Representative appearances of plasticizer–gelatin blended sheets. The plasticizers contained in the gelatin sheets at a plasticizer/gelatin ratio of 1.67 (w/w) were fructose (FRU), glucose (GLU), sucrose (SUC), glycerol (GLY), propylene glycol (PPG), and 1,3-buthylene glycol (BG). A gelatin sheet containing no plasticizers was also prepared (control).

Table 2
Textures of plasticizer–gelatin sheets evaluated using tactile sensation.

Content per gelatin	Plasticizer					
[w/w]	FRU	GLU	SUC	GLY	PPG	BG
1.67	+	+	−	++	−	++ *
1.33	−	+	−	++	−	++ *
1.00	−	−	−	++	−	+
0.67	−	−	−	+	−	+
0.33	−	−	−	+	−	−
0.13	−	−	−	−	−	−

The textures of the gelatin sheets were evaluated using tactile sensation, classified into three levels: slippery like hard plastics and dried gelatin membranes (−), smooth but exhibits slightly frictional resistance like food-wrapping plastic films (+), and tacky (++). The plasticizers mixed with gelatin were fructose (FRU), glucose (GLU), sucrose (SUC), glycerol (GLY), propylene glycol (PPG), and 1,3-buthylene glycol (BG). * The bleeding of BG from the surface of gelatin sheets was observed.

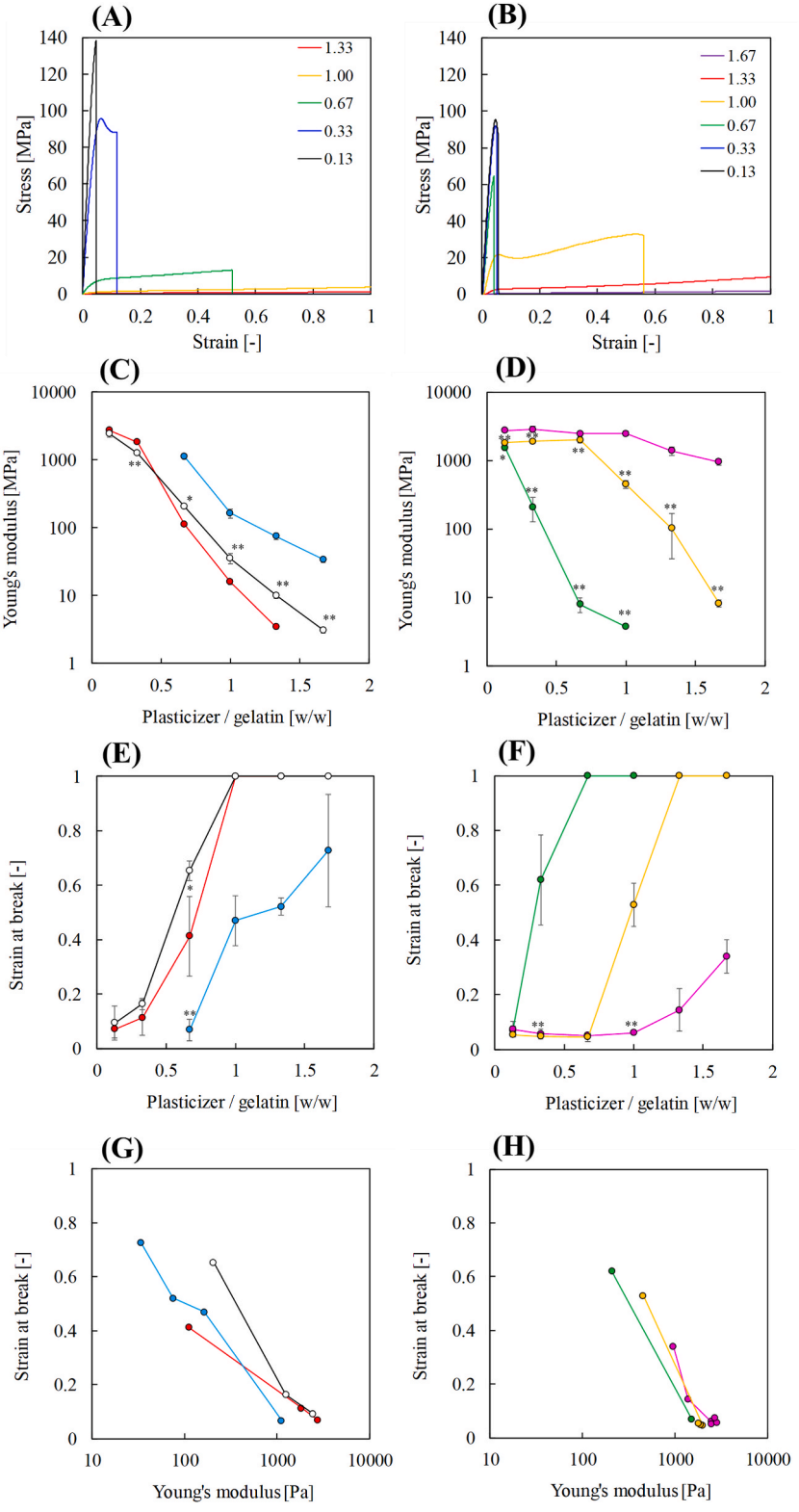
function of sugar and polyol contents, respectively. Overall, the Young's modulus tended to decrease as the plasticizer content increased, but the efficacy of each plasticizer was different. In the sugar group, the plasticizing effects of FRU and GLU were almost identical and significantly higher than those of SUC (Fig. 3C). The SUC_0.13 and _0.33 sheets were not mechanically tested because of their brittleness. The Young's moduli of the FRU–gelatin and GLU–gelatin sheets decreased by more than two orders of magnitude when the sugar/gelatin ratios increased from 0.13 to 1.67 (w/w), as shown in Fig. 3C. In the polyol group, the order of plasticizing effects was statistically determined as GLY > BG > PPG (Fig. 3D). The plasticizing effects of GLY were the highest among all the plasticizers employed in this study. Although the data are not shown in the figures, the Young's moduli of the GLY–gelatin sheets were significantly higher than those of the GLU–gelatin sheets ($p < 0.01$). The Young's moduli of the GLY–gelatin sheets decreased by almost three orders of magnitude even when the sugar/gelatin weight ratios increased from 0.13 to 1.0 (w/w). The gelatin sheets with higher GLY contents were too soft and tacky, making it difficult to cut or treat the specimens. In contrast, PPG showed negligible plasticizing effects in the content range investigated. The plasticizing effects of BG were intermediate between GLY and PPG and less than those of FRU and GLU. Fig. 3E and F shows the strain at break of the gelatin sheets as a function of sugar and polyol contents, respectively. The gelatin sheets having lower Young's moduli tended to exhibit higher strain at break and vice versa. The relationship between the logarithm of Young's modulus and strain at break converged to a single line without the data in which the strain at break reached the upper limit of the mechanical test (strain of 1.0) (Fig. 3G and H).

Based not only on the tensile mechanical properties of plasticizer–gelatin sheets but also on their texture, we selected FRU as a suitable plasticizer for a skin protection material from a physical aspect because FRU had the second highest plasticizing effects while the well-plasticized FRU–gelatin sheet exhibited smooth surface texture (Table 2). The plasticizing effect of GLY on gelatin was the highest (evidenced by the extensive literature conducted on it, particularly for food applications [36]). However, the tackiness of the GLY–gelatin sheets (Table 2) is unsuitable for use as a skin protection material. The mechanical properties and textures of the FRU–gelatin sheets were compared to those of MF to evaluate the potential of the sheets as skin protection materials (Fig. 4). The stress–strain curves of the FRU_1.00 sheet was similar to that of FM, whereas the FRU_1.33 sheet was much softer than FM (Fig. 4A). The Young's modulus of MF was determined to be 12.8 ± 1.2 MPa (mean ± SD; $n = 4$), which was significantly higher than that of FRU_1.33 (3.45 ± 0.23 MPa) and lower than that of FRU_1.00 (15.8 ± 1.1 MPa) ($p < 0.01$). Therefore, FRU_1.00 was selected for the preliminary tactile sensation evaluation on the human skin because it was well-plasticized and exhibited mechanical properties similar (but statistically different) to those of MF while exhibiting a slippery texture.

The mechanical properties of gelatin sheets were obtained for a total of 6 types of organic plasticizers in a wide range of plasticizer/gelatin ratios (0.13–1.67 (w/w)). Sugars (i.e., mono- and disaccharides) are edible and biologically safe chemical compounds; however, to the best of our knowledge, only SUC has been investigated among sugars as a plasticizer for plain gelatin [28] or gelatin–polysaccharide blends [37,38]. Moreover, sugars have been rarely used as plasticizers in other polysaccharides and proteins, except that FRU and xylitol were used as plasticizers for pullulan–alginate–carboxymethylcellulose blend films [26] and SUC for β-lactoglobulin films [27]. Our work demonstrated that the order of the plasticizing effects was GLY > FRU ≈ GLU > SUC > BG > PPG at the plasticizer/gelatin ratios ≤1.67 (w/w). This maximum content was much higher than those reported in the literature, generally <1.0 (w/w). At the upper limit of plasticizer/gelatin ratio (1.67 (w/w)), the plasticizer content was higher than that of gelatin as the base material. It is important to note that when a significant amount of plasticizer is required to soften the gelatin sheets, the physicochemical properties of the plasticizer may become evident. Our findings can contribute to the utilization of sugars as protein plasticizers in the food, pharmaceutical, and biomedical industries.

3.4. Textures of gelatin films on human skin

The physical assessment of the gelatin solution's ability to form a film on skin was conducted. After the topical application and spreading of the gelatin solutions on the skin, thin films were formed from the FRU_1.00 and CONT solutions on the human skin upon air-drying for the comparison of their textures and adhesive properties to those of the commercially available flexible film dressing. Fig. 4B shows the appearances of the FRU_1.00 and CONT films on the human skin. The former film well adhered to the skin, while the latter film vitrified as it dried, and the peripheral part spontaneously peeled off from the skin. Such properties of the CONT film could arise from the glassy state of gelatin at room temperature provided at a water content of less than 30 % [10] as well as the elevated



(caption on next page)

Fig. 3. Mechanical properties of plasticizer–gelatin sheets obtained by tensile tests.
(A and B) Typical stress–strain curves of fructose (FRU)–gelatin (A) and 1,3-buthylene glycol (BG)–gelatin sheets (B). The numbers indicate the plasticizer/gelatin weight ratios.
(C and E) Young's modulus (C) and strain at break values of sugar–gelatin sheets as a function of their weight ratio (E). Red circle, FRU; white circle, glucose (GLU); blue circle, sucrose (SUC). *, $p < 0.05$; **, $p < 0.01$. The data are presented as mean ± SD ($n = 4$).
(D and F) Young's modulus (D) and strain at break values of polyol–gelatin sheets as a function of their weight ratio (F). Green circle, glycerol (GLY); pink circle, propylene glycol (PPG); orange circle, BG. *, $p < 0.05$; **, $p < 0.01$. The data are presented as mean ± SD ($n = 4$).
(G and H) Relationship between the strain at break and Young's modulus of sugar–gelatin (G) and polyol–gelatin sheets (H) without the data of strain at break ≥1.0. The relation between the symbols and the specimens follows those in C–F.

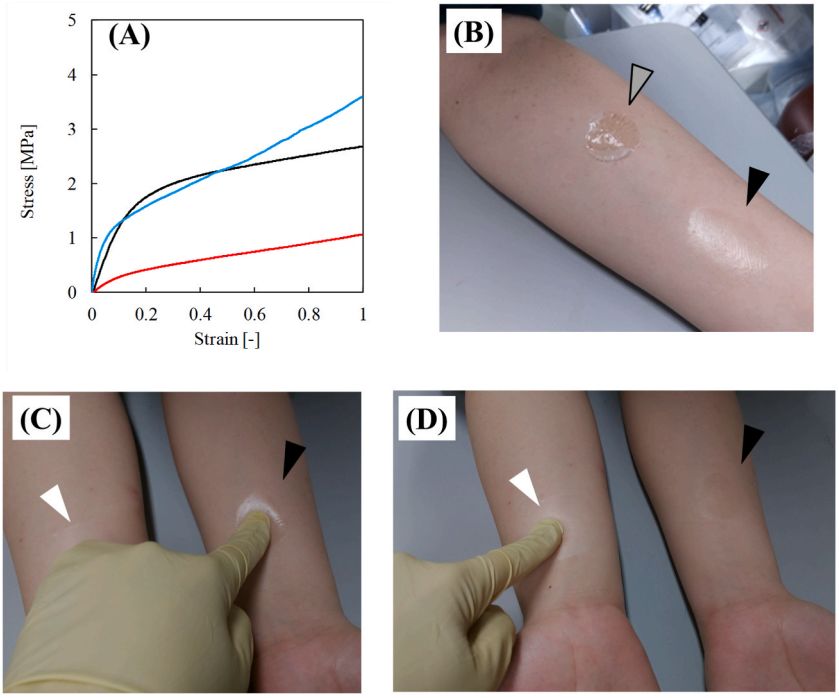


Fig. 4. Comparison of mechanical properties and textures between gelatin and the commercially available polyurethane-based flexible film dressing Multi Fix® (MF). (A) Stress–strain curves of fructose (FRU)–gelatin sheets and FM obtained by tensile tests. Red line, the FRU–gelatin sheet at a weight ratio of 1.33; blue line, the FRU–gelatin sheet at a weight ratio of 1.00 (FRU_1.00); black line, MF. (B) Appearances of the FRU_1.00 film (black arrow) and a plain gelatin film formed on the human skin (gray arrow). (C and D) Tactile sensation evaluation of the FRU_1.00 film (black arrow) and MF on the skin (white arrow).

stress that develops during the drying process of the gelatin solution [39].

The texture and adhesiveness of the FRU_1.00 film was compared to that of FM by pressing them with a finger (Fig. 4C and D). Both films were flexible and well adhered to the skin, thus deformed by the finger push together with the skin. According to these findings, our concept of creating a gelatin-based skin protection material can be considered feasible from a physical perspective. The primary objective of this preliminary experiment was to determine whether an FRU-gelatin solution can create a flexible and smooth film on the skin. However, this study did not investigate the stability of the film or its moisturizing effects on the skin after extended use (e.g., 24 h), which is a limitation.

3.5. *WVB of gelatin sheets*

WVB tests were performed for the plasticizer–gelatin sheets and MF with different thicknesses to evaluate their moisture retention properties instead of tests on human skin (Fig. 5). The FRU_1.00 sheets were selected to represent the plasticizer–gelatin sheets. The thickness of MF was 0.032 mm, but it was difficult to prepare thin films of FRU_1.00 (thickness <100 μm) with uniform thickness by stationary air-drying. The FRU_1.00 sheets exhibited no thickness-dependency of WVBblank/WVBexp. In contrast, the WVBblank/WVBexp value of MF increased almost linearly with the thickness of the test pieces (i.e., number of lamination).

We describe a possible limitation of the FRU–gelatin films concerning their WVBs. The WVB of MF increased with an increase in the thickness (i.e., number of lamination) (Fig. 5), suggesting that MF have channels through which water vapor can transmit. In contrast, the WVBs of the FRU_1.00 sheets did not exhibit thickness-dependency (Fig. 5). It is likely that the FRU_1.00 sheets contain certain amounts of water vapor and release it depending on atmospheric temperature and humidity. The FRU_1.00 sheets and films may

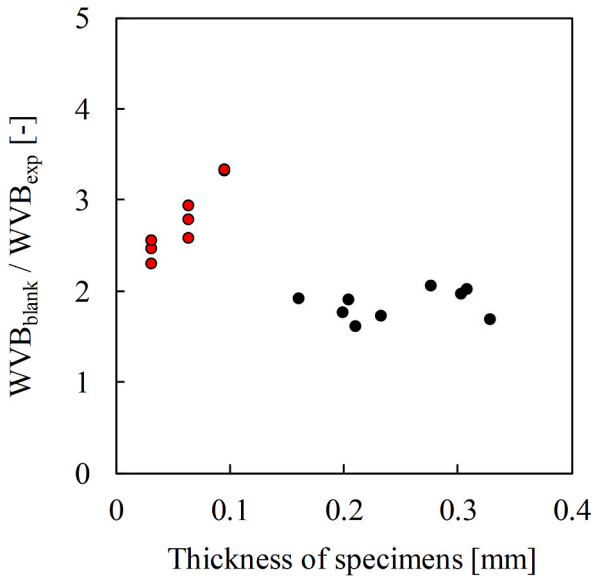


Fig. 5. Water vapor barrier properties of fructose (FRU)–gelatin sheets compared to those of the commercially available polyurethane-based flexible film dressing Multi Fix® (MF). The data from the individual specimens are shown in the figure. Black circle, FRU–gelatin sheets with different thicknesses at a weight ratio of 1.00; Red circle, MF prepared by laminating two, three, and four layers. WVBexp, the amount of water vapor that passes through the specimens; WVBblank, the data without the specimens.

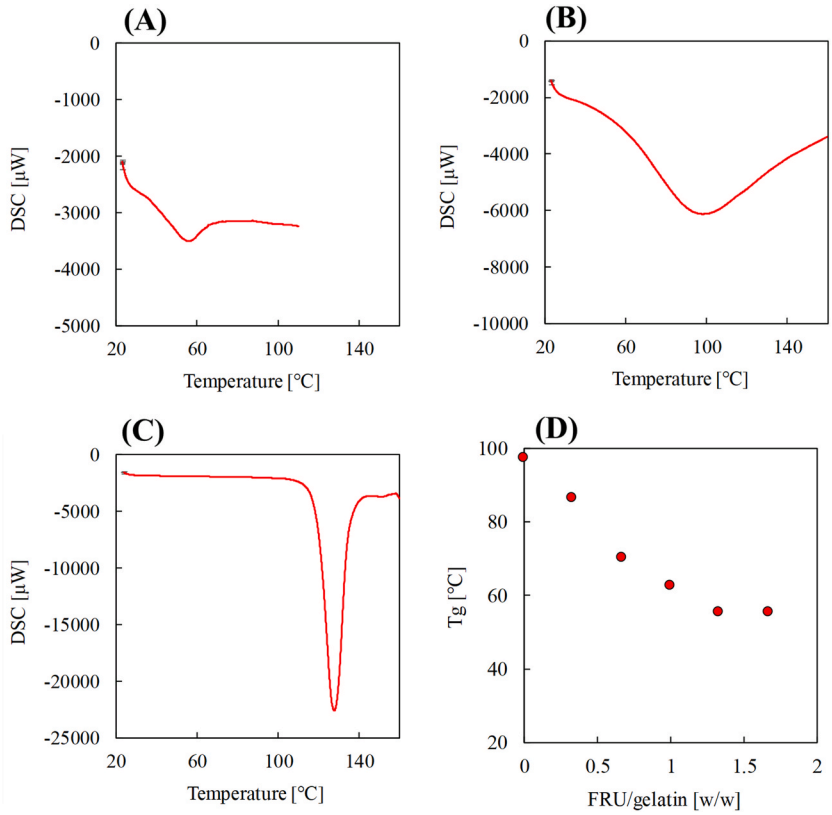


Fig. 6. Thermal properties of gelatin sheets evaluated using differential scanning calorimetry (DSC).
(A–C) DSC curves of the fructose (FRU)–gelatin sheet at a weight ratio of 1.67 (A), a plain gelatin sheet (B), and pure FRU powder (C). The measurements were conducted under heating at a rate of 10 °C/min. (D) Glass transition temperature (T_g) of FRU–gelatin sheets as a function of their weight ratio. T_g was determined from the peak temperature of each endothermic peak.

become too soft or tacky on the skin upon sweating or in high-humidity environments.

3.6. T_g of gelatin sheets

DSC analyses were performed for the FRU–gelatin sheets, CONT sheet, and FRU powder to evaluate the effects of FRU on the T_g of gelatin. Fig. 6A and B shows the DSC graphs of the FRU_1.67 and CONT sheets. The endothermic peak of the CONT sheet (97.5 °C) dramatically shifted to a lower temperature owing to the presence of FRU at an FRU/gelatin ratio of 1.67 (w/w) (55.4 °C). It was confirmed that those peaks did not overlap with that of FRU alone (Fig. 6C). The T_g of the FRU–gelatin sheets decreased as the FRU content increased (Fig. 6D).

Therefore, the plasticizing effects of FRU can be explained by the decrease in T_g. Many studies on the plasticizing effects of polyols have shown that the mechanical properties of the plasticized gelatin are well correlated with T_g [14,37,38,40]. The plasticizing effects of polyols can be attributed to their ability to locate between protein molecules, bind water, and disrupt intermolecular interactions [41], which aligns with the free volume theory, one of the four major plasticization theories of proteins [42]. Because of the decrease in T_g with increasing FRU content, the free volume theory is conceivable for the plasticizing effects of FRU as well as polyols.

The intention behind acquiring DSC data was to evaluate it alongside the mechanical data from specimens that had not been annealed. It is worth noting that the DSC data was obtained through the initial scan. Typically, DSC data for gelatin is acquired through a second scan [14,19], which eliminates the influence of the specimens' thermal history. The time intervals between specimen preparation and DSC measurements were kept consistent. However, the thermal history of the samples may vary and affect the Tg value.

3.7. Perspectives on plasticizer–gelatin mixed solutions

The main limitation of this study is that it mostly focused on physical data, which precludes discussion of the pharmacological and dermatological effects of the material. However, the use of a water-soluble gelatin sheet for skin protection offers the advantage of easy removal with warm water, making it a promising option for topically used noninvasive skin protection materials. The physical data of plasticizer-gelatin mixed solutions and plasticized gelatin sheets may prove useful for future investigations aimed at creating gelatin gels and sheets for pharmaceutical and dermatological purposes, such as novel, noninvasive skin protective films and groundbreaking wound-healing gels and sheets, which can be attributed to the scaffolding properties of gelatin [30].

4. Conclusion

Our study found that six types of sugars and polyols had varying plasticizing effects on dry gelatin, while exhibiting little impact on the rheological and gelation properties of the gelatin solutions. Specifically, the FRU-gelatin solution formed a flexible and smooth sheet, with mechanical properties similar to those of MF. In terms of physical properties, we determined that the optimal FRU-gelatin weight ratio for creating a skin protection material was 1.00. We conclude that, from a physical aspect, this material has potential as a skin protection material that transforms from a solution to a film on the skin.

Ethics statement

The tactile sensation evaluation of the gelatin sheets on human skin (female, age 24) was approved by the Business Ethics Review Committee of Tokyo Metropolitan Industrial Technology Research Institute (ME2021-5). Before starting examinations, we obtained the explicit consent concerning the examination from the subject.

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Data availability statement

The confidentiality of the data used in this research is ensured by the fact that the research is being conducted in collaboration with a private company, which intends to apply the material clinically based on the gathered information.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Shunji Yunoki: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition. **Asami Mogi:** Writing – review & editing, Visualization, Investigation. **Keizo Mizuno:** Writing – review & editing,

Supervision, Project administration, Conceptualization. **Yoshiyasu Nagakawa:** Methodology, Investigation, Formal analysis. **Yosuke Hiraoka:** Writing – review & editing, Supervision, Project administration, Methodology.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Shunji Yunoki reports financial support was provided by Japan Agency for Medical Research and Development. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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