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Effect of gelatin drying methods on its amphiphilicity

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Abstract:
Gelatin is a natural amphiphilic biopolymer that is widely used in food products, pharmaceuticals, and cosmetics. We studied the effect of spray and freeze drying on the solubility and amphiphilicity of gelatin samples.
The control sample was a commercially produced edible gelatin. The experimental samples were spray- and freeze-dried gelatins obtained by enzymatic-acid hydrolysis of cattle bone. Amino acid sequences were determined by matrix-activated laser desorption/ionization. Solubility was assessed visually. Bloom strength of the gelatin gels was measured by a texture analyzer. The ProtScale online service was used to predict the amphiphilic topology of gelatin proteins. Molecular weight distribution of proteins was carried out by electrophoresis in polyacrylamide gel in the presence of sodium dodecyl sulfate.
Spray drying reduced protein degradation and retained more α-chains, while freeze drying increased gelatin's hydrophobicity and decreased its solubility. The predicted topology of protein hydrophobicity based on the amino acid sequences was in line with our results on solubility. The freeze-dried gelatin had a 18% larger amount of low-molecular weight peptides, compared to the control and the spray-dried samples. This was probably caused by the cleavage of peptides during the drying process. Thus, freeze drying can lead to maximum degradation of gelatin components, which may be associated with a longer heat treatment, compared to spray drying.
Thus, spray drying is more suitable for gelatin, since this method improves the stability of its outer and inner structure, ensuring high hydrophilic properties.

Keywords: Drying, gelatin, protein, amino acid sequence, hydrophilicity, hydrophobicity, solubility

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INTRODUCTION

Gelatin is a protein substance that contains all essential amino acids except tryptophan. It is formed by cross-links between various polypeptide chains that developed after the destruction of the fibrous structure of collagen pre-treated with acid, alkaline, or enzymes. This protein-based hydrocolloid has a wide range of applications in various industries due to its unique structural stability, nutritional properties, and other physicochemical characteristics [1]. Particularly, hydrogels and modified gelatin-based composites are widely used in the food industry, biomedicine, pharmaceuticals, and cosmetology. Gelatin is also used in the production of food packaging materials due to its biocompatibility, biodegradability, non-immunogenicity, and ability to stimulate cell adhesion and proliferation. It can absorb 5–10 times as much water as its weight and is the main ingredient in hard and soft capsules for pharmaceuticals [2–6].

There is a high demand for gelatin in the modern market of food products and components, as well as in the pharmaceutical, medical, and cosmetic markets,

with an annual average of 326 000 tons produced worldwide. According to Grand View Research, the global gelatin market was worth $2.91 billion in 2020. It is estimated to grow by 8% per year and reach about $5 billion by 2025. Russia seeks to produce food gelatin domestically and therefore needs effective technological and biotechnological solutions [7–10]. Current research focuses on optimizing gelatin production technologies and searching for new sources of raw materials to replace the traditional ones (pig skins, bovine skins, and cattle bones).

Today, gelatin is still produced with the technologies developed several decades ago. The process contains the following stages: pre-treatment of raw materials, extraction of gelatin, processing of gelatin broths, gelatinization, and drying. The efficiency of collagen-to-gelatin conversion depends on extraction conditions (temperature, time, and pH), concentration, the quality of raw materials, and their pre-treatment methods.

Using chemical solvents for gelatin extraction can result in a higher gelatin yield along with more low-molecular-weight protein fragments that will affect the gel's strength and melting point. However, industrial production parameters are not always optimal, leading to a low gelatin yield. Therefore, we need to search for alternative solutions to optimize the process.

Drying is an important process to obtain gelatin with improved functional properties. These properties basically depend on the type of raw materials, pre-treatment methods, drying and extraction conditions, as well as the spatial structure of protein molecules and their state. Drying causes physicochemical changes in the structure and functions of proteins. For example, heating, which is part of the drying process, can break covalent and non-covalent bonds leading to changes in the protein structure. If significant, these changes can greatly affect gelatin's functional properties such as solubility, gelation, foaming, emulsification, as well as fat and water absorption. The extent of these changes is mainly determined by the drying methods and conditions [11–15].

Drying methods used in the production of protein ingredients (including gelatin) are convection drying, infrared drying, spray drying, and freeze drying.

Convection is the most common method of food drying. In convection drying, a stream of heated air is directed at a wet sample. The air here is both a heating agent and a dehydrator, since it carries away moisture vapor from the dryer. As a result of this lengthy process and elevated temperatures, the final product loses a significant amount of micronutrients and bioactive compounds. Although this method is simple to use, convection dryers have low productivity which can lead to uneven drying [16, 17].

Infrared heating with microwaves is a new method of heat treatment (drying) that extends shelf life, reduces drying time, and preserves food quality. The microwaves transfer water to the surface where it quickly evaporates under the influence of infrared radiation, which reduces the drying time [18, 19].

Spray drying is widely used in the food industry due to its simplicity and short drying time. This method allows for a good quality powdered product. However, spray drying causes particles to greatly shrink and become denser [20, 21].

Freeze drying is a process of removing water from a product by freezing it and then converting ice into steam. This process consists of three main stages: freezing, primary drying, and secondary drying. Freezing creates a solid matrix suitable for drying. Primary drying removes ice by sublimation, when the pressure in the system is reduced but the temperature remains the same. Secondary drying removes bound water reducing it to residual moisture.

Several studies indicate that protein denaturation during the formation of ice crystals can significantly change the protein structure. Therefore, when optimizing the freezing process, we should take into account the ice surface area, since it can contribute to protein denaturation caused by freezing [22–24].

In spray drying, evaporated material is sprayed through the nozzles of a conical-cylindrical apparatus (spray dryer) to obtain a product in the form of a powder or granules. This method is used to dry solutions or suspensions. Spray-dried products include powdered milk, food and fodder yeast, and egg powder. According to some studies, spray drying can effectively eliminate many of the shortcomings of protein and bioactive peptides, such as low bioavailability, high hygroscopicity, physical and chemical instability, as well as strong bitterness during and after storage [13]. It is also claimed that this method can improve gelatin's functional properties, compared to freeze or vacuum drying [25]. Assumingly, various drying methods affect the solubility and amphiphilicity of gelatin as a high-protein product, thereby changing its functional properties [26].

We studied the effect of spray and freeze drying on the solubility and amphiphilicity (hydrophobicity and hydrophilicity) of gelatin which we obtained in the previous study by enzymatic acid hydrolysis [7].

STUDY OBJECTS AND METHODS

The control sample was a commercially produced edible gelatin. The experimental samples were spray- and freeze-dried gelatins obtained by enzymatic-acid hydrolysis of cattle bone. For this, 3 kg of defatted beef bones was crushed to particles of 3.0 ± 0.5 mm in a laboratory chain grinder. The bones were obtained from a farm in Kuzbass (Russia). The crushed bones were placed in a solution of hydrochloric acid (1M HCl) which contained pepsin with an enzymatic activity of 300 000 units. The hydrolysis was carried out at 27 ± 2°C for 60 to 240 min, with a pH of 1.5–2.0. MS-01 magnetic stirrers (ELMI) were used throughout the experiment to stir the bone material at 100 rpm and 27 ± 2°C to ensure its uniform treatment with the

solution. The hydrolyzed material was centrifuged in a high-speed Avanti J-26S centrifuge (Beckman) to separate the mineral sediment from ossein. Then, the resulting ossein was washed with demineralized water and subjected to gelatin extraction. A detailed scheme of hydrolysis and gelatin extraction is described in our previous work [7].

Next, the gelatin broths were dried by the spray- and freeze-drying methods. The spray-dried gelatin was obtained in a B-290 Mini Spray Dryer (Buchi, Sweden) at 95°C and a rate of 3.0–3.2 mL/min. The freeze-dried gelatin was obtained in an INEY-6M freeze-drier. For this, gelatin broth was poured onto pallets in 1-cm layers and placed in the drying chambers. The chambers were closed with lids and the refrigerator was turned on. The unit entered the freezing mode within 15 min and when the evaporator temperature reached –35°C, the vacuum pump turned on to start the drying mode. The freeze-dried gelatin was then ground in an NS-2000 automatic laboratory mill.

The amino acid sequence of gelatin proteins, which is represented by a single letter code, was determined by matrix-activated laser desorption/ionization on a MALDI Biotyper (Bruker). Amino acid residues, isoelectric point, aliphatic index, molar absorption coefficient, as well as the surface area of the peptides, were determined by the *in silico* bioinformatic methods on the PepDraw online server. The gelatin samples' solubility was evaluated visually. For this, 500 mg of gelatin was mixed with 50 mL of distilled water and stirred actively (200 rpm) with MS-01 magnetic stirrers (ELMI). Dissolution was monitored at water temperatures of 25 and 50°C.

The Bloom strength of gelatin gels was determined on a ST-2 Strukturometr texture analyzer with a Bloom indenter. For this, 7.5 g of gelatin was placed in a glass of cold water (105 mL) and kept at 22°C max for 180 min. Next, the swollen gelatin was heated to 60°C in a water bath and stirred for 15 min until complete dissolution. The solution (6.67% concentration) was poured into a calibrated beaker and kept at 10.0 ± 0.1°C for 17 h. The prepared samples were then placed on the analyzer's table under the Bloom indenter for the study. The arithmetic mean of two determinations was taken as the final result.

The ProtScale online service was used to predict the topology of the hydrophobicity and hydrophilicity of gelatin proteins. In particular, this service allows us to compute and represent (in the form of a two-dimensional graph) the profile produced by any amino acid scale for a selected protein. The amino acid scale is defined by a numerical value assigned to each type of amino acid. ProtScale uses the Kyte and Doolittle scale that assigns individual values to 20 amino acids, namely *Ala*: 1.800, *Arg*: –4.500, *Asn*: –3.500, *Asp*: –3.500, *Cys*: 2.500, *Gln*: –3.500, *Glu*: –3.500, *Gly*: –0.400, *His*: –3.200, *Ile*: 4.500, *Leu*: 3.800, *Lys*: –3.900, *Met*: 1.900, *Phe*: 2.800, *Pro*: –1.600, *Ser*: –0.800, *Thr*: –0.700, *Trp*: –0.900, *Tyr*: –1.300, and *Val*: 4.200, –3.500, –3.500, –0.490.

The molecular weight distribution of proteins was carried out by polyacrylamide gel electrophoresis in the presence of an anionic detergent, sodium dodecyl sulfate (SDS-Na). For this, the dried gelatin samples were dissolved in deionized water at 60°C to create a 0.2% solution. The solution was then mixed with a loading buffer containing 5 μL of dithiothreitol (DTT) and subjected to heat denaturation in boiling water for 5 min. After that, 15-μL samples were loaded into polyacrylamide gels containing 6% of separating gel and 5% of stacking gel to perform electrophoresis. Then, the gels were stained with 0.1% Coomassie Blue R-250 in 25% isopropanol and 10% acetic acid for 2 h, followed by decoloring with 5% alcohol and 10% acetic acid. Next, 2-D gels were detected using the Gel Doc XR Plus Bio-RAD system.

RESULTS AND DISCUSSION

First, we spray- and freeze-dried the experimental samples of gelatin obtained by enzymatic acid hydrolysis. Next, we determined the amino acid sequence of all the dried gelatins (Table 1).

The proteins of the control, spray-dried, and freeze-dried samples are represented by peptide sequences of 85, 93, and 95 amino acids, respectively (Table 1).

These sequences allowed us to determine the amino acid composition (% or g/100 g of total amino acids) of the control and experimental samples. This is a critical indicator of gelatin quality largely depending on raw materials. Glycine and proline are the most important amino acids in gelatin. Collagen consists of three

Table 1 Amino acid sequences of gelatin samples (one-letter coding)*

Control	Experimental samples	
	Spray-dried gelatin	Freeze-dried gelatin
GGPAAGGPAYGGPILILAPAILA	SHILEILDVILDHILILDMILSHESHP	PILEVILEILESHILEMILHILMILS
PYILAAILADNPAANPAYNPILP	YCGDDGGYGPYPDDPGYDDGYH	HPSHPEEPEEEMPEMPPRPPRVR
NAAPNAYPNILPQGAPQGYSEA	EHPILMEMPPYQCCGQNYYNCDD	PVREPEHPHPILMPMPRPREYPY
ASEAYSEILTNAATNAYPATN	ENNPQQRRSVYAEVPYQCCVPGG	ESGQSQYNADEGNNPPPQQRS

*A – alanine; C – cysteine; D – aspartic acid; E – glutamic acid; F – phenylalanine; G – glycine; H – histidine; I – isoleucine; K – lysine; L – leucine; M – methionine; N – asparagine; P – proline; Q – glutamine; R – arginine; S – serine; T – threonine; V – valine; W – tryptophan; Y – tyrosine

identical or different polypeptide chains with a repeating pattern $(Gly\text{-}XY)_n$ (X and Y stand for any amino acid) and a high content of imino acids with a triple helical structure due to hydrogen bonds [27–29].

The composition and content of amino acids, especially imino acids, in gelatin have a significant impact on its structure and functional properties. In particular, the gel's supercoil structure is stabilized by both the hydrogen bonds forming between amino acid residues and the pyrrolidine rings of imino acids. A higher content of imino acids ensures a higher gel modulus, gelling temperature, and melting point [30].

The amino acid composition (% or g/100 g of total amino acids) of the control and experimental gelatin samples are presented in Table 2.

We found that the samples varied mostly in the content of alanine, accounting for 25.840% in the control sample and only 1% in the experimental samples.

None of the samples contained phenylalanine, lysine, or tryptophan. According to literature, the absence of tryptophan is what makes gelatin different from other hydrocolloids of animal origin. This amino acid is mainly present in membrane proteins and has aromatic residues in its structure.

Histidine, arginine, and threonine were not detected in the control sample.

Using the PepDraw online server, we determined the mass of amino acid residues defined as a sum of monoisotopic masses of all amino acid residues in the peptide. We also calculated the isoelectric point represented by a pH value at which the total charge of the peptide equals zero. This calculation shows the partial charge of the peptide at various pH values, starting from 0. Then, we determined the aliphatic index of the protein defined as a relative volume of aliphatic side chains (alanine, valine, isoleucine, and leucine). It can be considered a positive factor in increasing thermal stability of globular proteins.

The mass of amino acid residues in the control, spray-dried, and freeze-dried samples amounted to 13 173.86, 10 830.72, and 11 156.79, respectively.

The aliphatic index values in the control, spray-dried, and freeze-dried samples were 95.96, 87.53, and 70.74, respectively.

The isoelectric points in the control, spray-dried, and freeze-dried samples were 5.97, 4.89, and 5.96, respectively.

The molar absorption coefficients in the control, spray-dried, and freeze-dried samples were 8960.00, 12 800.00, and 3840.00 $M^{-1} \cdot cm^{-1}$, respectively.

The surface area values in the control, spray-dried, and freeze-dried samples were 21 223.00, 23 040.00, and 18 407.00, respectively.

We concluded that the control and the spray-dried samples had more thermostable proteins, since their aliphatic indexes (87 and 96, respectively) were higher than those of the freeze-dried sample (70). The samples' isoelectric points indicated a slightly acidic reaction, therefore their protein molecules were neutral at a pH value of 4.89 to 5.97.

Solubility is an important property of gelatin in food systems. In cold water, gelatin hydrates and swells, and at temperatures above 40°C, it forms a colloidal solution (sol). The solubility index depends on the method

Table 2 Amino acid contents in the control and experimental gelatin samples

Amino acid	Content of total amino acids, % or g/100 g		
	Control	Spray-dried gelatin	Freeze-dried gelatin
A – alanine	25.840	1.050	1.080
C – cysteine	ND	6.320	ND
D – aspartic acid	1.120	11.580	1.080
E – glutamic acid	3.370	6.320	16.130
F – phenylalanine	ND	ND	ND
G – glycine	8.990	9.470	2.150
H – histidine	ND	6.320	6.450
I – isoleucine	8.990	7.370	8.600
K – lysine	ND	ND	ND
L – leucine	8.990	7.370	8.600
M – methionine	ND	3.160	6.450
N – asparagine	10.110	4.210	3.230
P – proline	15.730	10.530	21.510
Q – glutamine	2.250	5.260	4.300
R – arginine	ND	2.110	7.530
S – serine	3.370	4.210	6.450
T – threonine	3.37	ND	ND
V – valine	ND	4.21	3.23
W – tryptophan	ND	ND	ND
Y – tyrosine	7.87	10.53	3.23

ND – not detected

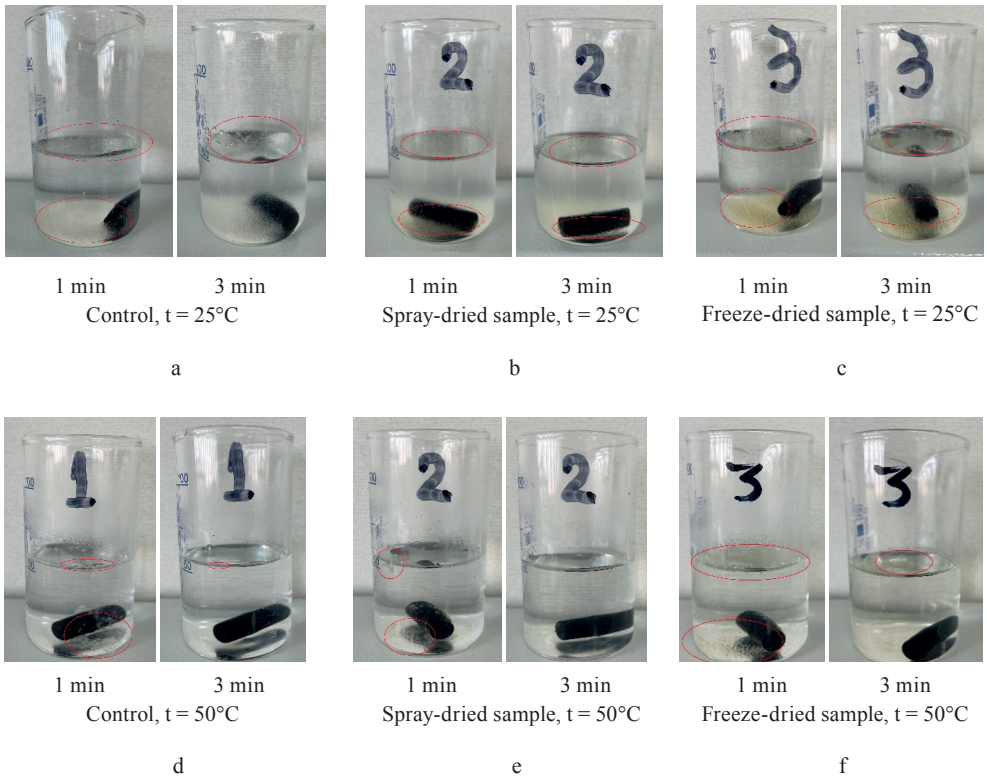

Figure 1 Dissolution of gelatin samples at 25 and 50°C for 1–3 min

of gelatin production. New methods are currently being developed to obtain water-soluble gelatin at temperatures below 40°C. Such gelatin usually has an amorphous powdery form.

Next, we visually assessed the degree of solubility of the gelatin samples at water temperatures of 25 and 50°C (Fig. 1).

As we can see, the control and the spray-dried samples showed higher protein solubility at 25 and 50°C than the freeze-dried sample. According to Fig. 1c and f, gelatin particles did not dissolve after 1 min of mixing at different temperatures, settling on the bottom and on the surface. After 3 min of mixing at 25 or 50°C, the freeze-dried sample still did not dissolve completely, its particles settling on the water surface (Fig. 1f). This could be due to the sample's mechanical grinding in a laboratory mill at the final stage of freeze-drying, which resulted in larger particles than those in the spray-dried gelatin and affected its solubility. We can also assume that spray drying exposes protein molecules to less thermal stress than freeze drying, which causes the highest degree of thermal and dehydration stress.

Next, we evaluated the Bloom strength of the gelatin gels (Fig. 2).

The Bloom value is an important parameter of gelatin's physical and mechanical properties used in food production. It is also used as a criterion in gelatin classifications.

The gel strength index depends on the protein content and the molecular weight of peptides formed in gelatin. In our study, this index was quite high in the control and spray-dried samples, amounting to 229.0 ± 0.5 and 224.0 ± 0.5 Bloom, respectively. The freeze-dried sample's index (186.0 ± 0.5 Bloom) was by 17 and 19% lower than for the control and spray-dried samples. Assumingly, the proteins of the freeze-dried gelatin had a lower molecular weight, which worsened its structural and mechanical properties. We can also assume that this sample might have more low-molecular weight (below 20 kDa) peptides.

Next, we determined the degree of protein hydrophilicity and hydrophobicity based on the amino acid sequences. Using the ProtScale online service (the Kyte and Doolittle scale), we predicted the topology of protein hydrophobicity and hydrophilicity for the control and experimental gelatin samples (Fig. 3).

On the Kyte and Doolittle scale, the peaks above 0 refer to hydrophobicity and those below 0 refer to hydrophilicity. As we can see in Fig. 3a, the control sample had higher hydrophilic properties since its peaks along the X axis ranged from 3 to 21, with a peptide sequence of PAAGGPAY GGPILILAPA I. Most of its peaks were for alanine, proline, isoleucine, and glycine. These amino acids had hydrophobic properties and 1 to 4 uncharged side radicals at pH = 6–7. In general, these peaks characterized a sequence of amino acids with hydrophobic properties.

The region from 22 to 53 had a sequence of LAPYILAAI LADNPAANPA YNPILPNAAP NAY,

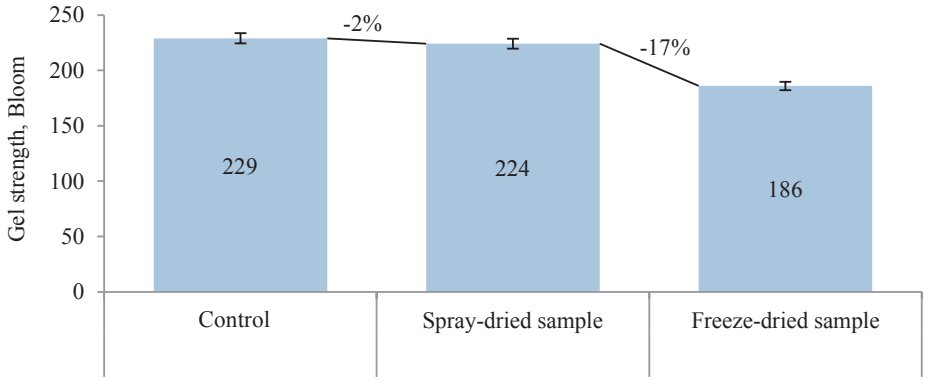

Figure 2 Bloom strength of gelatin gels

whereas the region from 56 to 85 was represented by PQGAPQGYSEAAS EAYSEILTNA ATNAY. Thus, 21 out of 85 amino acids had hydrophobic properties. According to Fig. 3a (control), leucine had maximum hydrophobicity of 2.585 units at point 17 and aspargin had highest hydrophilicity of –2.678 at 89.

The profile of the spray-dried sample is shown in Fig. 3b. As we can see, the peptide region from 5 to 21 represented by AGGPAY GGPILILAPA I and the region from 55 to 57 with a NIL sequence had hydrophobic properties. Most of the peaks were located above 0 and were represented by alanine, isoleucine, and proline. These amino acids had hydrophobic properties and 1 to 3 uncharged side radicals. Leucine (18) had a maximum hydrophobicity value of 2.500 units, while glutamine (77) had a maximum hydrophilicity value of –3.511. Thus, 20 out of 91 amino acids had hydrophobic properties.

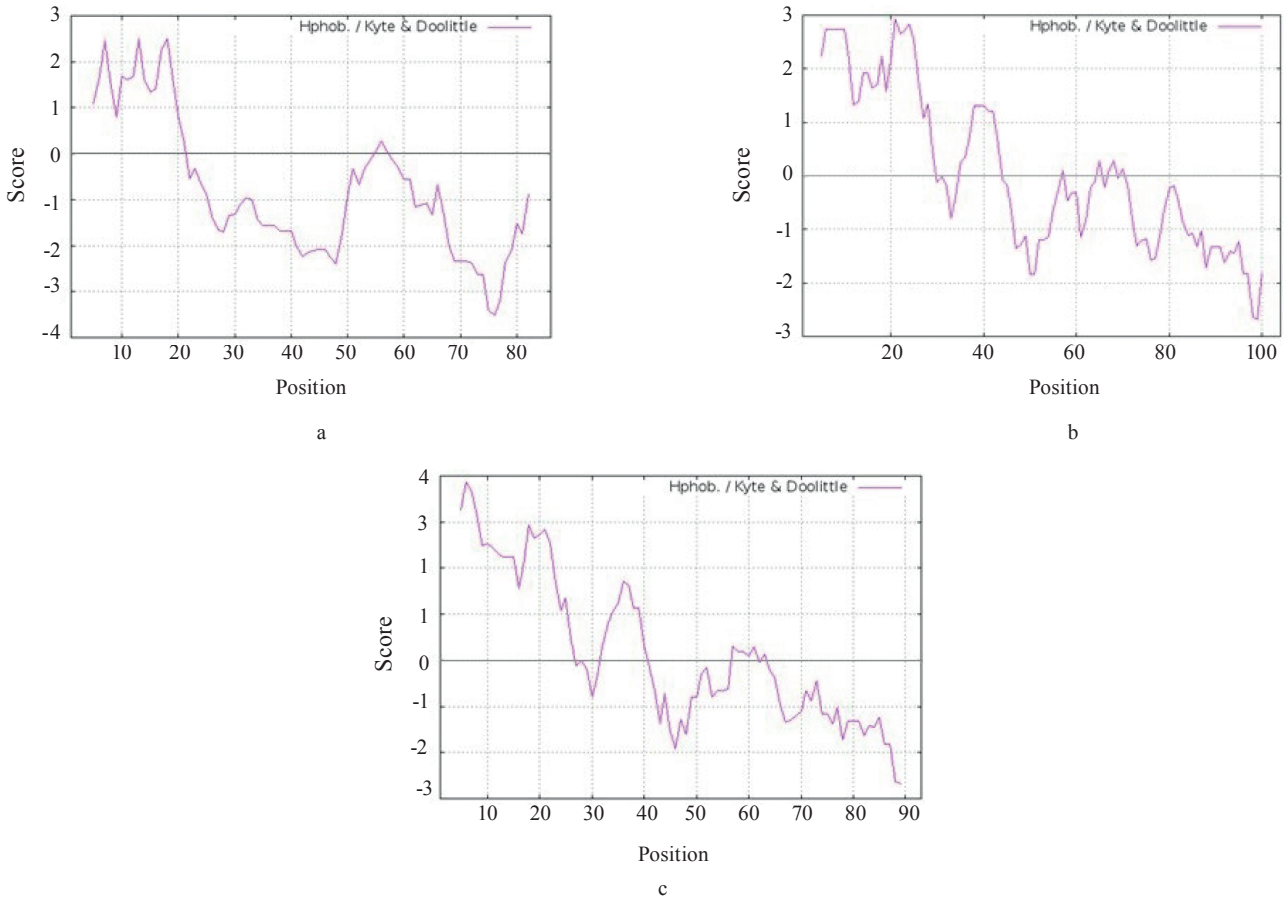

Figure 3 Predicted topology of protein hydrophobicity and hydrophilicity in the control and experimental gelatins (a – control; b – spray-dried sample; c – freeze-dried sample)

The profile of the freeze-dried sample is shown in Fig. 3c. As can be seen, the peptide region from 5 to 26 was represented by a sequence of VILEIL ESHILEMILH ILMILS, the region from 32 to 39 had a sequence of EEPEEEMP, and the one from 57 to 61 was represented by PHPI. Glutamic acid, isoleucine, and leucine had most peaks in the hydrophobicity area. They also had from 4 to 7 uncharged side radicals at pH = 6–7. Isoleucine (6) had maximum hydrophobicity of 3.856 units, while glutamine (76) had maximum hydrophilicity of –5.504 units. Thus, 36 out of 93 amino acids had hydrophobic properties. Our results were consistent with literature on the amphiphilic (hydrophobic and hydrophilic) properties of these amino acids [31–38].

The hydrophobicity values based on the amino acid sequences of the gelatin samples confirmed our data on their solubility. In particular, they proved that the method of drying affects the gelatin's structural and mechanical properties, as well as its physicochemical parameters. Spray drying can improve the proteins' functional properties compared to freeze drying. Therefore, we can conclude that different drying methods affect the solubility and amphiphilicity properties of gelatin, thereby changing its functional properties.

Finally, we analyzed the molecular weight distribution of proteins by polyacrylamide gel electrophoresis in the presence of an anionic detergent, sodium dodecyl sulfate (Fig. 4, Table 3).

According to the results, the control sample's protein fractions were more evenly distributed by molecular weight compared to the experimental samples. Its fractions between 50 and 100 kDa accounted for 72.6% and those below 20 kDa amounted to 6.1138% of the total content. The spray-dried sample showed a somewhat different molecular weight distribution. Its protein fractions between 40 to 100 kDa made up 73.8%, while those below 20 kDa accounted for 5.026315%. The freeze-dried sample had a completely different distribution of protein fractions. Most peptides were found at the level of 40 kDa (42.83855%). Yet, this sample had 30.214499% of proteins with a molecular weight below 20 kDa, which was by 20.23% more than in the control and by 16.64% more than in the spray-dried gelatin. This increase in low-molecular weight peptides by an average of 18% was most likely caused by the cleavage of peptides during freeze drying.

Our results showed that the degree of degradation of gelatin components can depend on the method of drying gelatin broths. Freeze drying can lead to maximum degradation, which may be associated with long heat treatment (5 h). This time is much longer compared to spray drying, although the process of freeze drying takes place at a lower temperature (60°C). Also, temperatures below 0°C cause gelation followed by freezing, which can also lead to structural changes in gelatin. In addition, a faster process of spray drying can slow down the degradation of gelatin proteins.

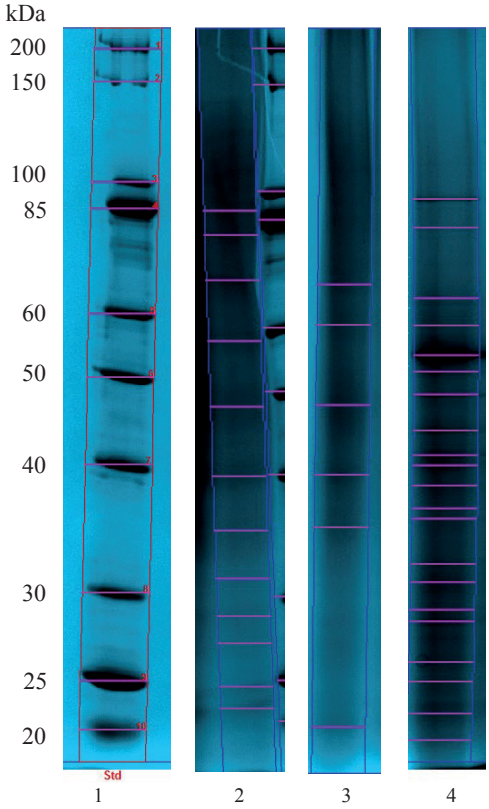

Figure 4 Electropherogram of the molecular weight distribution of gelatin samples (1 – marker; 2 – control, 3 – spray-dried sample, 4 – freeze-dried sample)

These results were consistent with those for gelatin solubility (Fig. 2). Therefore, spray drying is more suitable for maintaining the structure of gelatin and its functional properties.

CONCLUSION

We studied the effect of spray and freeze drying of gelatin broths on the solubility and amphiphilicity of gelatin. The results showed that spray drying can reduce the breakdown of gelatin proteins and retain more α-chains, while freeze drying increases the hydrophobicity of gelatin and decreases its solubility. The predicted topology of protein hydrophobicity, which was based on the amino acid sequences of the gelatin samples, confirmed the results on solubility. Particularly, the freeze-dried gelatin had 36 amino acids with hydrophobic properties out of 93, compared to 21 out of 85 in the control and 20 out of 91 in the spray-dried sample.

We found that the freeze-dried sample had by 18% more low-molecular weight peptides (below 20 kDa) compared to the control and the spray-dried samples. This was most likely caused by the cleavage of peptides during the drying process. Freeze drying can lead to maximum degradation of gelatin components due to long heat treatment. Temperatures below 0°C cause gelation followed by freezing, which can also cause

Table 3 Molecular weight distribution of gelatin samples

Molecular weight, kDa	Molecular weight distribution, %		
	Control	Spray-dried sample	Freeze-dried sample
200	1.316515	3.878759	6.386503
150	3.101737	5.118362	1.334564
100	19.4574	17.810301	1.301436
85	17.85029	13.035829	1.367691
60	14.53681	8.429303	42.83855
50	20.790700	21.727447	3.170772
40	10.111660	12.89394	3.459454
30	0.220568	6.732710	3.530442
25	2.909430	1.157420	3.452356
20	3.591122	4.189303	2.974374
Below 20	6.113800	5.026315	30.214499

structural changes in gelatin. By contrast, a faster spray drying process can, to a certain extent, slow down the degradation of gelatin proteins.

Thus, spray drying is more suitable for gelatin drying, since this method improves the stability of gelatin's outer and inner structure, which was confirmed by high hydrophilicity values of the spray-dried sample. Further research could search for optimal parameters and modes of spray drying for gelatin broths.

CONTRIBUTION

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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