OPTIMIZATION OF GELATIN EXTRACTION FROM SILVER CARP SKIN AND TEXTURAL, RHEOLOGICAL, AND SENSORY CHARACTERISTICS OF EXTRACTED GELATIN

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OPTIMIZATION OF GELATIN EXTRACTION FORM SILVER CARP SKIN AND TEXTURAL, RHEOLOGICAL, AND SENSORY CHRACTERISTICS OF EXTRACTED GELATIN

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Gelatin is an important multifunctional biopolymer, being the only protein widely used in foods, drugs and cosmetics; to improve elasticity, consistency, and stability. Although pork skin is still the most commonly used raw material for gelatin production, fish skin has been looked at as an alternative raw material, eliminating religious concerns shared by the Jewish and Muslim communities and providing an alternative way of using the waste from the fish processing industry. Silver carp, an Asian carp subspecies, accounts for over 2.5% of the world's total fish production with over 4 million tonnes annual production. The objective of this study was to optimize gelatin extraction from silver carp skin and to determine its textural, rheological, and sensory properties in comparison with commercially available gelatins from different sources. For the optimization part of the project, a two-step optimization procedure was used. First, those extraction parameters that significantly affect the quality of gelatin extracted were elucidated and second, the optimum levels of those parameters giving the best possible gelatin were determined. The optimum extraction conditions were 50 °C for water extraction with a 4:1 (v/w) water/skin ratio and a 0.1 N HCl acid pretreatment for 45 min. The predicted values for the most important gelatin parameters for these extraction conditions were 630 g gel strength, 6.3 cP viscosity, and 80.8% recovery of the gelatin in the original skin. The gelatin extracted from silver carp skin under optimum conditions was found to be similar to fish gelatins currently being exploited commercially and superior in some aspects such

as viscosity. The textural, rheological, and sensory measurements were strongly correlated with physiochemical parameters measured for various gelatin samples extracted from different raw materials. The rheological measurements were found to successfully discriminate between different gelatins. For the gelatin industry and future research studies, rheological measurements might be useful for routinely characterizing gelatin gels. Other fish species should also be studied as a raw material for gelatin production and a cost analysis might be useful to demonstrate the potential opportunities for further processing of waste from the fish processing industry into value-added products.

BIOGRAPHICAL SKETCH

Gokhan Boran was born in Trabzon, Turkey on May 10, 1977. He received a B.S. degree from the Department of Food Engineering, Ankara University in June, 2000 and a M.S. degree from the Department of Fisheries Science and Technology, Karadeniz Technical University in February, 2004. After getting his M.S. degree, he married his life time love, Handan. The same year he was selected by the Higher Education Council of Turkey to pursue his Ph.D. abroad. He was accepted by the Graduate School of Cornell University as a graduate student in the Field of Food Science in November, 2004 and he came to Cornell in the fall 2005 to pursue his Ph.D. degree in the Department of Food Science. During his studies, he enjoyed the scientific environment at Cornell and the environmental beauty of Ithaca throughout the year. He now has life time memories and friends from all over the world from Ithaca during the last 4 years. He has also become a father to a wonderful son, Alp, born on September 23, 2008.

Sevgili eşim Handan ve canım oğlum Alp’e...

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TABLE OF CONTENTS

BIOGRAPHICAL SKETCH…… …….... iii

DEDICATION..…… iv

ACKNOWLEDGMENTS..…..…. v

TABLE OF CONTENTS…….. vi

LIST OF FIGURES..…. xi

LIST OF TABLES..…….……..... xiii

CHAPTER ONE: FISH SKIN GELATIN: PHYSICOCHEMICAL, RHEOLOGICAL, AND SENSORY CHARACTERISTICS……..…… 1

Abstract……..…… 1

1.1 Introduction…….……...… 2

1.2 The Parent Molecule: Collagen…….. 3

1.3 The Structure and Composition of Gelatin……..… 7

1.4 Collagen-Gelatin Conversion…….. 9

1.5 The Mechanism of Gelation……..... 11

1.6 Unique Characteristics of Gelatin……...… 11

1.7 Gelatin Market and Raw Materials……. 12

1.8 Recent Studies on Fish Skin Gelatin……... 13

1.9 Quality of Fish Skin Gelatin Compared to Mammalian Gelatins…….... 13

1.10 Quality Parameters and Methodological Challenges…… 15

1.10.1 Water Content of Gelatin……... 15

1.10.2 Gel Strength……... 16

1.10.3 Viscosity…… 17

1.10.4 Rheological Properties of Gelatin…….. 20

1.10.5 Sensory Properties of Gelatin…… 21

1.11 Factors Affecting Gelatin Quality……. 22

1.11.1 Extraction Time and Temperature……. 22
1.11.2 Acid and/or Alkali Treatments……... 23
1.11.3 Amino Acid Composition…….. 24
1.11.4 The Effect of pH…… 24
1.11.5 Other Factors…….. 25
1.12 Conclusions……... 26
References……..…….. 27
CHAPTER TWO: OPTIMIZATION OF GELATIN EXTRACTION FROM SILVER CARP SKIN - PART I. SCREENING...……...……..... 32
Abstract……..32
2.1 Introduction……. 33
2.2 Materials and Methods...…….…..... 36
2.2.1 Silver Carp Skin……...… 36
2.2.2 Study Design…… 36
2.2.3 Gelatin Extraction…… 37
2.2.4 Gel Strength……. 42
2.2.5 Viscosity…….. 42
2.2.6 Melting Temperature…….. 43
2.2.7 Protein Concentration……. 44
2.2.8 Hydroxyproline Concentration…… 44
2.2.9 Protein and Gelatin Yield……..…45
2.2.10 Protein and Gelatin Recovery…… 46
2.2.11 Statistical Analysis……. 46
2.3 Results and Discussion...…….……... 47
2.3.1 Screening Analysis and Gel Strength……..… 47
2.3.2 Viscosity and Melting Temperature……. 51

2.3.3 Protein and Gelatin Yield…… 55
2.3.4 Protein and Gelatin Recovery…….. 55
2.4 Conclusions……..……... 58
References……..…59
CHAPTER THREE: OPTIMIZATION OF GELATIN EXTRACTION FROM SILVER CARP SKIN – PART II. OPTIMIZATION…….…….. 62
Abstract……..…… 62
3.1 Introduction……. 63
3.2 Materials and Methods..…… 65
3.2.1 Silver Carp Skin……..……. 65
3.2.2 Study Design…… 66
3.2.3 Gelatin Extraction…… 66
3.2.4 Gel Strength……. 69
3.2.5 Viscosity…….. 69
3.2.6 Melting Temperature……...… 69
3.2.7 Protein Concentration……. 69
3.2.8 Hydroxyproline Concentration…… 69
3.2.9 Protein and Gelatin Yield……..70
3.2.10 Protein and Gelatin Recovery…… 70
3.2.11 Statistical Analysis……. 70
3.3 Results and Discussion…….…….. 71
3.3.1 The Regression Model and Optimization…… 71
3.3.2 Gel Strength and Viscosity…….. 78
3.3.3 Protein and Gelatin Yield…… 80
3.3.4 Protein and Gelatin Recovery…….. 82
3.3.5 Melting Temperature……...… 82

3.4 Conclusions…… 86

References…… 87

CHAPTER FOUR: RHEOLOGICAL PROPERTIES OF GELATIN FROM SILVER CARP SKIN COMPARED TO COMMERCIALLY AVAILABLE GELATINS FROM DIFFERENT SOURCES…… 89

Abstract……..…… 89

4.1 Introduction……..…….. 90

4.2 Materials and Methods…… 92

4.2.1 Silver Carp Skin Gelatin…….. 92

4.2.2 Commercial Gelatin Samples…….. 93

4.2.3 Sample Preparations……. 93

4.2.4 Gel Strength and Texture Profile Analysis…….. 94

4.2.5 Viscosity…….. 95

4.2.6 Rheological Measurements…….. 95

4.2.7 SDS-PAGE…….. 96

4.2.8 Statistical Analysis……... 96

4.3 Results and Discussion…….…..... 97

4.3.1 Gel Strength and Viscosity…….. 97

4.3.2 Texture Profile Analysis (TPA)……... 100

4.3.3 Melting and Gelling Temperatures……..… 100

4.3.4 SDS-PAGE…….. 103

4.3.5 Rheological Measurements…….. 105

4.4 Conclusions……. 112

References……..……113

CHAPTER FIVE: THE EFFECT OF EXTRACTION CONDITIONS ON THE SENSORY AND INSTRUMENTAL CHARACTERISTICS OF FISH GELATIN GELS…..... 115

Abstract…….. 115

5.1 Introduction……..... 116

5.2 Materials and Methods……...…. 118

5.2.1 Silver Carp Skin Gelatin…….. 118

5.2.2 Sample Preparations……. 120

5.2.3 Gel Strength and Texture Profile Analysis……..…… 120

5.2.4 Viscosity…….. 121

5.2.5 Rheological Measurements……. 121

5.2.6 SDS-PAGE…….. 122

5.2.7 Sensory Analysis……. 123

5.2.8 Statistical Analysis…….. 125

5.3 Results and Discussion……... 125

5.3.1 Gel Strength and Texture Profile Analysis…….. 125

5.3.2 Viscosity……. 128

5.3.3 Rheological Measurements……. 130

5.3.4 SDS-PAGE……. 131

5.3.5 Sensory Analysis…… 131

5.4 Conclusions…….. 141

References……..... 142

CHAPTER SIX: OVERALL CONCLUSIONS AND SUGGESTIONS FOR FUTURE STUDIES……..…… 144

LIST OF FIGURES

Figure 1.1 Schematic representations of collagen molecule and collagen fibrils…..5

Figure 1.2 Standard ‘bloom jar’……..…….. 18

Figure 2.1 Flowchart for gelatin extraction…….. 40

Figure 2.2 Gelatin sheets obtained from silver carp skin……..… 41

Figure 2.3 Force against deformation plots…….. 50

Figure 2.4 Gel strength of the screening samples……. 52

Figure 2.5 Viscosity of the screening samples……. 53

Figure 2.6 PY, GY, PR, and GR values for the screening samples…….. 54

Figure 2.7 Delta plotted against temperature of three samples……. 56

Figure 2.8 Melting temperature of screening samples…….. 57

Figure 3.1 Surface profile plots of the three dependent responses……... 77

Figure 3.2 Gel strength of the optimization samples…… 79

Figure 3.3 Viscosity of the optimization samples…… 81

Figure 3.4 PY, GY, PR, and GR values for the optimization samples……. 83

Figure 3.5 Percentage of gelatin in each protein sample extracted…….. 84

Figure 3.6 Melting temperature of the optimization samples……... 85

Figure 4.1 Effect of heating on phase angle……. 101

Figure 4.2 Effect of cooling on phase angle……. 102

Figure 4.3 SDS-PAGE of gelatins from different sources…… 104

Figure 4.4 Time sweep for gelatin gels from various sources……..… 106

Figure 4.5 Frequency sweep for gelatin gels from various sources……. 107

Figure 4.6 Strain sweep for gelatin gels from various sources……. 109

Figure 4.7 Stress sweep for gelatin gels from various sources……. 110

Figure 4.8 Creep-recovery for gelatin gels from various sources……. 111

Figure 5.1 SDS-PAGE of gelatin samples extracted from silver carp skin…….. 132

Figure 5.2 Qantitative descriptive analysis for samples GSH and GSL……... 134

Figure 5.3 Quantitative descriptive analysis for samples VSH and VSL…… 135

Figure 5.4 Quantitative descriptive analysis for samples MTH and MTL……... 136

Figure 5.5 The changes of the overall flavor intensity among the samples…….. 138

Figure 5.6 The changes of the sensory firmness among the samples……... 139

LIST OF TABLES

Table 1.1 Gel strength measured using different instruments…….. 19

Table 2.1 Independent variables and their levels in screening……. 38

Table 2.2 Fractional factorial screening design…… 39

Table 2.3 The corresponding p-values for the main and interaction effects…….… 48

Table 3.1 Independent variables and their levels in optimization……...……. 67

Table 3.2 Unblocked central composite rotatable design……. 68

Table 3.3 Analysis of variance (ANOVA) for the regression model……... 72

Table 3.4 Regression coefficients for the model…….. 73

Table 3.5 Optimization parameters used in the prediction profiler…….. 75

Table 3.6 Optimization results obtained by using the prediction profiler…… 76

Table 4.1 Comparison of some textural properties……... 98

Table 4.2 Correlations between some of the instrumental measurements…… 99

Table 5.1 Extraction conditions for gelatin samples extracted……. 119

Table 5.2 Gel strength, viscosity, and melting temperature of gelatin samples…... 126

Table 5.3 Comparison of rheological and textural properties……... 127

Table 5.4 Correlations of the instrumental measurements…… 129

Table 5.5 Correlations of the sensory attributes……... 140

CHAPTER ONE

FISH SKIN GELATIN: PHYSICOCHEMICAL, RHEOLOGICAL, AND SENSORY CHARACTERISTICS

Abstract

Gelatin is a multi-functional ingredient used as a gelling agent, stabilizer, thickener, emulsifier, and film former in foods, pharmaceuticals, cosmetics and photographic films. As a thermo-reversible hydrocolloid with a relatively narrow gap between its melting and gelling temperature, gelatin provides unique advantages over carbohydrate-based gelling agents. Gelatin is mostly produced from pig skin, and cattle hides and bones and some by-products from the chicken and fish processing industries. Fish skin has recently gained substantial attention from researchers as fish skins have a significant potential for the production of high quality gelatin. Gelatin quality is industrially determined by gel strength, viscosity, melting/gelling temperatures, the water content and microbiological safety. For gelatin manufacturers, yield from a particular raw material is also important. Recent experimental studies have shown that these quality parameters might vary greatly depending on the biochemical characteristics of the raw materials, the manufacturing processes applied, and the experimental settings used with the quality control tests. In this review of fish skin gelatins the gelatin quality achieved from different fish species is reviewed along with the experimental procedures used to determine gelatin quality. In addition, the chemical structure of collagen and gelatin, the collagen-gelatin conversion, the gelation process, and gelatin marketing are discussed.

1.1 Introduction

Gelatin is a term used for a class of protein fractions that have no existence in nature. Gelatin is derived from collagen, which is a natural structural protein, predominantly found in the connective tissues of animals (Balian and Bowes 1977; Belitz and others 2004; DeMan 1999). Gelatin is one of the most widely used biopolymers and is added to foods, drugs, cosmetics, photography products, and other products including paints, matches, and fertilizers as a gelling agent, foam stabilizer, and structure enhancer (Gudmundsson 2002; Karim and Bhat 2009; Yang and others 2007; Zhou and Regenstein 2004). Gelatin is able to form a high viscosity solution in warm water, which sets to a gel on cooling. The chemical composition of gelatin is, in many respects, similar to that of collagen, its parent molecule. Gelatin is not composed of one size of collagen fraction or peptide chain but is a combination of several fractions varying in size, including the whole α-chain of tropocollagen and parts of α-chains of different lengths (Eastoe and Leach 1977). Gelatin gels have relatively lower melting temperatures compared to the gels of other gelling agents (Williams 2007). Gelatin gels generally have a melting temperature below 35 °C, i.e., below human body temperature, which makes gelatin unique in terms of its sensory aspects, especially flavor release, which is particularly desired for some food applications (Baziwane and He 2003; Choi and Regenstein 2000; Boran and Regenstein 2009). Other gelling agents such as starch, alginate, pectin and agar are carbohydrates and their gels cannot melt below body temperature and most have much higher melting temperatures (Williams 2007).

Gelatin is obtained from the skins and bones of pigs and cattle, but mostly from pig skin. However, there are other alternative raw materials used in gelatin manufacturing including by-products from the chicken and fish processing industries. Fish skin has received attention from researchers as an alternative raw material having

a potential for the production of high quality gelatin. Therefore, recent studies with fish skin gelatin have focused on the evaluation of different fish species as an alternative raw material for gelatin production and the quality of extracted gelatins in comparison with commercial gelatins from conventional sources (Boran and Regenstein 2009).

In this review the recent studies related to fish skin gelatin are reviewed and discussed. Methodological challenges are also discussed to help to understand and possibly eliminate method related problems in future studies. In addition, the most critical factors affecting gelatin quality are discussed. For this purpose, the chemical structure of collagen and gelatin is first reviewed in detail to take a closer look at the possible factors affecting gelatin. Second, the conversion process of collagen into gelatin and the gelation mechanism are discussed to show which driving forces are involved in gelation, which factors might affect the sol-gel and gel-sol transitions, and how these factors might affect the final product, gelatin. Third, the methods being currently used to characterize gelatin are reviewed.

1.2 The Parent Molecule: Collagen

Collagen is the most abundant protein in the animal body (DeMan 1999). Collagen is part of the connective tissue in muscles, many organs, skin, bones, teeth, and tendons. Collagen fibrils have a regular periodicity of 64 nm, which can be increased to 400 nm under tension (DeMan 1999). Collagen molecules are arranged head-to-tail, with a 35 nm gap between molecules, in a staggered bundle. Charged and uncharged residues are found to be periodically clustered along the sequence of collagen about every 230 residues, which is around 64 nm, although this distance may vary among different tissues. This suggests that the collagen molecules are aligned such that the maximum electrostatic and hydrophobic interactions occur between

different molecules (Figure 1.1). Collagen constitutes 20-25% of the total protein in mammals and has a unique amino acid composition, which includes hydroxyproline and hydroxylysine (Belitz and others 2004). Its molecular structure is mainly the multiple repetition of a 'glycine-x-y' sequence, where 'x' is often proline, and 'y' is often hydroxyproline. Collagen has a unique triple helix structure that is based on a special helix of three polypeptide chains. Each polypeptide chain is left handed and has three amino acids per turn. These three polypeptide chains, called α-chains, are super-twisted around each other and form a superhelix that is right handed (Nelson and Cox 2005). The basic structural unit of the collagen superhelix is called tropocollagen. It has a molecular weight of approximately 330 kDa, with a length of approximately 300 nm and a diameter of 1.5 nm (Belitz and others 2004). When hydrolyzed, the collagen can give three different fractions: independent α-chains, a β-chain that is two α-chains linked to each other by covalent bonds, and a γ-chain that is three α-chains linked to each other by covalent bonds. These fractions differ in molecular size: α-chains corresponds to a molecular weight of 80-125 kDa, β-chains corresponds to a molecular weight of 160-250 kDa, and γ-chains corresponds to a molecular weight of 240-375 kDa, which is very similar to the molecular size of collagen (Imeson 1997).

Collagen typically contains about 35% glycine, 11% alanine, and 21% proline and hydroxyproline, the amount of which varies among the species although the high content of proline and hydroxyproline is characteristics of collagen structure regardless of the source (Balian and Bowes 1977). The hydroxyproline is a rare amino acid in nearly all other proteins so its presence can be used to determine the amount of collagen or gelatin (Engel and Bachinger 2005). Another protein containing hydroxyproline is elastin, but the amount of hydroxyproline in elastin is very low and the amount of elastin in most tissues is also very low when compared to that of

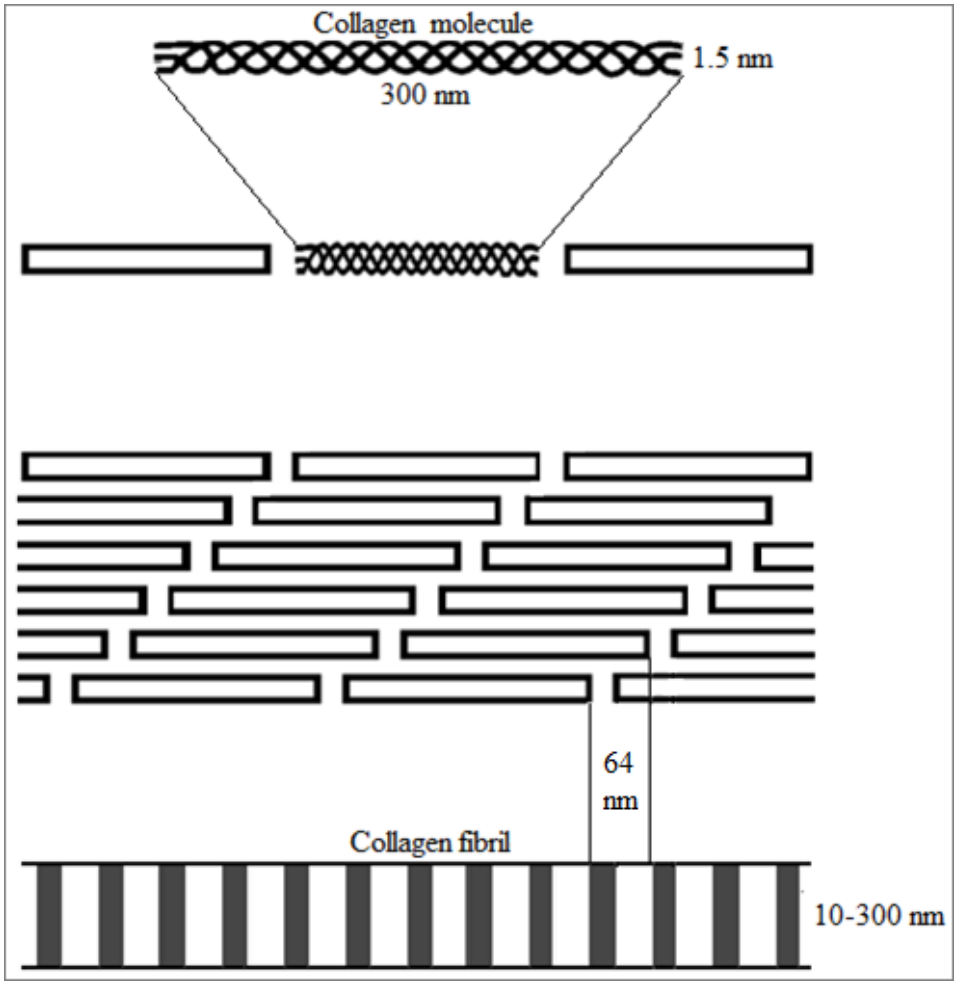

Figure 1.1 Schematic representations of collagen molecule and collagen fibrils.

collagen (Nelson and Cox 2005). Collagen is generally considered to be an incomplete protein since the concentration of some essential amino acids are low in collagen and consequently, in gelatin (Nelson and Cox 2005; Belitz and others 2004). However, when eaten as part of a meal, the contribution of gelatin needs to be considered. The amounts of the aromatic and sulphur containing amino acids are low (0-0.6%) in collagen, i.e., tryptophan and cysteine are mostly absent in collagen (Balian and Bowes 1977). Cysteine is usually absent in collagen, therefore there are usually no disulfide bonds involved in collagen structure although there are some collagens that have cysteine (Engel and Bachinger 2005). For those, disulfide bonds are also involved in the formation of intermolecular crosslinks (disulfide bonds) and stabilizing the structure. The structure of collagen provides an explanation of why glycine is the most abundant amino acid and why proline and hydroxyproline are found so often in collagen. Only glycine residues can fit into the very tight central core between the individual α-chains while proline and hydroxyproline residues permit sharp twists of the collagen helix allowing for the three amino acids per turn (Nelson and Cox 2005).

The collagen molecule is primarily stabilized by hydrogen bonds between the backbone amino group of glycine and the backbone carboxyl group of a residue in the x position of a neighboring α-chain, which is usually a proline. Proline in the y position is generally hydroxylated into hydroxyproline, which also plays an important role in the formation of intra and intermolecular hydrogen bonds. Therefore, hydroxyproline is important for both the structure of the collagen molecule and of collagen fibrils (Brinckmann 2005). During maturation or aging, collagen fibers strengthen and are further stabilized primarily by covalent bonds. Lysine, hydroxylysine, and histidine residues are heavily involved in the formation of these covalent bonds, i.e., aldimine bonds between lysine and lysine or hydroxylysine (Balian and Bowes 1977; Engel and Bachinger 2005; Eyre and Wu 2005; Belitz and

others 2004; Nelson and Cox 2005) that lead to the formation of desmosine and isodesmosine, which are unusual in that they involve the participation of four amino acids in the reaction. All the fibril-forming collagen types (type I, II, III, V, XI, XXIV, and XXVII collagens) are cross-linked through a mechanism based on the reactions of aldehydes derived from lysine (or hydroxylysine) side chains. Histidine might also participate in the formation of a trivalent crosslink by reacting with an aldimine bond formed between a lysine aldehyde and hydroxylysine residue.

With respect to tissue source, type I collagen is the most widely occurring collagen found in skin, tendon, bone, cornea, lung, and the vasculature while type II has a more specific tissue distribution being limited essentially to cartilage and type III is found in relatively elastic tissues such as embryonic skin, lung and blood vessels (Hulmes 2008). For most non-fibrillar collagens (type IV, VI, and VII collagens as examples) disulfide bonds may be the only source of intra and intermolecular covalent bonds. There are usually no lysine mediated crosslinks in these collagens (Eyre and Wu 2005). The best known non-fibrillar collagen is type IV collagen, which is a basement membrane collagen that forms specialized structures found at tissue boundaries, fat, muscle and nerve cells. Collagen VI, on the other hand, is important in maintaining tissue integrity (Hulmes 2008).

1.3 The Structure and Composition of Gelatin

Collagen containing tissues are treated with acid and/or alkali followed by a heat treatment in the presence of water to break the structure of collagen fibrils irreversibly to obtain gelatin (Eastoe and Leach 1977). While the molecular weight of the collagen molecule is about 330 kDa, gelatin is considered to be all collagen fractions with a molecular weight higher than an arbitrary minimum of 30 kDa. The collagen fractions with a lower molecular weight are not considered to be gelatin but

rather gelatin hydrolysates as they are not able to form a gel although they may participate in gel formation (Eastoe and Leach 1977). A heat treatment of about 40 °C breaks hydrogen and possibly electrostatic bonds in newly formed collagen molecules releasing single α-chains but this is insufficient to break the crosslinks and covalent bonds in the collagen structure of mature collagen (Eastoe and Leach 1977). With treatments at higher temperatures, on the other hand, those covalent bonds including intermolecular crosslinks and peptide bonds break down and therefore, smaller α-chain fractions could be obtained (Eastoe and Leach 1977). The position of the bond breaks determines the molecular weight, and the number of polypeptide chains. As amino acid sequence and composition of collagens from different sources vary greatly, bond breaks appear to be random and this random bond breakdown is the main cause of molecular heterogeneity in gelatin (Eastoe and Leach 1977).

The raw materials used in gelatin production contain a variety of substances that are the source of organic and/or inorganic impurities in gelatin. Non-collagen protein fractions, lipids, nucleic acids and other cell components are among the organic impurities. Inorganic impurities include naturally present minerals such as calcium, sodium, potassium, and iron along with those derived from substances added for gelatin preparation, i.e., acid and/or alkali with their impurities (Eastoe and Leach 1977). Finally, commercial gelatin products contain a substantial amount of water usually as the second largest component in the whole and its amount varies greatly based on the drying process applied, the nature of the raw material, and temperature and relative humidity of storage. Generally speaking, the water in gelatin is between 9-14% with occasional samples outside this range (Eastoe and Leach 1977).

1.4 Collagen-Gelatin Conversion

There are several methods used by the industry to manufacture gelatin from collagen. The main purpose of the gelatin production process is to convert collagen that is insoluble in water into gelatin that is soluble in water, while obtaining maximum yield and good functional properties (Hinterwaldner 1977). In general, gelatin is obtained using a sequence of three processing steps: pretreatments to remove non-collagen impurities, water extraction to convert collagen into gelatin, and finally a refinement and recovery step to get highly purified dried gelatin. In the first step, raw materials are water washed to remove obvious impurities and then treated with alkali and/or acid to weaken the collagen structure by breaking intramolecular crosslinkages including covalent and hydrogen bonds and to release other impurities. In the second step, the actual water extraction is performed at warm temperatures for an appropriate period of time. In the last step, extracted gelatin is subjected to several separation methods including filtration, evaporation, and deionization followed by drying and grinding (Hinterwaldner 1977). Gelatins are classified according to whether an acid or an alkali is used in the pre-extraction step. If an acid solution is used as the solvent, type-A gelatin (acid process) is obtained. In case of alkali solvent, type-B gelatin (alkali process) is obtained (Hinterwaldner 1977). Type-A gelatin's isoelectric point is higher compared to that of type-B gelatin as a milder acid process does not remove the amide nitrogen of gelatin, therefore, the resulting gelatin's isoelectric point might be as high as 9.4. If a more severe acid treatment is required, then some of the amide groups are hydrolyzed and the isoelectric point would be similar to that of the original collagen molecule, which generally lies between 6 and 8. Type-B gelatin's isoelectric point might be as low as 4.8 as the alkali process results in the loss of amide groups (Eastoe and Leach 1977). In the acid process, the bones and skins are treated in a vessel containing a dilute solution of acid for a predetermined period of time. Then,

the acid is washed out with cold water. In the alkali process, the demineralized bones (demineralization is mostly done with acid solutions to remove calcium and other salts from the bone to prepare the collagen rich bone material known as ossein) are placed in liming pits and soaked in a lime suspension for longer than 60 days. For the hides or skins, a caustic soda solution is used for a shorter period of time. After this treatment, the raw material is washed thoroughly to remove any residual lime. The acid pretreatment is mostly used for skin while the alkali pretreatment is used mostly for bones (Petersen and Yates 1977). The actual extraction method used for both acid and alkali pretreated raw materials are similar. The main extraction step is done using hot water at controlled temperatures, mostly higher than 40°C and it is the most important step in gelatin production. In the industry, the extraction step is actually multiple extractions performed with gradually increasing temperatures beginning from 50-60 °C and going up to the boiling temperatures, usually in 5-10 °C temperature increment. Gelatins are collected so that the lower temperature fractions have minimal degradation and the higher temperature fractions have more variable molecular weights (Hinterwaldner 1977). The dilute gelatin solution from the extraction process is clarified using lamellar separators (this equipment is built as a set of plates or discs that are arranged at such an angle that the solids can slide off into the sludge chamber, thus achieving clarification) and filtered using self-cleaning centrifugal filters or cellulosic filters. After that, gelatin solutions are deionized by passing through ion exchangers and concentrated, usually in a multiple effect vacuum evaporator. The concentrated solution is then sterilized by hot air in batch driers, cooled or chilled to rapidly form a gel. Then, the gel obtained is extruded to get gelatin noodles followed by a final drying and grinding process. After all these treatments, gelatin granules or powder are obtained. Acid or alkali pretreatments designed to destroy or weaken crosslinkages between α-chains or tropocollagen molecules need to be adjusted in

terms of not only concentration but also treatment time to avoid extensive degradation of collagen, which might result in lower quality gelatin, but at the same time enough degradation is needed to be able to get a higher yield and acceptable gel strength (Holzer 1996).

1.5 The Mechanism of Gelation

Hydrogen bonds certainly play an important role in gelation (Johns and Courts 1977). Gelation can be considered as gelatin regaining collagen structure, but this would not be exactly correct because the conversion of collagen into gelatin is an irreversible process although gelatin can partially regain collagen structure by recovering crosslinkages. The greater the amount of crosslinkages recovered, the higher the gel strength and viscosity along with the melting and gelling temperatures (Belitz and others 2004). The concentration of α-chains and cooling rate are the most important factors for gelation. At high concentrations, intermolecular bond formation would occur with multiple strands, while the same process is more like to occur with intramolecular bonds within a single strand at low concentrations. Similarly, slow rates of cooling allow more intra and intermolecular crosslink formation, while rapid cooling does not allow that to happen (Belitz and others 2004).

1.6 Unique Characteristics of Gelatin

Gelatin is a gelling agent that is able to form thermo-reversible gels, which means that when the gelatin gel is heated, it softens and turns into a liquid. Then, it is able to return back to the gel form when the solution is cooled again (Stainsby 1977). Being able to melt below human body temperature makes its use very favorable in the food industry since gelatin is able to melt and release flavor when it is taken into the mouth, which may be desired in terms of sensorial properties of food products (Choi

and Regenstein 2000). Another important characteristic of gelatin is that its gel strength is relatively higher than most of the common gelling agents, which are usually carbohydrates and obtained from vegetable sources (Badii and Howell 2006). The gap between melting and gelling temperature of gelatin gels is smaller than that of other gelling agents, which is desired for particular applications, i.e. food applications including jellies and custards (Jones 1977).

1.7 Gelatin Market and Raw Materials

The world's total gelatin production is close to 350,000 tons annually, accounting for a market size of over 2 billion USD. About 45% of the world's total gelatin production is obtained from pork skin, followed by bovine hides with almost 30% (Karim and Bhat 2009) and 23% of gelatin is obtained from bovine and porcine bones. Other sources including chicken and fish only account for 1.5% of the world's annual gelatin production. In Europe, pork skin is the most abundantly used raw material for gelatin production, accounting for around 80% of the total, followed by cattle skin with 15% of the total gelatin production. The remaining 5% is from pork and cattle bones, fish and chicken. Recent studies have shown that fish skin, especially, might be an alternative for gelatin production. Fish skin gelatin may provide a better alternative for some applications because of its relatively lower gel strength and melting temperature compared to pork skin gelatin. These characteristics are generally highly desired in some food systems for ease of flavor release leading to better sensory characteristics (Boran and Regenstein 2009; Choi and Regenstein 2000). In addition, obtaining valuable by-products from the fishery industry and reducing waste have made it an attractive research topic. Many fish species have been investigated as a raw material for gelatin extraction and the properties of gelatin obtained from these sources have also been examined.

1.8 Recent Studies on Fish Skin Gelatin

In the last decade gelatin extraction from fish skin has been intensively investigated. The physicochemical, textural, rheological, and sensory properties of extracted fish gelatin have also been studied in comparison with mammalian gelatin. The overall results suggest that fish skin might be an alternative raw material for high quality gelatin production, eliminating religious concerns shared by the Jewish and Muslim communities and also providing an alternative way to use some fishery by-products (Boran and Regenstein 2009). Some of the fish species investigated include Atlantic salmon (Arnesen and Gildberg 2007), cod (Gudmundsson and Hafsteinsson 1997), sin croaker and short fin scad (Cheow and others 2007), Alaska pollock (Zhou and Regenstein 2004), big eye snapper and brown stripe red snapper (Jongjareonrak and others 2006), yellow fin tuna (Cho and others 2005), Nile perch (Muyonga and others 2004), black and red tilapia (Jamilah and Harvinder 2002), grass carp (Kasankala and others 2007), and silver carp (Boran and Regenstein 2009).

1.9 Quality of Fish Skin Gelatin Compared to Mammalian Gelatins

Arnesen and Gildberg (2007) studied the skins of Atlantic salmon and Atlantic cod for gelatin production and reported that Atlantic salmon skin gelatin had higher gel strength and gelling temperature than Atlantic cod skin gelatin. The gel strength of the salmon and cod were found to be 108 and 71 g, respectively, while their gelling temperatures were 12 and 10 °C, respectively. Arnesen and Gildberg (2007) also reported that the gel strength of the gelatins obtained increased with storage time and higher extraction temperature resulted in lower gel strength. Gudmundsson and Hafsteinsson (1997) also studied cod skin as a raw material for gelatin production, reporting that the proline and hydroxyproline content of cod (a cold water species) skin gelatin (~18%) was lower compared to that of tilapia (a warm water species) skin

gelatin (~25%), resulting in relatively lower gel strength and viscosity. According to their results, tilapia skin gelatin gave 260 g bloom strength while cod skin gelatin had 180 g bloom under the best extraction conditions reported. Choi and Regenstein (2000) compared various gelatin samples from different sources in terms of their physicochemical and sensory properties and reported that Alaska pollock gelatin had lower gel strength along with lower melting temperature compared to that of pork skin gelatin. Alaska pollock gelatin melted at 24 °C while the pork skin gelatin melted at 29 °C (Choi and Regenstein 2000). They also compared the sensory properties of gelatin gels prepared from Alaska pollock and pork skin gelatins and reported that a low melting temperature and gel strength might be useful in creating products with a faster and stronger flavor release. Chiou and others (2006) studied Alaska pollock and Alaska pink salmon for gelatin production and the quality of the gelatin obtained in comparison with pork skin gelatin. They reported that Alaska pollock and Alaska pink salmon skin gelatins had lower melting and gelling temperatures along with lower gel strength compared to that of pork skin gelatin due to the lower proline and hydroxyproline content of skin gelatins obtained from these fish species. They reported that the pollock and salmon skin gelatins had gelling temperatures of 7 and 5 °C, respectively, while pork skin was reported to have a gelling temperature of 24 °C, which was attributed to the high content of proline and hydroxyproline of pork skin gelatin (Chiou and others 2006). Kasankala and others (2007) studied grass carp skin as an alternative raw material for gelatin production and reported that the hydroxyproline content of grass carp skin gelatin (11.27%) was slightly higher than that of bovine skin gelatin (11.17%) and a little lower than that of pork skin gelatin (13.17%). They also reported high gel strength, melting and gelling temperatures for grass carp skin gelatin compared to that of gelatins obtained from other fish species. According to their results, carp skin gelatin had a gelling temperature around 19 °C

and a melting temperature around 26 °C, which was a little lower than that of pork skin (25 and 31 °C) and bovine gelatins (21 and 30 °C) (Kasankala and others 2007). Boran and Regenstein (2009) also reported similar results for skin gelatin obtained from silver carp, another Asian carp species, i.e., it had high gel strength (600 g for optimized gelatin) possibly due to the high hydroxyproline content (~11%). Therefore, it does appear that the assumption that there is a strong connection between the content of hydroxyproline and proline and the physicochemical properties of gelatins continues to hold with the more recent research with fish gelatins.

1.10 Quality Parameters and Methodological Challenges

1.10.1 Water Content of Gelatin

Both commercial gelatin powders and those produced on a small scale for research purposes have an amount of water that varies due to the differences in processing and drying methods (Eastoe and Leach 1977). Water content of gelatin is important for both ease and duration of storage as high water content favors microbial spoilage. In addition, higher water containing gelatin formulations can be sold for less. The drying method is the major factor affecting the water content of gelatin products. Heat drying and freeze drying are two of the most common methods used to remove water from gelatin preparations. Heat drying is generally done at low temperatures between 40 and 60 °C from several hours to several days (Hinterwaldner 1977). Freeze drying might be a much faster method compared to heat drying and may be able to remove water while causing less damage to the gelatin.

The gelatin powder obtained is generally not tested for its water content, and even when determined, this information is not generally included in the calculations when preparing samples for testing, i.e., the gelatin is simply weighed out. This can lead to a lack of agreement between data from different sources. To prevent confusion

and to get comparable data, water content of gelatin powders should be determined and included in the calculations to make sure that the actual gelatin amount is the same in each sample being compared for their characteristics. As a theoretical example, 100 mL of 10% (w/v) gelatin solution is prepared by dissolving 10 g of dried gelatin in 90 mL water. Assuming 10% of the dried gelatin is water then, the actual gelatin concentration of the solution would be 9%. Another dried gelatin sample including 5% water, with the same preparation method, would give a 9.5% gelatin concentration. After maturation, making a direct comparison of these two gelatin samples for gel strength would be erroneous as the actual gelatin concentration of the samples is different.

1.10.2 Gel Strength

Gel strength is one of the most important quality characteristics used in the gelatin industry to differentiate gelatins. As measuring gel strength is very popular, there is a standard method. According to the standard method (Gelatin Manufacturers Institute of America, GMIA), gel strength must be measured at 10 °C on a gelatin sample prepared at 6.67% concentration (w/v). Dissolving gelatin in water is not standardized and there are variations in the procedures used, i.e., different temperatures, duration before cooling, with or without stirring, etc. Maturation time and temperature are standardized and are generally followed, i.e., 16-18 h at 10 °C. A particular jar is used for this measurement, called a “bloom jar” (Figure 1.2), it requires about 155 mL gelatin solution that corresponds to about 10 g of gelatin. However, this particular jar cannot regularly be used in many scientific studies as it requires a substantial amount of sample, which is often limited in scientific studies. Therefore, many scientists use other containers that differ in size and shape leading to significant differences in the results, making the data incomparable among the

different studies. The test settings are also standardized: The force required for a 4 mm penetration into the gel of a 12.7 mm diameter probe lowered onto the sample at a speed of 1 mm/s is given as gel strength in g. There are different instruments that can be used for this purpose and different instruments give different results (Table 1.1).

1.10.3 Viscosity

Viscosity is generally measured using tubular glass viscometers as they are relatively inexpensive and easy to use compared to expensive and complicated computer controlled instruments. Although the advanced viscosity instruments might provide higher reproducibility and accuracy, the tubular glass viscometers also give high precision and they are low cost, easy to use, and convenient. Compared to gel strength, viscosity is not as well correlated with textural properties and the molecular structure of the gelatins obtained as viscosity is mostly affected by molecular weight distribution.

Gelatin samples with high molecular weight fractions give high viscosity but that does not necessarily mean that their gel strengths would also be high. Gelatin samples from fish skin, for example, give unexpectedly high viscosity while giving low gel strength compared to that of pork skin gelatin due to the carefully controlled extraction conditions and consequently the presence of higher molecular weight protein fractions (Boran and Regenstein 2009). Arnesen and Gildberg (2007) reported that Atlantic salmon and Atlantic cod skin gelatin had higher viscosities than pork skin gelatin while giving lower gel strengths than pork skin gelatin. Generally, fish skin gelatins are expected to have a lower viscosity compared to that of gelatins obtained from porcine and bovine sources with similar molecular weight distributions.

Figure 1.2 Standard ‘bloom jar’ provided by Texture Technologies Corporation (Scarsdale, NY).

Table 1.1 Gel strength of a commercial gelatin measured using different instruments and probes in either standard bloom jar or 15 mL capacity small plastic jar.

Measurement details	Average	SD
Standard bloom jar, TAXT2 texture analyzer, Spherical probe	242	5.7
Standard bloom jar, TAXT2 texture analyzer, Cylindrical probe	523	2.1
Standard bloom jar, Stevens texture analyzer, Spherical probe	213	1.2
Standard bloom jar, Stevens texture analyzer, Cylindrical probe	466	3.8
Small plastic jar, TAXT2 texture analyzer, Spherical probe	320	9.2
Small plastic jar, TAXT2 texture analyzer, Cylindrical probe	814	13.6
Small plastic jar, Stevens texture analyzer, Spherical probe	294	2.5
Small plastic jar, Stevens texture analyzer, Cylindrical probe	746	9.6

SD: standard deviation. Same sample (Knox Gelatin, Kraft Foods Global, Inc., Glenview, IL, U.S.A.) used for the measurements: 6.67% gelatin, dissolved at 50 °C for 30 min in distilled water, matured at 4 °C for 16-18 h. The measurements are done at 4 °C using the following settings: 4 mm penetration with 12.7 mm diameter probe (either spherical or cylindrical) with 1 mm/s penetration speed. Gel strength is given as g force required penetrating the probe onto the sample (N=3).

1.10.4 Rheological Properties of Gelatin

Rheological methods have recently gained importance and have found applications in determination of gelatin quality. Rheological measurements of both melting and gelling temperatures give highly reproducible results. A temperature sweep test is performed for this purpose. Heating or cooling is required to determine the melting and gelling temperature, respectively. The gelatin gel sample is prepared at a certain concentration and matured at a certain temperature for a certain period of time to standardize the procedure to discriminate the samples based on their chemical differences (Chiou and others 2006; Cho and others 2006; Fernandez-Diaz and others 2003; Kasankala and others 2007). Prior to rheological determinations of melting and gelling temperatures, the droplet method was used as a standard method for determining the melting temperature. However, the rheological methods have replaced this older method (Wainewright 1977), which was less precise and more laborious. Other rheological tests including time sweep, frequency sweep, stress sweep and strain sweep have also found applications in determination of gelatin quality as they allow researchers to discriminate the gelatin gels according to their strength and elasticity. Stress and strain sweep tests are used to determine the linear viscoelastic region of the gels. Frequency sweep tests are useful to determine if the gelatin gels change with changing frequency of stress applied. Time sweep tests are used to determine if the gelatin gels' viscoelastic properties changes with time at a controlled temperature and at a set level of stress applied. Recent literature on fish gelatin includes some examples of these tests used to make comparisons among gelatin samples from different sources. Chiou and others (2006) used temperature sweep tests to determine the melting and gelling temperature of gelatin gels. They also used time sweep test to show the increasing elastic modulus at different temperatures with increasing concentration of glutaraldehyde added to the gelatin gels. Gudmundsson (2002) used frequency sweep

tests successfully to differentiate the gelatin gels based on their elastic moduli and the temperature sweep tests to determine the melting temperature of blended fish gelatin gels. Zhou and Regenstein (2007) used temperature sweep tests to compare the melting temperatures of gelatin gels from different sources. In another study, Zhou and others (2006) used strain sweep and frequency sweep tests to compare the gelatin gels from different sources based on their viscoelastic properties. Recent studies gave good examples of how rheological measurements had strong correlations with conventional parameters including gel strength and viscosity (Gudmundsson 2002; Gilsenan and Ross-Murphy 2000; Zhou and others 2006).

1.10.5 Sensory Properties of Gelatin

Very few studies have been done in the recent published literature on the sensory analysis of gelatin. Perception of sensory characteristics of gelatin has mostly been studied with gel samples prepared with water alone or fruit juices to analyze the sensory characteristics including firmness, cohesiveness, viscosity, melting rate, sweetness, sourness, etc. A study done by Choi and Regenstein (2000) is, to date, the only example reporting a quantitative descriptive analysis of gelatin gels prepared with fruit juice, comparing Alaska pollock skin and pork skin gelatin gels, which have different melting temperatures. They investigated the effects of melting temperature on the sensory characteristics of gelatin gels with similar gel strength. According to their results, the melting temperature had significant effects on several sensory characteristics of gelatin gels. They reported that the Alaska Pollock skin gelatin gels gave higher flavor and aroma intensities, melted faster and had lower viscosity than pork skin gels, which was consistent with the low melting temperature of the fish skin gelatin gels. Other textural parameters including firmness and cohesiveness were not significantly different (Choi and Regenstein 2000). More such sensory work is needed

to establish how extraction conditions of gelatin production affect the sensory perception of gels. Gelatins from different sources might be compared using other food systems other than fruit juice gels in terms of their sensory properties.

1.11 Factors Affecting Gelatin Quality

There are several factors that significantly affect the properties of gelatin (Cho and others 2006). The raw materials used in gelatin manufacture have obvious effects on gelatin, mostly originating from differences in the amino acid composition of the collagen of the raw material. Also, variations in processing conditions such as extraction time, extraction temperature, and concentration of acid or alkali dramatically affect the product (Zhou and Regenstein 2005; Boran and Regenstein 2009; Cho and others 2006; Hinterwaldner 1977). For example, longer extraction temperatures and/or higher extraction temperatures cause excessive damage to the collagen molecule and the resulting gelatin forms a weak gel and has low viscosity. Similarly, excessive concentrations of acid and/or alkali cause degradation of collagen structure giving a gelatin with lower values.

1.11.1 Extraction Time and Temperature

Different temperatures and times are used in gelatin manufacturing but most extractions are between 45 and 60 °C. Temperatures from 50 until 80 °C can promote intramolecular bond formation between strands and consequently gelatin with stronger gelling ability can be obtained (Djagny and others 2001). Higher temperatures over 80 °C, however, result in fracturing of intramolecular chains giving gelatin having a weaker gelling ability. Lower extraction temperatures, on the other hand, lead to low yields but a superior quality. Similarly, longer extraction times give better yield while the extracted material suffers from low strength and viscosity due to excessive damage

to collagen fractions with longer heating. Therefore, it is necessary to balance both extraction temperature and duration of the extraction, to get the best possible outcome. For this purpose, a few conventional optimization studies have been done on gelatin extraction from skins of different fish species (Zhou and Regenstein 2004; Kasankala and others 2007; Boran and Regenstein 2009).

1.11.2 Acid and/or Alkali Treatments

The gel strength of gelatin is greatly influenced by the concentration of acid and/or alkali, the duration of the acid and/or alkali treatment, and possibly the treatment temperature. A previous study by Gudmundsson and Hafsteinsson (1997) showed that high concentrations of alkali or acid increased the gelatin yield while decreasing gel strength. These results have also been confirmed by Zhou and Regenstein (2005). Another study done by Cho and others (2006) showed that alkali concentration up to 1.5% increased gelatin yield significantly. Zhou and Regenstein (2004) confirmed that the concentrations of acid or alkali have a significant effect on gelatin yield, gel strength, and viscosity. Acid treatment is also important for the sensory aspects of gelatin, appearance and smell, as the acid treatment effectively removes odors and color that originate from the raw material (Zhang and others 2007, Boran and Regenstein 2009). Alkali treatment is, similarly, important and responsible for removal of possible impurities from the raw material and also for weakening the collagen structure, leading to higher yield and superior quality. In addition, alkali treatment causes glutamine and asparagine to lose their amine groups, converting them to glutamic and aspartic acid residues, respectively, lowering the isoelectric point of collagen (Johns and Courts 1977). Therefore, both acid and alkali treatments need to be optimized for pH, duration and temperature of extraction.

1.11.3 Amino Acid Composition

Chiou and others (2006) showed that differences in amino acid composition have significant effects on melting and setting temperatures of gelatin obtained from different sources. According to their results, the higher proline and hydroxyproline content of pork gelatin correlated with stronger gels having higher gelling temperatures. Proline and hydroxyproline are, however, not the only amino acids having significant effects on gelatin structure. The content of glutamic acid, aspartic acid, lysine, hydroxylysine, arginine, and histidine are also important in crosslink formation and electrostatic interactions. As collagen usually lacks cystine, there are no disulfide bonds in the collagen structure. Collagen is mostly stabilized by hydrogen bonds formed between side chains of the amino acids and water in addition to the twisted structure enforced by the high content of proline and hydroxyproline along with intra and intermolecular crosslinks (Engel and Bachinger 2005).

1.11.4 The Effect of pH

The main extraction step can be done at neutral, acid or alkali conditions. Acid or alkali treatments are useful for a more effective extraction, increasing the yield and shortening the extraction time. Zhou and Regenstein (2005) showed that acidic conditions are more favorable for higher gelatin yield. However, acidic conditions also cause low gel strength, which is not desired in most gelatin applications. The isoelectric point of collagen is around 6-6.5, depending on the amino acid composition, specifically the content of acidic and basic amino acids of collagen, which vary both due to source and to processing conditions. The isoelectric point of purified collagen is difficult to measure because collagen is difficult to isolate in its natural form as it is not readily soluble in water at room temperature and when it is dissolved with the help of heat treatment, collagen loses its natural state. Therefore,

the isoelectric point measured does not reflect the physiologically isoelectric point of the collagen but many researchers agree on the value of 7.0 for the isoelectric point of collagen under physiological conditions (Johns and Court 1977). Neutral extracts of untreated tissues of pork and rabbit skin, for example, had isoelectric points in the range of pH 5.6 and 6.8, respectively (Johns and Courts 1977). A pH that is higher or lower than the isoelectric point results in higher extraction yield as collagen is less tightly bound at pH values different from its isoelectric point. The net charge of the collagen molecule is zero at the isoelectric point where there are equal number of positive and negative charges on the molecule allowing it to form the maximum number of intermolecular salt bonds and electrostatic interactions, which strengthen and stabilize the structure of the collagen. According to the application in which gelatin will be used, the effect of pH on gelatin needs to be carefully considered and the pH of the extraction solution needs to be adjusted to get a high quality gelatin. For example, as type A gelatin has a higher isoelectric point, its use is favorable in those applications that require low pH at which the gelatin would be conducive to forming gel networks. Similarly, as type B gelatin has a low isoelectric point, it is used in those applications that require a high pH at which the gelatin is readily available for formation of gel network.

1.11.5 Other Factors

There are many other factors affecting gelatin properties. Going into detail for each one of them is beyond the scope of this paper. To be brief, every processing step, especially if heat is involved, has an effect on gelatin properties including yield, gel strength, melting and setting temperatures, and viscosity. Raw materials are also important with respect to purity and ease of processing. Freshness and storage of raw materials, any possible microbial contamination or the presence of microbial or natural

enzymes, the actual type of acid or alkali used are other factors that can affect the final gelatin.

1.12 Conclusions

Previous studies done on gelatin have shown that there are clear connections between gelatin's functional properties and the extraction conditions. While higher extraction temperatures and durations result in higher yield, the gelatin obtained is of poorer quality due to damage to the collagen fractions. Similarly, higher acid and/or alkali concentrations result in higher yield along with purer material, but the gelatin obtained lacks necessary functional properties. Therefore, an optimization of manufacturing process of gelatin is needed to get a final product with desired properties.

REFERENCES

Arnesen JA, Gildberg A. 2007. Extraction and characterization of gelatine from Atlantic salmon (*Salmo salar*) skin. Bioresource Technol. 98: 53–57.

Badii F, Howell NK. 2006. Fish gelatin: Structure, gelling properties and interaction with egg albumen proteins. Food Hydrocolloid. 20: 630-40.

Balian G, Bowes JH. 1977. The structure and properties of collagen. In: The science and technology of gelatin. Ward AG, Courts A, editors. New York: Academic Press. p 1-27.

Baziwane D, He Q. 2003. Gelatin: The paramount food additive. Food Rev Int. 19(4): 423-35.

Belitz HD, Grosch W, Schieberle P. 2004. Food chemistry. 3rd revised edition. New York: Springer. p 579-86.

Boran G, Regenstein JM. 2009. Optimization of gelatin extraction from silver carp skin. J Food Sci (accepted).

Brinckmann J. 2005. Collagens at a glance. In: Collagen: Primer in structure, processing and assembly. Brinckmann J, Notbohm H, Müller PK, editors. New York: Springer. p 1-6.

Bustillos RJA, Olsen CW, Olson DA, Chiou B, Yee E, Bechtel PJ, McHugh TH. 2006. Water vapor permeability of mammalian and fish gelatin films. J Food Sci. 71(4): E202-E207.

Cheow CS, Norizah MS, Kyaw ZY, Howell NK. 2007. Preparation and characterization of gelatins from the skins of sin croaker (*Johnius dussumieri*) and short fin scad (*Decapterus macrosoma*). Food Chem. 101: 386-91.

Chiou BS, Bustillos RJA, Shey J, Yee E, Bechtel PJ, Imam SH, Glenn GM, Orts WJ. 2006. Rheological and mechanical properties of cross linked fish gelatins. Polymer. 47: 6379-86.

Cho SM, Gu YS, Kim SB. 2005. Extracting optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins. Food Hydrocolloid. 19: 221-9.

Cho SH, Jahncke ML, Chin KB, Eun JB. 2006. The effect of processing conditions on the properties of gelatin from skate (*Raja kenojei*) skins. Food Hydrocolloid. 20: 810-6.

Choi SS, Regenstein JM. 2000. Physicochemical and sensory characteristics of fish gelatin. J Food Sci. 65(2): 194-9.

DeMan JM. 1999. Proteins: Animal proteins. In: The principles of food chemistry. Gaithersburg, Md.: Aspen Publishers, Inc. p 147-9.

Djagny KB, Wang Z, Xu S. 2001. Gelatin: A valuable protein for food and pharmaceutical industries: Review. Crit Rev Food Sci. 41(6): 481-92.

Eastoe JE, Leach AA. 1977. Chemical constitution of gelatin. In: The science and technology of gelatin. Ward AG, Courts A, editors. New York: Academic Press. p 73-105.

Engel J, Bachinger HP. 2005. Structure, stability and folding of the collagen triple helix. In: Collagen: Primer in structure, processing and assembly. Brinckmann J, Notbohm H, Muller PK, editors. New York: Springer. p 8-24.

Eyre DR, Wu JJ. 2005. Collagens crosslinks. In: Collagen: Primer in structure, processing and assembly. Brinckmann J, Notbohm H, Müller PK, editors. New York: Springer. p 208-25.

Fernandez-Diaz MD, Montero P, Gomez-Guillen MC. 2001. Gel properties of collagens from skins of cod (*Gadus morhua*) and hake (*Merluccius merluccius*) and their modification by the coenhancers magnesium sulfate, glycerol and transglutaminase. Food Chem. 74: 161-7.

Fernandez-Diaz MD, Montero P, Gomez-Guillen MC. 2003. Effect of freezing fish skins on molecular and rheological properties of extracted gelatin. Food Hydrocolloid. 17: 281-6.

Gilsenan PM, Ross-Murphy SB. 2000. Rheological characterization of gelatins from mammalian and marine sources. Food Hydrocolloid. 14: 191-5.

Gomez-Guillen MC, Sarabia AI, Solas MT, Montero P. 2001. Effect of microbial transglutaminase on the functional properties of megrim (*Lepidorhombus boscii*) skin gelatin. J Sci Food Agric. 81: 665-73.

Gudmundsson M, Hafsteinsson H. 1997. Gelatin from cod skins as affected by chemical treatments. J Food Sci. 62(1): 37-9 and 47.

Gudmundsson M. 2002. Rheological properties of fish gelatins. J Food Sci. 67(6): 2172-6.

Haug IJ, Draget KI, Smidsrod O. 2004. Physical and rheological properties of fish gelatin compared to mammalian gelatin. Food Hydrocolloid. 18: 203-13.

Hinterwaldner R. 1977. Technology of gelatin manufacture. In: The science and technology of gelatin. Ward AG, Courts A, editors. New York: Academic Press. p 315-61.

Holzer D. 1996. Gelatin production. United States Patent No: 5,484,888.

Hulmes DJS. 2008. Collagen diversity, synthesis, and assembly. In: Collagen, structure and mechanics. Fratzl P, editor. New York: Springer. p 16-22.

Imeson A. 1997. Thickening and gelling agents for food. New York: Springer. p 146.

Jamilah B, Harvinder KG. 2002. Properties of gelatins from skins of fish: Black tilapia (*Oreochromis mossambicus*) and red tilapia (*Oreochromis nilotica*). Food Chem. 77: 81-4.

Jongjareonrak A, Benjakul S, Visessanguan W, Tanaka M. 2006. Skin gelatin from big eye snapper and brown stripe red snapper: Chemical compositions and

effect of microbial transglutaminase on gel properties. Food Hydrocolloid. 20: 1216-22.

Johns P, Courts A. 1977. Relationship between collagen and gelatin. In: The science and technology of gelatin. Ward AG, Courts A, editors. New York: Academic Press. p 138-68.

Jones NR. 1977. Uses of gelatin in edible products. In: The science and technology of gelatin. Ward AG, Courts A, editors. New York: Academic Press. p 366-92.

Karim AA, Bahat R. 2009. Fish gelatin: Properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocolloid. 23(3): 563-76.

Kasankala LM, Xue Y, Weilong Y, Hong SD, He Q. 2007. Optimization of gelatin extraction from grass carp (*Catenopharyngodon idella*) fish skin by response surface methodology. Bioresource Technol. 98(17): 3338-43.

Muyonga JH, Cole CGB, Duodu KG. 2004. Extraction and physico-chemical characterization of Nile perch (*Lates niloticus*) skin and bone gelatin. Food Hydrocolloid. 18. p 581-92.

Nelson DL, Cox MM. 2005. Lehninger's principles of biochemistry. Fourth edition. New York: WH Freeman and Company. p 127-9.

Nielsen PM. 1995. Reactions and potential industrial applications of transglutaminase: Review of literature and patents. Food Biotech. 9(3): 119-56.

Petersen BR, Yates JR. 1977. Gelatin extraction. United States Patent No: 4,064,008.

Stainsby G. 1977. The gelatin gel and the sol-gel transformation. In: The science and technology of gelatin. Ward AG, Courts A, editors. New York: Academic Press. p 179-206.

Zhang S, Wang Y, Herring JL, Oh JH. 2007. Characterization of edible film fabricated with channel catfish (*Ictalurus punctatus*) gelatin extract using selected pretreatment methods. J Food Sci. 72(9): C498-503.

Zhou P, Regenstein JM. 2004. Optimization of extraction conditions for pollock skin gelatin. J Food Sci. 69(5): 393-8.

Zhou P, Regenstein JM. 2005. Effects of alkaline and acid pretreatments on Alaska pollock skin gelatin extraction. J Food Sci. 70(6): 392-6.

Zhou P, Mulvaney SJ, Regenstein JM. 2006. Properties of Alaska pollock skin gelatin: A comparison with tilapia and pork skin gelatins. J Food Sci. 71(6): 313-21.

Yang H, Wang Y, Jiang M, Oh JH, Herring J, Zhou P. 2007. 2-step optimization of the extraction and subsequent physical properties of channel catfish (*Ictalurus punctatus*) skin gelatin. J Food Sci. 72(4): C188-C195.

Yi JB, Kim YT, Bae HJ, Whiteside WS, Park HJ. 2006. Influence of transglutaminase induced crosslinking on properties of fish gelatin films. J Food Sci. 71(9): 376-83.

Williams PA (Editor). 2007. Handbook of industrial water soluble polymers. New York: Wiley-Blackwell. p 75-6.

Wainewright FW. 1977. Physical tests for gelatin and gelatin products. In: The science and technology of gelatin. Ward AG, Courts A, editors. New York: Academic Press. p 529.

CHAPTER TWO

OPTIMIZATION OF GELATIN EXTRACTION FROM SILVER CARP SKIN: PART I. SCREENING

Abstract

Fish skins are a by-product of the fish processing industry that can be successfully processed into gelatin. This study was designed to optimize extraction parameters to obtain the highest yield, gel strength, and viscosity for skin gelatin from silver carp (*Hypophthalmichthys molitrix* Valenciennes 1844). Nine parameters were selected as independent variables and the three above were the dependent variables. A fractional factorial design (2 levels, resolution III, 2^{9-5}) was chosen to screen the effects of the independent variables. Extraction temperature and acid concentration were found to significantly affect the protein yield ($P<0.05$). Protein yield varied between 4.5 and 20.3%, while gel strength varied between 85 and 875 g, and viscosity varied between 2.1 and 13.9 cP. The hydroxyproline content and hydroxyproline/protein ratio of the skin were 1.7 and 6.5%, respectively. The hydroxyproline content of the gelatin for the sample giving the highest hydroxyproline/protein ratio was 10.9%. This sample was arbitrarily called pure gelatin and the purity of the remaining samples were calculated to be between 24.4 and 88.2%. The protein content of the skin was 26.0%. The highest protein and gelatin recovery were 78.1 and 98.8% of the total available, respectively. The latter, gelatin recovery, is proposed to be used instead of protein yield. The data suggest that skin protein is not solely collagen with about 40% of it being non-collagen protein and/or non-protein nitrogen. Furthermore, the screening data suggest that the yield, viscosity and gel strength of gelatin from silver carp skin are akin to those of fish gelatins currently being exploited commercially.

2.1 Introduction

Gelatin is a pure protein obtained by partial hydrolysis of collagen through destruction of crosslinkages between polypeptide chains of collagen along with some level of breakage of polypeptide bonds (Belitz and others 2004). It is the only hydrocolloid widely used in the food, drug, and cosmetics industries that is not a carbohydrate (Nelson and Cox 2005). The chemical composition of gelatin is similar to that of the parent collagen and like collagen, gelatin's molecular structure is mainly multiple repetitions of a gly-x-y sequence, where x is often proline (pro), and y is often hydroxyproline (hyp) (Balian and Bowes 1977; Ergel and Bachinger 2005). Collagen has a molecular weight of approximately 330 kDa while gelatin is considered as all collagen fractions that exceed an arbitrary minimum molecular weight of 30 kDa (Eastoe and Leach 1977). The collagen fractions with a lower molecular weight are not considered to be gelatin but are rather considered gelatin hydrolysates, as they are not able to form a gel. The presence of hyp is almost exclusively unique to collagen and can be used to determine the amount of collagen or gelatin (Nelson and Cox 2005).

One of the most important characteristics of gelatin is the low melting temperature of its hydrocolloid gel that makes its use very favorable in the food industry (Choi and Regenstein 2000). Another important characteristic of gelatin is that its gel strength is relatively higher than most of the common gelling agents, which are usually carbohydrates obtained from vegetable sources (Badii and Howell 2006). Carbohydrate-based gelling agents have much higher melting temperatures along with less gel strength. A unique disadvantage of gelatin, however, is its challenges with regard to kosher and halal status, since almost half of the world's gelatin production is obtained from pig skin while the rest comes from animals that have not been religiously slaughtered. In addition, vegetarians also have objections to its usage since

gelatin is derived from animal hides and bones, although some vegetarians do accept ingredients derived from fish (Choi and Regenstein 2000).

Recent studies have shown that fish skin might be an alternative raw material for gelatin production since it provides a relatively good quality gelatin and also eliminates religious concerns that the Muslim and Jewish communities have. In addition, the idea of using fish processing industry by-products for value-added products has attracted substantial attention from researchers. Therefore, many fish species have been investigated as raw materials for gelatin extraction and the properties of gelatin obtained from these sources have also been examined. There are, however, limited studies (Cho and others 2004; Cho and others 2005; Kasankala and others 2007; Yang and others 2007; Zhou and Regenstein 2004) done using formal optimization procedures for gelatin extraction, which is an important tool for understanding how processing conditions affect the final product and for being able to get products with desired characteristics since the formal optimization procedures determine the most significant factors affecting the final product and optimize those factors for the best possible outcome. Thus, this study is designed to optimize gelatin extraction from silver carp (*Hypophthalmichthys molitrix* Valenciennes 1844) skin.

Asian carp species are native to Asia, including seven subspecies that have been introduced into the U.S.A. Silver carp were first brought into the U.S.A. in 1973 by a private fish farmer in Arkansas as a potential biological control agent to improve water quality in municipal sewage treatment lagoons and aquaculture ponds, and also as a food fish (Conover and others 2006). Silver carp skin was chosen as the raw material for this project for a number of reasons. First, very few studies have been done on gelatin extraction from Asian carp species. Second, the total world production of Asian carp species (silver, black, bighead, common and grass carp) was over 13.5 million tonnes (about 8.6% of the total fish production in the world) in 2005 and silver

carp alone was 4.2 million tonnes (about 2.7% of the total fish production in the world) of the total (FAO 2006). In the U.S.A., the commercial harvest of silver carp, an invasive species, is increasing in parts of the Mississippi River Basin. The combined annual commercial harvest of bighead and silver carps from the Mississippi and Illinois rivers increased from <600 kg per year between 1988 and 1992 to >50,000 kg per year since 1997. The reported combined commercial harvest of these species in 2003 was nearly 60,000 kg from the Mississippi River alone and exceeded 338,000 kg in the Illinois River (Conover and others 2006). Thus, Asian carp species are harvested in sufficient quantity for commercial gelatin production. On-going fishing pressure on these species is considered highly desirable by the conservation/natural resource departments in many of the Mid-Western states as it slows the further expansion of these species. Asian carps are usually processed into skinless fillets (mainly for gefilte fish, a European Jewish fish ball-like product), so there is an abundant amount of raw skins available.

The main objective of this study was to determine extraction conditions significantly affecting the characteristics of gelatin extracted from silver carp skin based on three dependent variables, namely protein yield (PY), gel strength (GS) at 4 °C and viscosity (V) at 60 °C. These dependent variables are considered to be the most important quality parameters in the gelatin industry (Zhou and Regenstein 2004). In addition, melting temperature (MT) was also determined while other parameters including gelatin yield (GY), protein recovery (PR) and gelatin recovery (GR) were calculated.

2.2 Materials and Methods

2.2.1 Silver Carp Skin

The frozen skins of silver carp were provided by Schafer Fisheries (Thomson, IL, U.S.A.) with overnight shipping to Ithaca with frozen gel packs included. Upon arriving at the laboratory, frozen skins were immediately washed with running cold tap water. The meat residues and scales, which were around 2/3 of the skin's weight, were removed using a knife. After washing the cleaned skins with running cold tap water, they were refrozen and stored at -20 °C for up to two months until further processing and use. Frozen skin samples were cut into small pieces (about 2-3 cm squares) while they were still frozen. Then, they were thawed overnight at 4 °C and used for extraction. About 50 g of skin were used for each different treatment. All reagents were analytical grade and obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.) or Fisher Scientific (Pittsburgh, PA, U.S.A.).

2.2.2 Study Design

For screening, a 2^{9-5} fractional factorial design (resolution III) was chosen (NIST 2007). The independent variables selected were acid pretreatment temperature (1) and duration (2), alkali pretreatment temperature (3) and duration (4), extraction temperature (5) and duration (6), the concentrations of alkali (7) and acid (8), and finally the water/skin ratio (9) at two different levels for each of these variables (Table 2.1.). Three dependent variables (responses) were determined to evaluate the effects of the independent variables on the gelatin extracted. The methodology permits the optimization to include all three dependent variables simultaneously.

2.2.3 Gelatin Extraction

Skin samples were put in 500 mL Pyrex erlenmeyer flasks and treated with alkali (NaOH) and then acid (HCl) solutions (5:1, v/w) at varying concentrations and temperatures for varying periods of time according to the experimental design (Table 2.1 and 2.2). After each alkali and acid treatment, skin samples were washed with distilled water (5:1, v/w) three times at ambient temperature and filtered through four layers of cheesecloth and squeezed dry by hand. After these treatments, water extraction was done in a waterbath (Isotemp Digital, model 205, Fisher Scientific) at varying temperatures and water/skin ratios for varying periods of time. After the extraction, gelatin solutions were filtered through four layers of cheesecloth to remove the skin residues. Then, the volume of the gelatin solutions was measured using Pyrex graduated cylinders. Prior to extraction, Pyrex erlenmeyer flasks were sealed with two layers of Parafilm (Structure Probe, Inc., West Chester, PA, U.S.A.). After putting the flasks in the waterbath, 15 min was allowed to bring samples to the previously set temperature of the waterbath before starting the timing. After extraction, appropriate amounts of gelatin solutions were used for determination of protein and hyp concentration. The rest of the solutions were put in aluminum loaf pans (22.9 cm long, 12.7 cm wide, 7.6 cm deep; Pactiv Corp., Lake Forest, IL, U.S.A.) that had been covered with non-stick aluminum foil (Reynolds Kitchens, Richmond, VA, U.S.A.) to dry in an oven (Sheldon Manufacturing Inc., Cornelius, OR, U.S.A.) at 60 °C until the gelatin sheets were obtained, which usually took about 72 h (Figure 2.1). Gelatin sheets (Figure 2.2) were carefully separated from the aluminum foil to prevent aluminum contamination.

Table 2.1 Independent variables and the levels of independent variables (2-levels and 9-factors fractional factorial design, 2^{9-5}, resolution III).

		Level	
Independent variable	Symbol	-	+
Alkali concentration (N)	A	0.1	1
Alkali pretreatment temperature (ºC)	B	4	24
Alkali pretreatment time (min)	C	45	90
Acid concentration (N)	D	0.1	1
Acid pretreatment temperature (ºC)	E	4	24
Acid pretreatment time (min)	F	45	90
Extraction temperature (ºC)	G	40	60
Extraction time (min)	H	120	240
Water/skin ratio (v/w)	J	4	6

‘+’ represents higher and ‘-’ represents lower levels.

Table 2.2 Fractional factorial screening design (2^{9-5}, resolution III) in a randomized order.

		Independent variables								
Standard order	Run order	A	B	C	D	E	F	G	H	J
1	2	-	-	-	-	-	-	-	-	+
2	12	+	-	-	-	+	-	+	+	-
3	6	-	+	-	-	+	+	-	+	-
4	10	+	+	-	-	-	+	+	-	+
5	7	-	-	+	-	+	+	+	-	-
6	14	+	-	+	-	-	+	-	+	+
7	13	-	+	+	-	-	-	+	+	+
8	15	+	+	+	-	+	-	-	-	-
9	3	-	-	-	+	-	+	+	+	-
10	11	+	-	-	+	+	+	-	-	+
11	1	-	+	-	+	+	-	+	-	+
12	4	+	+	-	+	-	-	-	+	-
13	16	-	-	+	+	+	-	-	+	+
14	5	+	-	+	+	-	-	+	-	-
15	9	-	+	+	+	-	+	-	-	-
16	8	+	+	+	+	+	+	+	+	+

'+' represents the higher and '-' represents the lower level of that variable (NIST, 2007). See Table 1 for letter identification.

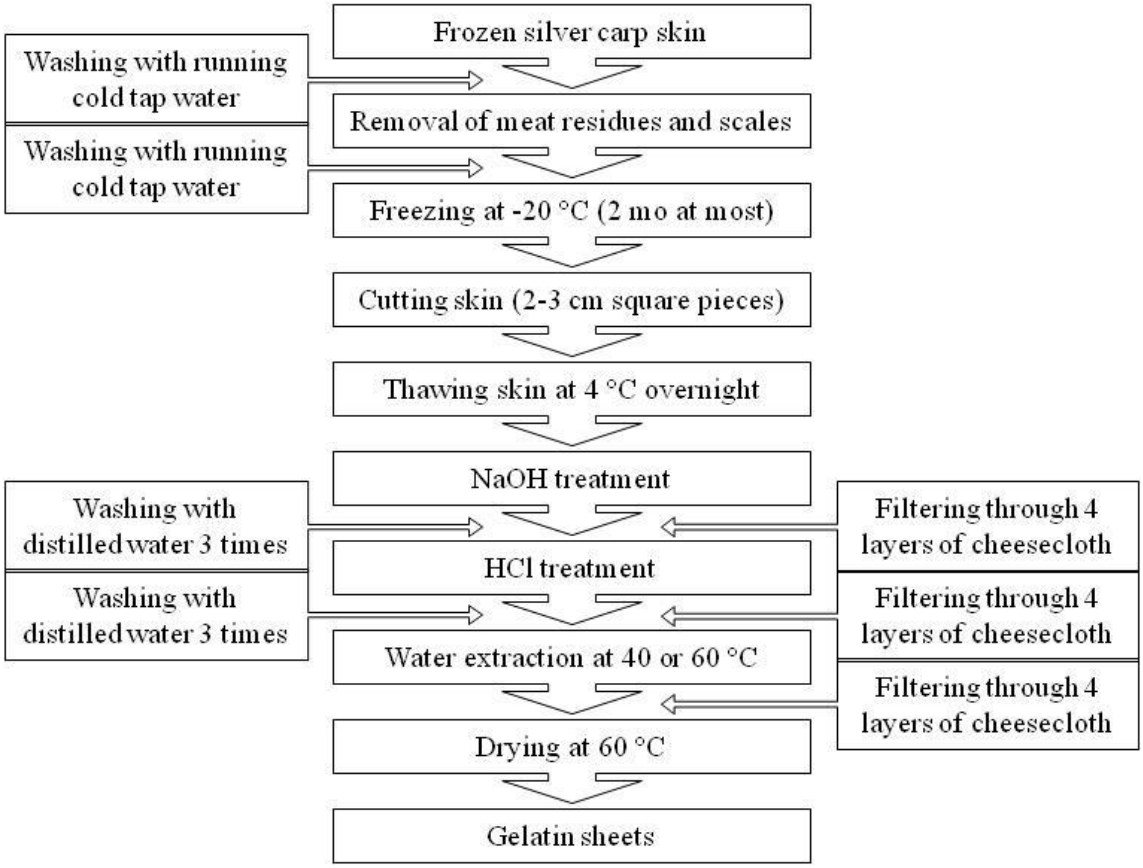

Figure 2.1 Flowchart for gelatin extraction.

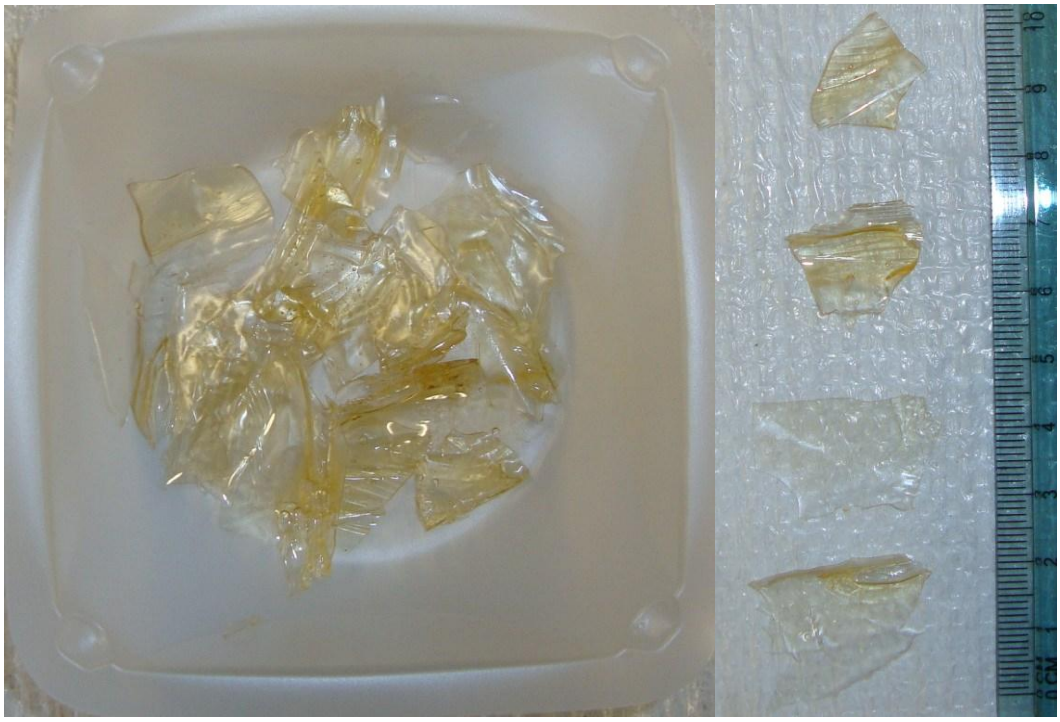

Figure 2.2 Gelatin sheets obtained from silver carp skin.

2.2.4 Gel Strength

Dried gelatin sheets (about 1 mm thick) were broken into small pieces and used to prepare 6.67% (w/v) gelatin solutions. Gelatin sheets were dissolved in distilled water using a waterbath at 60 °C for 30 min with occasional stirring using a spatula. Then, 15 mL of gelatin solutions were transferred into small screw-cap plastic jars (36 mm in dia, 16 mm in height, flat bottom), which were tightly capped and refrigerated at 4 °C for 16-18 h for maturation. Matured samples were immediately tested for gel strength at 4 °C while still in the plastic jars. The elapsed time between taking the samples out of the refrigerator and performing the test was less than 30 s. Gel strength measurement was done using a TA-XT2 texture analyzer (Texture Technologies Corp., Scarsdale, NY, U.S.A.).

The head penetration speed was 1 mm/s. The force required for the head (cylindrical plastic probe, 12.7 mm diameter, flat bottom) to penetrate 4 mm into the sample was taken as the gel strength in g.

2.2.5 Viscosity

Gelatin solutions (6.67%, w/v) were prepared as described above. Viscosity was measured by using a calibrated Cannon Fenske routine viscometer (size 200; Cannon Instrument Co., State College, PA, U.S.A.) in a waterbath at 60 °C. Ten mL of gelatin solutions were transferred into the viscometer and allowed to stand for 15 min to equilibrate to temperature. Then, the efflux time was recorded using a stopwatch for each sample and viscosity in units of cP was calculated according to the formulas given below. The density of the gelatin samples were determined by weighing 5 mL of gelatin samples in triplicates and the density was found to be 1.099±0.009 g/mL averaging all of the samples. The viscometer constant at 60 °C was

0.10143 (mm^2/s^2) as calculated by linear interpolation from given constants at 40 and 100 °C in the calibration document provided by the manufacturer.

Kinematic viscosity (mm^2/s) = efflux time (s) × viscometer constant (mm^2/s^2)

Viscosity (cP) = kinematic viscosity (mm^2/s) × density (g/mL)

2.2.6 Melting Temperature

Melting temperature was determined by using an AR-1000N rheometer (TA Instruments, Newcastle, DE, U.S.A.). A temperature sweep was done from 5 to 40 °C at a scan rate of 1 °C/min, frequency of 1 Hz and oscillating stress of 3.0 Pa with parallel plate geometry (25 mm diameter). Gelatin solutions were prepared as described above and then transferred (2.4 mL) into the small plastic cups after applying one drop of mineral oil (Walgreen Co., Deerfield, IL, U.S.A.) to the inner surface of the plastic cups using a small brush so as to prevent samples from sticking and/or fracturing. After maturation as described above, the rheometer previously cooled to 5 °C was loaded with the gel samples, which were 2 mm thick. The gel sample was glued (Loctite Super Glue, Henkel Consumer Adhesives Inc., Avon, OH, U.S.A.) to the bottom plate of the rheometer and the top plate was crosshatched to minimize slippage. The excess sample was trimmed with a sharp knife to fit the size of plate (25 mm diameter). The exposed outer gel surface was covered with a thin layer of mineral oil to prevent moisture loss during the measurements. G' (Pa), G" (Pa), and delta (δ, degrees) were determined. Melting temperature was calculated by interpolation and was taken as the cross-over point of G' and G" where tan δ becomes 1 and δ becomes 45°.

2.2.7 Protein Concentration

The protein concentration (PC) of gelatin solutions was determined using the Biuret method as described by Gornall and others (1949). Bovine serum albumin (BSA; Sigma-Aldrich) was used as the reference protein in the range of 0 to 1 mg/mL (Zhou and Regenstein 2006). In addition, the BSA was calibrated based on the absorbance of BSA at 280 nm (absorbance of BSA at 280 nm is 6.66 for a 1% BSA solution) with the absorbance at 320 nm subtracted as a background scattering correction (Regenstein and Regenstein 1984). The protein concentration of the skin was calculated based on nitrogen determined by the Kjeldahl method (Barbano and others 1990) and by using a conversion factor of 5.4 (Muyonga and others 2004).

2.2.8 Hydroxyproline Concentration

The hydroxyproline (hyp) concentration of the skin and gelatin solutions was determined by the method of Woessner (1961) using L-hydroxyproline (Sigma-Aldrich) as the standard. Standard solutions were prepared at several sequential concentrations between 0 and 2 µg/mL based on powder weight. For skin, about 2 g of skin was put into a Pyrex screw cap test tube for hyp hydrolysis. Then, 10 mL of 6 N HCl was added and the test tube was tightly closed and mixed using a Vortex mixer (Fisher Scientific). In the case of the gelatin solutions, however, 2 mL of gelatin solution were transferred into the test tube. Then, 3 mL of 10 N HCl were added to get a final concentration of 6 N HCl. The mixtures were kept in an oven (Fisher Scientific) at 130 °C for 3 h with an additional 15 min to allow the tubes to reach the preset temperature. After hydrolysis, the content of the tubes was transferred into a 100 mL Pyrex erlenmeyer flask and the tubes' contents were washed into the flask with distilled water. This solution was neutralized by using 2.5 N NaOH after adding 5-6 drops of methyl red (Sigma-Aldrich) indicator solution (0.02 g methyl red

dissolved in 60 mL ethyl alcohol and 40 mL distilled water). Finally, the solution was diluted to 50 mL adding distilled water to standardize the dilution factor for each sample. According to the PC results previously obtained, an appropriate amount (about 100 µL) of solution was transferred to a test tube and diluted to 2 mL with distilled water. After preparing the standards and the samples, 1 mL of chloramine T solution (0.05 M chloramine T solution was prepared from 98% chloramine T trihydrate, ACS grade, Sigma-Aldrich) was added to each tube to initiate the hyp oxidation. After mixing, the tubes were left to stand for 20 min. Then, 1 mL of perchloric acid solution (3.15 M perchloric acid solution was prepared by diluting 27 mL of 70% perchloric acid, ACS grade, Fisher Scientific) was added to each tube to destroy chloramine T. The contents were mixed again and the tubes were allowed to stand for 5 min. After that, 1 mL of *p*-dimethylaminobenzaldehyde solution (20% *p*-dimethylaminobenzaldehyde solution was prepared from 99% *p*-dimethylaminobenzaldehyde, ACS grade, Sigma-Aldrich) was added to each tube as before and the tubes were kept in a waterbath at 60 °C for 20 min for color development. Finally, the absorbances were read at 557 nm by using a spectrophotometer (SmartSpec Plus, Bio-Rad Laboratories, Hercules, CA, U.S.A.) and the hyp concentration of the samples was calculated using the standard curve (Woessner 1961). The percentage of hyp in the protein was calculated using the following formula:

$$\text{Hyp\%} = [\text{hyp conc. (mg/mL)} / \text{protein conc. (mg/mL)}] \times 100$$

2.2.9 Protein and Gelatin Yield

Protein yield (PY) was calculated by comparing the amount of protein extracted with the amount of skin used. Gelatin yield (GY) is calculated based on the

hyp concentration of the extracted solutions and that of the skin using a factor of 9.1 (10.9% hyp in the best gelatin) for the conversion of hyp to gelatin. This may slightly overestimate the amount of gelatin in the skin and/or in the solution as the percentage of hyp might increase with further purification. GY was calculated by comparing the amount of gelatin extracted with the amount of skin used.

2.2.10 Protein and Gelatin Recovery

In addition to PY and GY, protein recovery (PR) was calculated by comparing the amount of protein extracted with the amount of the protein in the skin. And gelatin recovery (GR) was calculated by comparing the amount of the gelatin extracted with the amount of gelatin (collagen) in the skin.

2.2.11 Statistical Analysis

Fractional factorial designs are usually best suited for studies where it is necessary to study more than five independent variables for screening purposes. Resolution 3 fractional factorial designs are generally preferred for this purpose as they minimize the cost of the experiments (NIST 2007). As nine parameters given above were selected as independent variables, a fractional factorial design (2^{9-5}, resolution 3) was chosen. The screening test results were analyzed using JMP statistics software (Version 7; SAS Institute, Cary, NC, U.S.A.). The screening test allows for an analysis of all the independent variables according to all three responses at once to determine which independent variable(s) affect responses at the significance level selected, which was 0.10 to include as many variables that might be relevant in the follow-up optimization study. All experiments were performed in triplicate.

2.3 Results and Discussion

2.3.1 Screening Analysis and Gel Strength

The screening analysis showed that the most important variable was the extraction temperature, which significantly affected PY ($P<0.05$) and GS ($P<0.10$). The interaction effect of extraction temperature and acid concentration was also significant for PY ($P<0.05$). In addition, the main effect of the water/skin ratio was significant for PY ($P<0.10$) and the main effect of acid concentration was significant for V ($P<0.10$). To include as many independent variables as possible, the acid pretreatment time in addition to the extraction temperature, the acid concentration, and the water/skin ratio is included in the optimization study as the acid pretreatment time had significantly ($P<0.10$) affected the gelatin yield and gelatin recovery (Table 2.3). Higher extraction temperatures gave lower gel strength while increasing the protein yield. Higher acid concentrations and water/skin ratios also gave higher protein yield while higher acid pretreatment times decreased the gelatin yield and gelatin recovery. All other main and interaction effects were not significant ($P>0.10$). P-values are given in Table 2.3 showing whether the main and/or the interaction effects of the independent variables were significant. The significant results obtained were mainly for PY, which may suggest that some of the differences for the other dependent variables, GS and V, might have been lost because the drying process might have neutralized the differences between the samples. A correction for the water content of the dried gelatin samples has not been done due to the limitations of the amount of each sample obtained.

The results confirm that extraction conditions significantly affect the gelatin process. Figure 2.3 illustrates the gel strength of three representative samples, one has average gel strength and the other two have the highest gel strengths, showing how elasticity and gel strength differed among the samples. All three samples shown were

Table 2.3 The corresponding p-values for the main and interaction effects of independent variables on the dependent variables obtained from the screening analysis.

Term	Gel strength	Viscosity	Protein yield	Protein recovery	Gelatin yield	Gelatin recovery
Extraction temp.	**0.098	0.314	*0.044	*0.048	**0.081	**0.075
Water/skin ratio	0.185	0.294	**0.084	**0.090	**0.062	**0.060
Acid conc.	0.297	**0.097	0.131	0.133	**0.057	**0.054
Acid pret. time	0.740	0.473	0.183	0.187	**0.073	**0.069
Extraction temp. × Acid conc.	0.462	0.575	*0.019	*0.021	*0.020	*0.019

*Significant at the level of P<0.05. **Significant at the level of P<0.10.

completely elastic although sample 1 and 8 were almost perfectly elastic as they instantly recovered when the force was removed while sample 15 was not perfectly elastic as its recovery took longer. Sample 15 had higher gel strength than samples 1 and 8 while being completely but not perfectly elastic. This might suggest that the average molecular weight of the collagen fractions in sample 15 was higher due to the milder extraction in terms of the amount of gelatin obtained as confirmed by the results for PY. In contrast, the average molecular weight of the collagen fractions in samples 1 and 8 must be lower due to the harsher extraction conditions. Samples 1 and 8 also gave higher PY than sample 15, confirming that the extraction conditions for sample 15 was milder. The average GS obtained for silver carp skin gelatin suggests that silver carp skin has enough GS to be a raw material for gelatin production.

GS might be considered as the most important quality characteristic required by the gelatin industry and should be reasonably high (Zhou and Regenstein 2004). There are many studies reporting on the gel strength of gelatin extracted from various fish species but, they are not always comparable due to the differences in the preparations of the samples, the experimental settings, and the equipments used. In this study, the highest gel strength measured was over 850 g for sample 15 and it was 550 g for sample 8 as the 2nd highest (Figure 2.4). Besides having high gel strength, sample 8 gave the highest viscosity (Figure 2.5) and melting temperature although its protein yield was lower than average (Figure 2.6), which may suggest that the extraction was relatively mild in dissolving collagen but what was extracted was of particularly high quality. A relatively mild treatment might also lead to high molecular weight collagen fractions in the solution, thus accounting for the high elasticity and relatively strong GS. Extraction conditions largely affected all of the dependent variables (GS, V and PY) and gave reasonably high values as shown in Figure 2.4, 2.5, and 2.6,

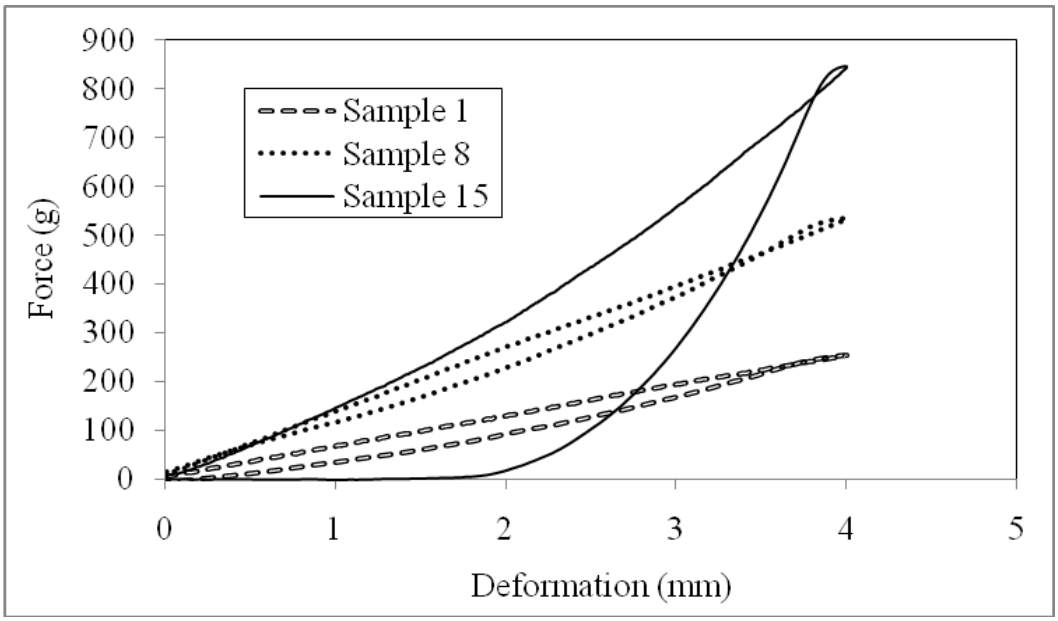

Figure 2.3 Force (g) against deformation (penetration of the probe into the gel, mm) plots of three samples.

respectively. GS varied between 85 and 876 g among the samples while viscosity varied between 2.1 and 13.9 cP and protein yield varied between 4.5 and 20.3%.

2.3.2 Viscosity and Melting Temperature

Viscosity of the gelatin samples varied, but mostly were under 7 cP except for sample 8 (Figure 2.5). This is in agreement with the values previously reported by Zhou and Regenstein (2004) for skin gelatin extracted from Alaska pollock, which was between 1.56 and 6.62 cP depending on the extraction applied. The average viscosity of gelatin samples extracted from silver carp skin was similar to that of pork skin gelatin, suggesting that silver carp skin might be used as an alternative raw material in place of pork skin for production of high viscosity gelatin.

The melting temperature of three representative samples is illustrated in Figure 2.7 as plots of delta (in angular degrees) against temperature (°C), showing that the melting temperature is significantly affected by the extraction conditions and varied between 14.0 and 28.3 °C among the samples.

These samples had a relatively sharp increase in delta as the temperature was increased, indicating a rapid transition and phase change although sample 8 gave a little more gradual phase change transition, which might be due to differences in the molecular weight of the collagen fractions and the heterogeneity of the molecular structure of the sample. The melting temperatures of the samples are given in Figure 2.8 as the cross-over point of the elastic or storage modulus (G') and the viscous or loss modulus (G") as described by Kasankala and others (2007). The highest melting temperature measured was 28.3 °C for sample 8 and was a little higher than the value of 26.8 °C for grass carp skin gelatin reported by Kasankala and others (2007). They also reported that the melting temperature of porcine and bovine gelatin was 31.5 and 30.0 °C, respectively, suggesting that silver carp skin might replace pork skin as it did

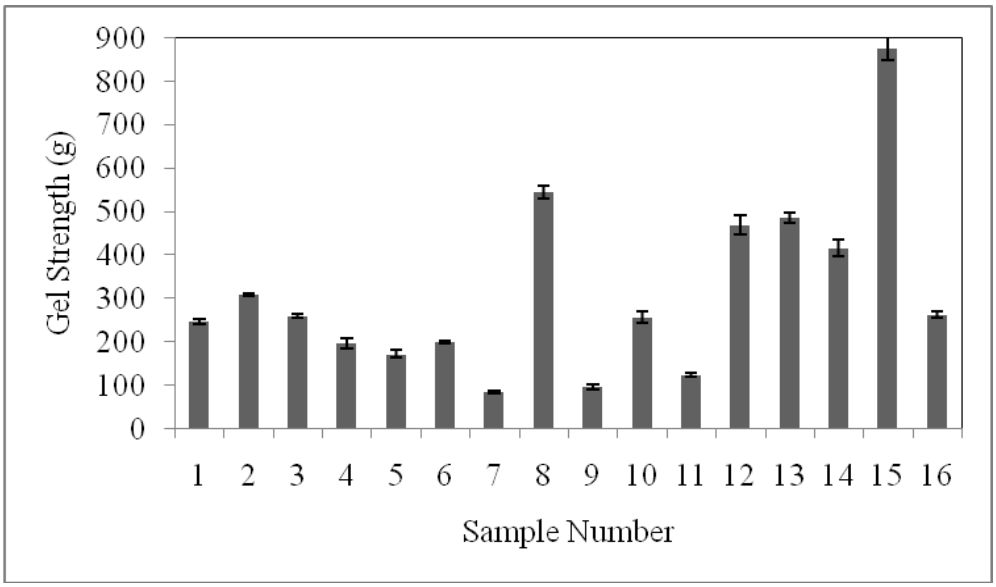

Figure 2.4 Gel strength (g) of the screening samples extracted under different combinations of extraction conditions according to the experimental design (the bars represent plus or minus one standard deviation obtained from triplicate measurements).

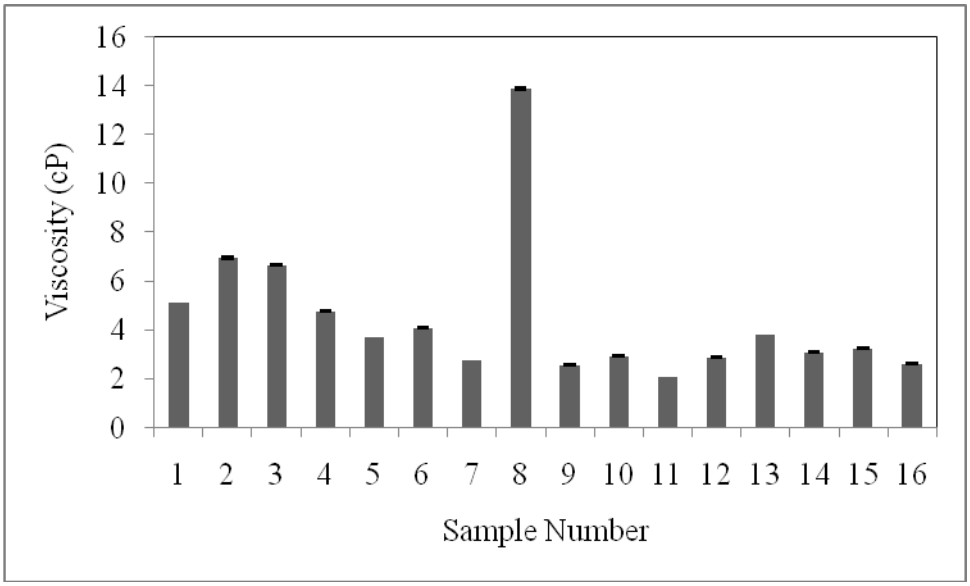

Figure 2.5 Viscosity (cP) of the screening samples extracted under different combination of extraction conditions according to the experimental design (the bars represent plus or minus one standard deviation obtained from triplicate measurements).

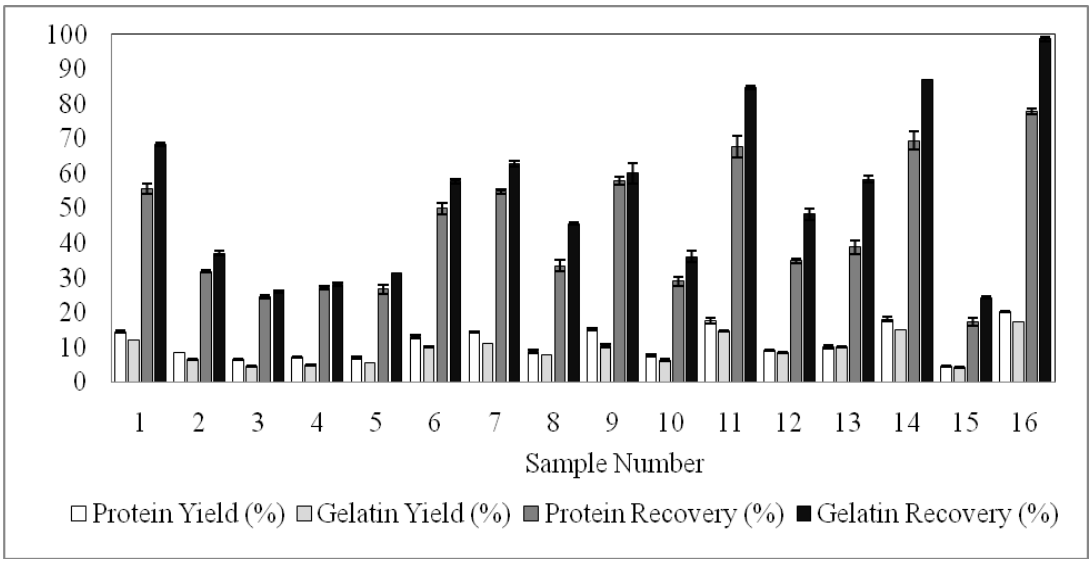

Figure 2.6 PY, GY, PR, and GR values for the screening samples extracted under different combinations of extraction conditions according to the experimental design (the bars represent plus or minus one standard deviation obtained from triplicate measurements).

not melt even at a relatively warm room temperatures (24-25 °C) but as in general, it melts below human body temperature (36.5 °C), which is desired for many food applications as well as pharmaceuticals.

2.3.3 Protein and Gelatin Yield

PY is considered to be one of the most important parameters by the gelatin industry because of its potential economic importance. In many of the previous studies, PY and GY were used interchangeably although they are, in fact, different. While PY indicates how much protein is extracted, GY indicates how much gelatin is extracted. The PY varied between 4.5 and 20.3% depending on the extraction conditions (Figure 2.6). In addition to PY, GY was calculated and varied between 3.7 and 15.3% (Figure 2.6), which indicated as expected that the extracted protein is not solely collagen or gelatin. The hyp concentration of silver carp skin was about 1.7%, which corresponds to a skin collagen amount of 15.4%. The protein concentration of the skin was found to be 26.0% (Kjeldahl) suggesting that about 40% of the skin protein is non-collagen protein and/or non-protein nitrogen.

2.3.4 Protein and Gelatin Recovery

PY and GY give values for how much protein and gelatin was obtained at the end of the extraction without relating to the initially available protein and gelatin. Therefore, PR and GR are calculated to evaluate the efficiency of the extraction process relative to the starting materials and the results are shown in Figure 2.6. Among these four parameters, GR is best suited to evaluate the efficiency of the extraction, as this parameter compares the initial amount of gelatin in the skin with the amount of gelatin extracted. Sample 16, for instance, while giving 20% PY, also had a PR of 78%, showing that a greater part of the initial protein present is actually

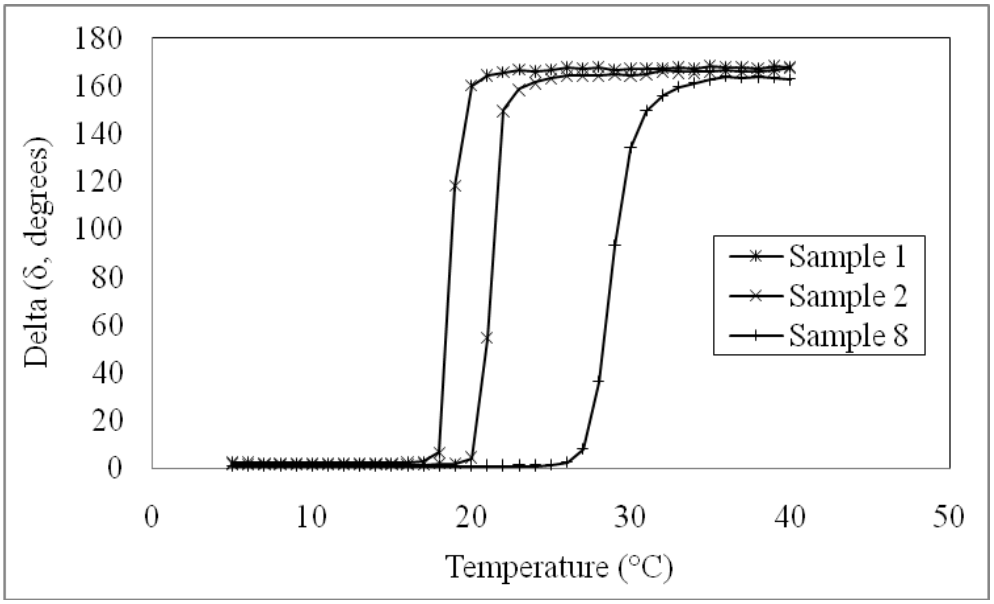

Figure 2.7 Delta (δ, degrees) plotted against temperature (°C) of three samples showing an estimate of melting temperature in °C.

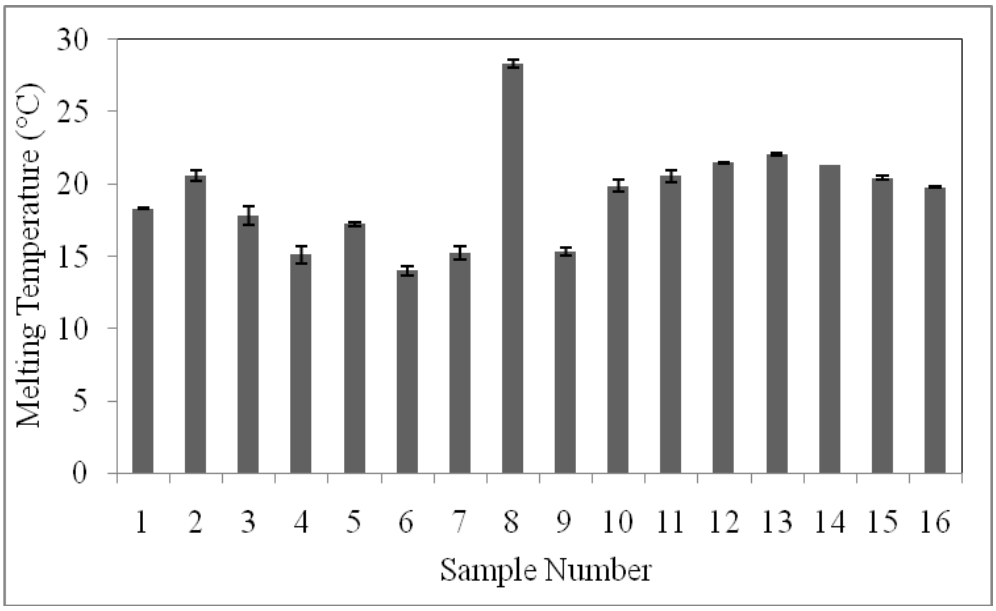

Figure 2.8 Melting temperature of screening samples (the bars represent plus or minus one standard deviation obtained from triplicate measurements).

extracted and the process is efficient. However, the GR was 98%, while the corresponding GY was 15%, showing that almost the entire gelatin in the skin was extracted using these conditions although about 25% of the protein is not gelatin. Thus, the GR is an indication of how efficient the process is at extracting gelatin.

In addition to PY; PR, GY, and GR were also substituted into the screening test and according to the results, PY and PG gave similar results with similar p-values for the main and interaction effects of the extraction parameters, while the results based on GY and GP were also similar to each other but, GY and GR gave different results from PY and PR. In addition to the extraction temperature, the water/skin ratio, and the HCl concentration; the HCl treatment time was also found to be significant ($P<0.10$). Therefore, the HCl treatment time should also be included in the follow-up optimization study, which will focus on GR rather than PY, PR or GY.

2.4 Conclusions

This study suggests that silver carp skins might be successfully used in gelatin production, giving relatively high protein yields, viscosity and gel strength. The results also suggest that the hyp content of gelatin extracted from silver carp skin is high compared to that of gelatins extracted from various fish species previously studied. GR is proposed as an alternative parameter to be used along with PY and/or GY, as it gives a better sense of the efficiency of the extraction process. In addition, the extraction temperature is confirmed as being the most significant factor affecting the quality of gelatin although other processing factors such as the acid concentration, the acid pretreatment time, and the water/skin ratio are also important thus they are included as independent variables to determine their optimum levels to obtain high quality gelatin from silver carp skin.

REFERENCES

Badii F, Howell NK. 2006. Fish gelatin: Structure, gelling properties and interaction with egg albumen proteins. Food Hydrocolloid. 20: 630-40.

Balian G, Bowes JH. 1977. The structure and properties of collagen. In: The science and technology of gelatin. Ward AG, Courts A, editors. New York: Academic Press. p 1-27.

Barbano DM, Clark JL, Dunham CE, Fleming JR. 1990. Kjeldahl method for determination of total nitrogen content of milk: Collaborative study. J AOAC. 73: 849-59.

Belitz HD, Grosch W, Schieberle P. 2004. Food chemistry. Third revised edition. New York: Springer. p 579-86.

Cho SM, Gu YS, Kim SB. 2005. Extraction optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins. Food Hydrocolloid. 19: 221-9.

Cho SM, Kwak KS, Park DC, Gu YS, Ji CI, Jang DH, Lee YB, Kim SB. 2004. Processing optimization and functional properties of gelatin form shark (*Isurus oxyrinchus*) cartilage. Food Hydrocolloid. 18: 573-9.

Choi SS, Regenstein JM. 2000. Physicochemical and sensory characteristics of fish gelatin. J Food Sci. 65(2): 194-9.

Conover G, Simmonds R, Whalen M, editors. 2006. Draft management and control plan for Asian carps in the United States. Asian carp working group. Aquatic nuisance species task force. Washington DC. 190 p.

Eastoe JE, Leach AA. 1977. Chemical constitution of gelatin. In: The science and technology of gelatin. Ward AG, Courts A, editors. New York: Academic Press. p 73-105.

Ergel J, Bachinger HP. 2005. Structure, stability and folding of the collagen triple helix. In: Collagen: Primer in structure, processing and assembly. Brinckmann J, Notbohm H, Muller PK, editors. New York: Springer. p 23-4.

FAO Fisheries and Aquaculture Department. 2006. The total fish production statistics of the world. Fishery information, data and statistics unit. Rome.

Gornall AG, Bardawill CJ, David MM. 1949. Determination of serum proteins by means of the biuret reaction. J Biol Chem. 177: 751-66.

Kasankala LM, Xue Y, Weilong Y, Hong SD, He Q. 2007. Optimization of gelatin extraction from grass carp (*Catenopharyngodon idella*) fish skin by response surface methodology. Bioresource Technol. 98(17): 3338-43.

Muyonga JH, Cole CGB, Duodu KG. 2004. Extraction and physico-chemical characterization of nile perch (*Lates niloticus*) skin and bone gelatin. Food Hydrocolloid. 18: 581-92.

Nelson DL, Cox MM. 2005. Lehninger's principles of biochemistry. Fourth edition. New York: WH Freeman and Company. p 127-9.

National Institute of Standards and Technology (NIST). 2007. E-handbook of statistical methods. (NIST/SEMATECH). http://www.itl.nist.gov/div898/handbook/.

Regenstein JM, Regenstein CE. 1984. Food protein chemistry: An introduction for food scientists. New York: Academic Press. p 62-6.

Woessner JF Jr. 1961. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Arch Biochem Biophys. 93: 440-7.

Yang H, Wang Y, Jiang M, Oh J, Herring J, Zhou P. 2007. 2-step optimization of the extraction and subsequent physical properties of channel catfish (*Ictalurus punctatus*) skin gelatin. J. Food Sci. 72(4): C188-195.

Zhou P, Regenstein JM. 2004. Optimization of extraction conditions for pollock skin gelatin. J Food Sci. 69(5): 393-8.

Zhou P, Mulvaney SJ, Regenstein JM. 2006. Properties of Alaska pollock skin gelatin: A comparison with tilapia and pork skin gelatins. J Food Sci. 71(6): 313-21.

Zhou P, Regenstein JM. 2006. Determination of total protein content in gelatin solutions with the Lowry or Biuret assay. J Food Sci. 71(8): 474-9.

CHAPTER THREE

OPTIMIZATION OF GELATIN EXTRACTION FROM SILVER CARP SKIN: PART II. OPTIMIZATION

Abstract

Gelatin is a hydrolyzed version of collagen mainly used in foods, pharmaceuticals, cosmetics, and photographic films. Among other sources, fish skins have been intensively investigated as alternative raw materials for gelatin production as fish skin is rich in collagen. This follow-up study was designed to determine optimum conditions for gelatin extraction from silver carp (*Hypophthalmichthys molitrix* Valenciennes 1844) skin to obtain the highest gel strength, viscosity, and gelatin recovery. Four parameters were selected as independent variables based on the data obtained in the screening and the three above were the dependent variables. A central composite rotatable design (4-factor and 5-level with 6 central points) was chosen to model the process and to optimize the level of independent variables. Gel strength varied between 88 and 764 g while viscosity varied between 2.2 and 8.9 cP and gelatin recovery varied between 19.5 and 93.2%. The hydroxyproline content of the gelatin for the sample giving the highest hydroxyproline/protein ratio was 10.9%. This sample was arbitrarily called pure gelatin and the purity of the remaining samples were calculated to be between 71.8 and 97.0%. The optimum extraction conditions were 50 °C for the extraction temperature, 0.1 N HCl for the acid concentration, 45 min for the acid pretreatment time, and finally 4 (v/w) for the water/skin ratio. The predicted responses for these extraction conditions were 630±74 g gel strength, 6.3±0.8 cP viscosity, and 80.8±8.3% gelatin recovery. The results obtained suggest that silver carp skin might be an alternative raw material for high quality gelatin production.

3.1 Introduction

Gelatin is a commonly used biopolymer obtained by thermo-hydrolysis of collagen through destruction of crosslinkages between polypeptide chains of collagen along with some level of breakage of polypeptide bonds (Balian and Bowes 1977; Belitz and others 2004). Although there are some carbohydrate based hydrocolloids used in foods, pharmaceuticals, and cosmetics, gelatin is the only protein based hydrocolloid used in these products, providing unique advantages including sol-gel and gel-sol transitions both under the human body temperature (Stainsby 1977). Gelatin functions as a gelling agent and structure enhancer in foods to improve elasticity, consistency, and stability (Zhou and Regenstein 2004). Moreover, gelatin's unique characteristics make its usage advantageous in many other products including drugs, cosmetics, photographic films, paints, and fertilizers (Gudmundsson 2002; Yang and others 2007). Gelatin is generally obtained from mammals, mainly from porcine and bovine sources. Among other alternative sources, fish and chicken by-products have been intensively investigated as alternative raw materials for gelatin production and the results obtained were promising. Fish skin has been shown to be an alternative raw material for gelatin production as it gives a relatively good quality gelatin, provides an opportunity of converting by-products of the fish processing industry into value-added products and also eliminates religious concerns that the Muslim and Jewish communities have regarding pork gelatin and beef gelatin from non-religiously slaughter animals (Zhou and Regenstein 2004).

There are several methods used by the gelatin industry to convert collagen into gelatin. The main purpose of gelatin extraction is conversion of water-insoluble collagen into gelatin that is soluble in water, while obtaining maximum yield and superior rheological and textural properties (Hinterwaldner 1977). In general, gelatin is obtained in three steps: pretreatment, extraction, and purification. In the first step,

raw materials are washed to remove impurities and then treated with alkali and/or acid to weaken collagen's structure by breaking intermolecular crosslinkages including covalent and hydrogen bonds. In the second step, the actual extraction is carried out with water at warm temperatures for appropriate periods of time to prevent extensive heat damage. In the last step, the gelatin extracted is subjected to several separation methods that might include filtration, evaporation, ion exchange chromatography, and drying to remove residues such as skin pieces, water, and ions (Hinterwaldner 1977). Many factors in a particular combination of extraction/conversion process affect the rheological, textural, and sensory characteristics of gelatin (Cho and others 2006). The extraction parameters such as extraction time and temperature, concentrations of acid and/or alkali, and skin/water ratio are some of the factors affecting the gelatin extracted (Cho and others 2006; Hinterwaldner 1977; Zhou and Regenstein 2004). The raw material used in gelatin production also has an obvious impact, mostly originating from differences in the amino acid composition of the gelatin's parent molecule, collagen. The effects of each factor should be considered very carefully to design a process giving high quality gelatin with desired characteristics for a particular application.

There have been only a few studies (Cho and others 2004; Cho and others 2005; Kasankala and others 2007; Yang and others 2007; Zhou and Regenstein 2004) done using formal optimization procedures for gelatin extraction, which is an important tool for understanding how processing conditions affect the final product and for being able to get products with desired characteristics since the formal optimization procedures generates regression models describing how and to what extend the extraction parameters affect the final outcome. Therefore, this study is designed to optimize gelatin extraction from silver carp (*Hypophthalmichthys molitrix* Valenciennes 1844) skin.

The main objective of this study was to optimize gelatin extraction from silver carp skin building on a previously done screening study, based on optimizing 3 important variables, namely gel strength (GS) at 4 °C and viscosity (V) at 60 °C, and gelatin recovery (GR). In the screening process, 4 out of 9 independent factors were found that significantly affected the gelatin extracted. To optimize the level of these four specifically identified factors, a central composite rotatable design was used. GS and V are considered to be the most important quality parameters in the gelatin industry (Zhou and Regenstein 2004). In addition, GR was proposed to be an alternative parameter to be used along with protein and/or gelatin yield in evaluation of the extraction process as GR might give a better sense on how efficient the extraction process actually is by directly comparing the amount of gelatin extracted to the amount of gelatin in the raw material prior to the extraction. Melting temperature (MT) was also determined so it could be used in a planned follow-up study to investigate interrelationships between the extraction conditions and the sensory and textural characteristics of the gelatin extracted.

3.2 Materials and Methods

3.2.1 Silver Carp Skin

The frozen skins of silver carp were provided by Schafer Fisheries (Thomson, IL, U.S.A.) with overnight shipping to Ithaca with frozen gel packs included. Upon arriving at the laboratory, frozen skins were processed as described in the screening study. About 50 g of skin were used for each different treatment. All reagents were analytical grade and obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.) or Fisher Scientific (Pittsburgh, PA, U.S.A.).

3.2.2 Study Design

The four factors determined during the screening procedure were set as independent variables in the optimization procedure. These independent variables were extraction temperature (A), acid concentration (B), acid treatment time (C), and the water/skin ratio (D). These variables were investigated at five different levels (Table 3.1) using a 4-factor and 5-level central composite rotatable design. All variables were studied at 5 levels covering the levels used in screening. The levels of independent variables were calculated according to the coefficients given by the central composite rotatable design and 6 central point determinations were performed (Table 3.1). Three dependent variables (responses), namely gel strength, viscosity, and gelatin recovery were determined to be used to model the extraction process, to evaluate the effects of the independent variables on the gelatin extracted, and to determine the optimum levels of the independent variables.

3.2.3 Gelatin Extraction

Skin samples were put in 500 mL Pyrex erlenmeyer flasks and treated with alkali (0.55 N NaOH) solution for 67.5 min at ambient temperature (24±2 °C) and then with acid (HCl) solutions (5:1, v/w) at varying concentrations for varying periods of time at ambient temperature according to the experimental design (Table 3.1 and 3.2). After each alkali and acid treatment, skin samples were washed 3 times with distilled water (5:1, v/w) at ambient temperature and filtered through 4 layers of cheesecloth and squeezed dry by hand as described in the screening study. After these treatments, water extraction was done in a waterbath (Isotemp Digital, model 205, Fisher Scientific) at varying temperatures and water/skin ratios according to the experimental design for 3 h. After the extraction, gelatin solutions

Table 3.1 Independent variables and the levels of independent variables (4-factor and 5-level) used in the unblocked central composite rotatable design.

		Level				
Independent variable	Symbol	-2	-1	0	+1	+2
Extraction temperature (ºC)	A	30	40	50	60	70
Acid concentration (N)	B	0	0.1	0.55	1	1.45
Acid pretreatment time (min)	C	22.5	45	67.5	90	112.5
Water/skin ratio (v/w)	D	3	4	5	6	7

Table 3.2 A 4-factor, 5-level and 6-central point unblocked central composite rotatable design with experimental (Exp) and predicted (Pre) results for the dependent variables.

		IV				GS		V		GR	
SO	RO	A	B	C	D	Exp	Pre	Exp	Pre	Exp	Pre
1	21	-1	-1	-1	-1	764	709	8.9	7.5*	71.7	70.0
2	28	+1	-1	-1	-1	603	607	4.8	5.1	88.8	84.7
3	7	-1	+1	-1	-1	655	614	4.2	3.9	58.1	48.7*
4	30	+1	+1	-1	-1	270	273	2.5	2.4	84.3	90.5
5	13	-1	-1	+1	-1	546	495	5.7	5.8	77.1	71.4
6	16	+1	-1	+1	-1	401	348	4.7	4.1	88.3	86.8
7	8	-1	+1	+1	-1	473	528	2.7	2.7	38.3	41.0
8	6	+1	+1	+1	-1	152	141	2.2	1.9	80.6	83.5
9	4	-1	-1	-1	+1	585	536	5.5	5.8	79.1	71.4
10	26	+1	-1	-1	+1	578	516	5.2	4.5	85.3	85.1
11	2	-1	+1	-1	+1	295	340	2.7	2.5	45.2	49.2
12	12	+1	+1	-1	+1	88	80	2.2	2.1	89.1	90.0
13	14	-1	-1	+1	+1	639	628	6.2	5.6	75.4	71.8
14	17	+1	-1	+1	+1	580	561	4.8	5.0	81.8	86.3
15	27	-1	+1	+1	+1	623	559	3.1	2.8	41.2	40.5
16	1	+1	+1	+1	+1	205	253	2.4	3.1	77.8	82.0
17	29	-2	0	0	0	581	633	3.5	4.4	19.5	29.4*
18	23	+2	0	0	0	211	226	2.5	2.3	93.2	85.6
19	3	0	-2	0	0	575	690*	7.5	8.3	73.2	82.1*
20	9	0	+2	0	0	334	287	3.0	2.9	63.0	56.5
21	18	0	0	-2	0	469	517	3.5	4.2	83.5	88.4
22	22	0	0	+2	0	458	477	3.4	3.4	84.3	81.8
23	5	0	0	0	-2	372	413	3.0	3.7	72.3	76.4
24	19	0	0	0	+2	325	352	3.3	3.2	78.1	76.3
25	10	0	0	0	0	355	357	3.5	3.5	71.1	72.0
26	11	0	0	0	0	358	357	3.5	3.5	70.1	72.0
27	15	0	0	0	0	350	357	3.6	3.5	73.5	72.0
28	20	0	0	0	0	357	357	3.6	3.5	71.3	72.0
29	24	0	0	0	0	362	357	3.5	3.5	74.0	72.0
30	25	0	0	0	0	358	357	3.7	3.5	71.9	72.0

IV: Independent variables, GS: Gel strength, V: Viscosity, GR: Gelatin recovery, A: Extraction temperature, B: Acid concentration, C: Acid pretreatment time, D: Water/skin ratio, SO: Standard order, RO: Run order. The superscript of '*' denotes an observation with a significantly different prediction from the actual.

were processed as described in the screening study until the gelatin sheets were obtained.

3.2.4 Gel Strength

The sample preparations and gel strength measurements were done as described in the screening study.

3.2.5 Viscosity

The sample preparations and viscosity measurements were done as described in the screening study.

3.2.6 Melting Temperature

The sample preparations and melting temperature measurements were done as described in the screening study.

3.2.7 Protein Concentration

The protein concentration (PC) of gelatin solutions was determined using the Biuret method as described in screening according to the method of Gornall and others (1949). The protein concentration of the skin was calculated based on total nitrogen determined by the Kjeldahl method (Barbano and others 1990) and by using a Kjeldahl conversion factor of 5.4 (Muyonga and others 2004).

3.2.8 Hydroxyproline Concentration

The hydroxyproline (hyp) concentration of the skin and gelatin solutions was determined as described in the screening study according to the method of Woessner (1961).

3.2.9 Protein and Gelatin Yield

Protein yield (PY) and gelatin yield (GY) were calculated as described in the screening study.

3.2.10 Protein and Gelatin Recovery

In addition to PY and GY, protein recovery (PR) and gelatin recovery (GR) were calculated as described in the screening study.

3.2.11 Statistical Analysis

Four extraction parameters (determined to be significant by the screening procedure) were studied at five levels covering the levels studied in the screening experiments. For this purpose, a central composite rotatable design (4-factor, 5-level, and 6-center point) was chosen. The levels of the factors were calculated based on the coefficients given in the central composite rotatable design (Table 3.1) (NIST 2007). The JMP statistics software (Version 7; SAS Institute, Cary, NC, U.S.A.) was used to analyze the optimization data to define a regression model and to produce ANOVA tables and surface profile plots for all 3 responses. Response surface methodology (RSM) was used to optimize the extraction parameters. RSM is a mathematical modeling technique that relates independent and dependent variables and establishes regression models that describe the interrelations between input parameters and output responses (Yang and others 2007). In general, the desirability function is used as an indicator of how closely the goal (i.e., minimizing or maximizing the response or matching a target response) is achieved by the model. The desirability level for each response is set manually and this affects the overall desirability of the results. The prediction profiler of the JMP statistics software was used to obtain the highest individual desirability for each response, the highest overall composite desirability and

the highest values for each response based on the settings given. MS Office Excel 2007 (Microsoft Corp., Redmond, WA, U.S.A.) was used to draw the relevant graphics to illustrate the data obtained, excluding the surface profile plots. All experiments were performed in triplicate.

3.3 Results and Discussion

3.3.1 The Regression Model and Optimization

The experimental results of the optimization study are given in Table 3.2 along with the results predicted by the regression model obtained by using JMP statistics software. The statistical analysis performed let to the observations that there were both positive and negative correlations between dependent variables. There was a relatively strong positive correlation (0.77) between GS and V while the correlation was -0.27 between GS and GR and 0.12 between GR and V. This was even lower than the correlation between GS and V. Thus, optimization was done considering these correlations, balancing all responses to be able to obtain one of the best possible outcomes. The regression model gave R^2 values over 0.90 for each response sufficiently explaining the variation in the results. Some of the observations were significantly different from the predicted values. Among the 30 samples in the optimization, 1 sample had a significantly different experimental result from the predicted result for both GS and V. For GR, however, 3 samples had significantly different experimental results from the predicted results. The R^2 value for GS was 0.93 while it was 0.91 for V and 0.92 for GR, which indicates that the regression model developed sufficiently explains the system (Table 3.3). The coefficients of linear, quadratic, and interaction terms are given in Table 3.4 along with p-values showing which terms contributed significantly to the responses ($P<0.05$).

Table 3.3 Analysis of variance (ANOVA) for the regression model.

		GS		V		GR	
SV	DF	SS	P value	SS	P value	SS	P value
Model	14	744398	<0.0001	65	<0.0001	7422	<0.0001
Error	15	51895	-	7	-	660	-
Total	29	796293	-	72	-	8082	-
R^2	-	93.48%		90.65%		91.83%	
R^2_{adj}	-	87.40%		81.92%		84.21%	

R^2 is used for discussion of the model's power in explaining the variation in the experimental data obtained. R^2_{adj} is just given for comparison purposes. GS: Gel strength, V: Viscosity, GR: Gelatin recovery, SV: Source of variation, DF: Degrees of freedom, SS: Sum of squares.

Table 3.4 Regression coefficients for the model

Term	GS	V	GR
Intercept	356.772*	3.538*	72.005*
A	-101.797*	-0.507*	14.052*
B	-100.701*	-1.360*	-6.394*
C	-10.049	-0.184	-1.646
D	-15.196	-0.129	-0.025
A*A	18.236	-0.049	-3.626*
A*B	-59.859*	0.219	6.772*
B*B	32.881*	0.519*	-0.684
A*C	-11.440	0.181	0.188
B*C	31.916*	0.122	-2.287
C*C	34.995*	0.067	3.271*
A*D	20.175	0.281	-0.241
B*D	-25.489	0.074	-0.231
C*D	76.390*	0.374*	-0.241
D*D	6.378	-0.013	1.092

GS: Gel strength, V: Viscosity, GR: Gelatin recovery, A: Extraction temperature, B: Acid concentration, C: Acid pretreatment time, D: Water/skin ratio. The superscript of '*' denotes significant difference, i.e., from zero at $P<0.05$.

Optimization was done according to the settings given in Table 3.5 using the prediction profiler of the JMP statistics software. Optimum extraction conditions were obtained for a 50 °C extraction temperature, 0.1 N the concentration of HCl, 45 min acid pretreatment time, and a 4 (v/w) water/skin ratio. The corresponding predictions along with 95% confidence intervals on these predictions for the independent variable under these extraction conditions were 630±74 g gel strength, 6.3±0.8 cP viscosity, and 80.8±8.3% gelatin recovery (Table 3.6). The corresponding protein yield was also predicted by the model and it was found to be 15.1±1.8%. As illustrated in Figure 3.1, a lower acid concentration and a shorter acid pretreatment time would give higher GS and V along with higher GR according to the relations determined in the model. In addition, the color of the gelatin would be darker as the acid treatment was done at lower concentrations and shorter times as this procedure is essentially responsible for removing the color from the gelatin prior to extraction as observed informally in this study and also reported with data by Zhang and others (2007). Nevertheless the color of the samples had not been studied as a dependent variable. However, because of the fact that the acid treatment helps with neutralizing the color of the gelatin extracted, the acid concentration and acid treatment time were held to reasonable levels, so the final product will have a reasonable GR along with reasonably high GS and V. These values can be manipulated by the investigators, so there are other possible optimizations that can be done. Surface plots given in Figure 3.1 summarize some of the interrelations between the independent and dependent variables. According to the data obtained, increasing the extraction temperature at selected levels does not affect GS very much, while the acid concentration is at the lowest level (i.e., the -2 level of acid concentration, which is just distilled water). However, the combination of high acid concentration and high extraction temperature decreased GS dramatically along

Table 3.5 Optimization parameters used in the prediction profiler.

Response	Goal	Low	Middle	High	Importance
GS (g)	Maximize	100	500	750	1
V (cP)	Maximize	2	6	9	1
GR (%)	Maximize	20	60	95	1
Desirability	-	0.01	0.60	0.99	-

Based on the experimental data obtained, low values were paired with low desirability, high values were paired with high desirability and average values were paired with medium desirability. GS: Gel strength, V: Viscosity, GR: Gelatin recovery.

Table 3.6 Optimization results obtained by using the prediction profiler.

IV				Predicted responses		
A	B	C	D	GS (g)	V (cP)	GR (%)
50 °C	0.1 N	45 min	4 v/w	630 ± 74	6.3 ± 0.8	80.8 ± 8.3
Individual desirability				0.78	0.64	0.83
Composite desirability					0.75	

IV: Independent variables, A: Extraction temperature, B: Acid concentration, C: Acid pretreatment time, D: Water/skin ratio, GS: Gel strength, V: Viscosity, GR: Gelatin recovery.

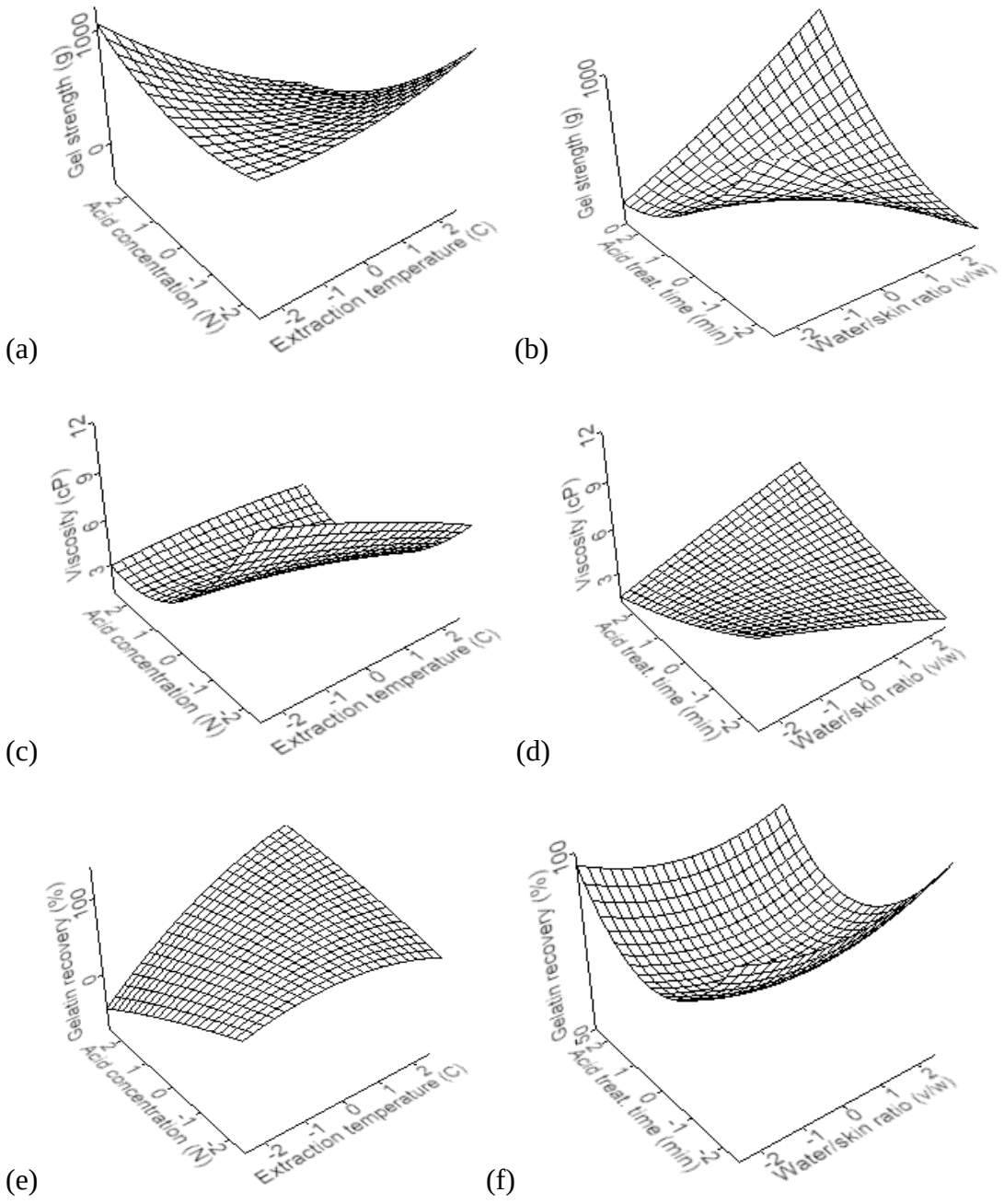

Figure 3.1 Surface profile plots of the three dependent responses plotted against the independent variables, obtained using JMP statistics software: (a-b) Gel strength (g), (c-d) Viscosity (cP), (e-f) Gelatin recovery (%). -2, -1, 0, 1, and 2 represent the level of the independent variables.

with V. On the other hand, GR increased with increasing acid concentration and extraction temperature at the levels studied. Decreasing the water/skin ratio did not affect GS much when the acid pretreatment time was increasing. However, decreasing both the water/skin ratio and acid treatment time increased GS significantly. Minimizing both the acid pretreatment time and the water/skin ratio might also maximize V. The middle levels for both the acid pretreatment time and the water/skin ratio resulted in a low GR and either decreasing or increasing the level of these factors resulted in higher GR (Figure 3.1).

3.3.2 Gel Strength and Viscosity

GS might be considered as the most important quality characteristic required by the gelatin industry and should be reasonably high (Zhou and Regenstein 2004). There are many studies reporting on the gel strength of gelatin extracted from various fish species but, they are not always comparable due to the differences in the preparations of the samples, the experimental settings used, and the equipment used. In this study the highest gel strength measured was over 750 g and on average GS was 431 g (Figure 3.2). These results are relatively high compared to that of pork skin or fish skin gelatins measured with standard methods but this does not necessarily mean that the GS of silver carp skin gelatin is higher than that of pork skin gelatin due to the differences in the methodology used. Nevertheless, the results obtained suggest that silver carp skin gelatin extracted under optimum conditions might be of high quality in terms of GS and V. There are very few studies directly comparing fish skin gelatin with pork skin gelatin in terms of quality. As an example, the results reported by Zhou and others (2006) indicated that the GS of Alaska pollock skin gelatin extracted under optimum conditions (Zhou and Regenstein 2004) might be as good as that of low molecular weight commercial pork skin gelatins. While low molecular weight pork

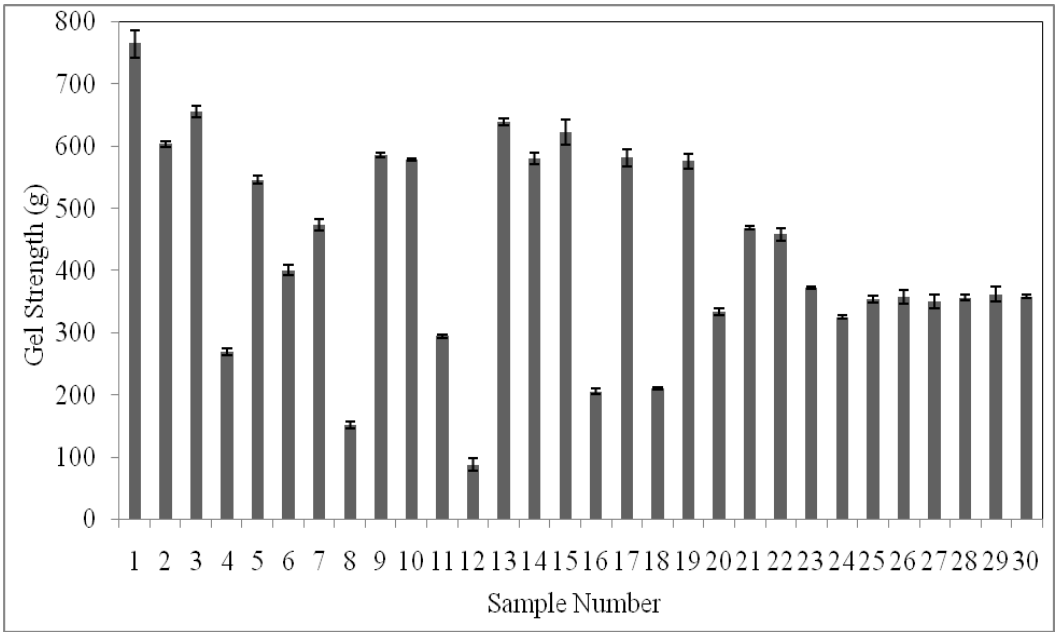

Figure 3.2 Gel strength (g) of the optimization samples extracted using different extraction conditions according to the experimental design (the bars represent plus or minus one standard deviation, obtained from triplicate measurements and the samples are shown in the standard order).

skin gelatin gave a GS of 110 g, Alaska pollock skin gelatin gave a GS of 98 g with the standard GS method (6.67% gelatin concentration, 16-18 h maturation at 10 °C, measured at 10 °C; the force in g with a 4 mm penetration is achieved with a 12.7 mm diameter flat bottom plunger while the sample is in a standard bloom jar). Unfortunately, the results obtained in this study are not completely comparable to those reported by Zhou and others (2006). However, silver carp skin gelatin extracted under optimum conditions is anticipated to give an even higher GS than that of Alaska pollock skin gelatin considering the values obtained and the methodology used. Viscosity (V) of the gelatin samples varied widely, but mostly were under 7 cP except for sample 1 and 19 (Figure 3.3). The average viscosity obtained from 30 gelatin samples was found to be 4.0 cP, i.e., similar to that of pork skin gelatin (Zhou and others 2006), suggesting that silver carp skin might successfully be used as an alternative raw material in place of pork skin for production of high viscosity gelatin. According to the results reported by Zhou and others (2006), low bloom pork skin gelatin gave 22 mP (2.2 cP) and high bloom pork skin gave 47 mP (4.7 cP) viscosity that is quite similar with the average viscosity reported in this study for silver carp skin gelatin but lower than that of some of the higher viscosity samples.

3.3.3 Protein and Gelatin Yield

PY and GY were calculated based on the protein and hyp concentrations of the extracted gelatin solutions. PY and/or GY were used interchangeably in many of the previously done studies although they are, in fact, different. PY indicates how much protein is extracted while GY indicates how much gelatin is extracted. Among the optimization samples, PY varied between 3.0 and 18.4% depending on the extraction conditions (Figure 3.4). GY, however, varied between 3.0 and 14.4%, which indicated that the extracted protein is not solely collagen or gelatin as expected. The gelatin

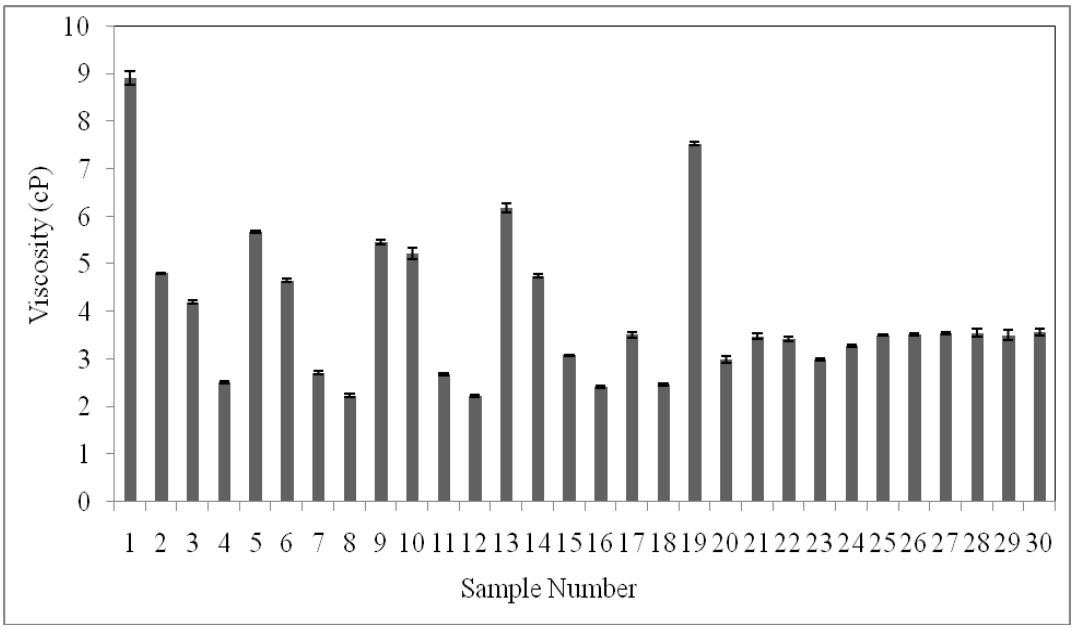

Figure 3.3 Viscosity (cP) of the optimization samples extracted using different extraction conditions according to the experimental design (the bars represent plus or minus one standard deviation, obtained from triplicate measurements and the samples are shown in the standard order).

percentage of protein samples extracted varied between 70% and almost 100% (Figure 3.5). However the almost 100% gelatin samples were very low in yield, average in MT and V, and high in GS. The percentage gelatin of samples suggests that even the poorest extraction increased the percentage of gelatin in extracted protein compared to the gelatin (collagen) percentage of the skin protein, which was about 60%.

3.3.4 Protein and Gelatin Recovery

PY and GY give values for how much protein and gelatin was obtained at the end of the extraction without relating to the initially available protein and gelatin. Therefore, PR and GR were calculated to evaluate the efficiency of the extraction process relative to the starting materials and the results are shown in Figure 3.4. Among these four parameters, GR is best suited to evaluate the efficiency of the extraction, as this parameter compares the initial amount of gelatin in the skin with the amount of gelatin extracted. Sample 18, for instance, while giving 18% PY, also had a PR of 71%, showing that a greater part of the initial protein present is actually extracted and the process is pretty efficient. However, the GR was 93%, while the corresponding GY was 14%, showing that almost the entire gelatin in the skin was extracted using these conditions although about 22% of the protein in this sample is not gelatin. Therefore, GR should be used as a dependent variable in place of PY as a more direct indication of the efficiency of the extraction process.

3.3.5 Melting Temperature

The melting temperature (MT) of samples varied between 18.0 and 25.4 °C and the results are shown in Figure 3.6. The melting temperatures of samples are given as the cross-over point of the elastic or storage modulus (G') and the viscous or loss modulus (G") as described by Kasankala and others (2007). The highest melting temperature

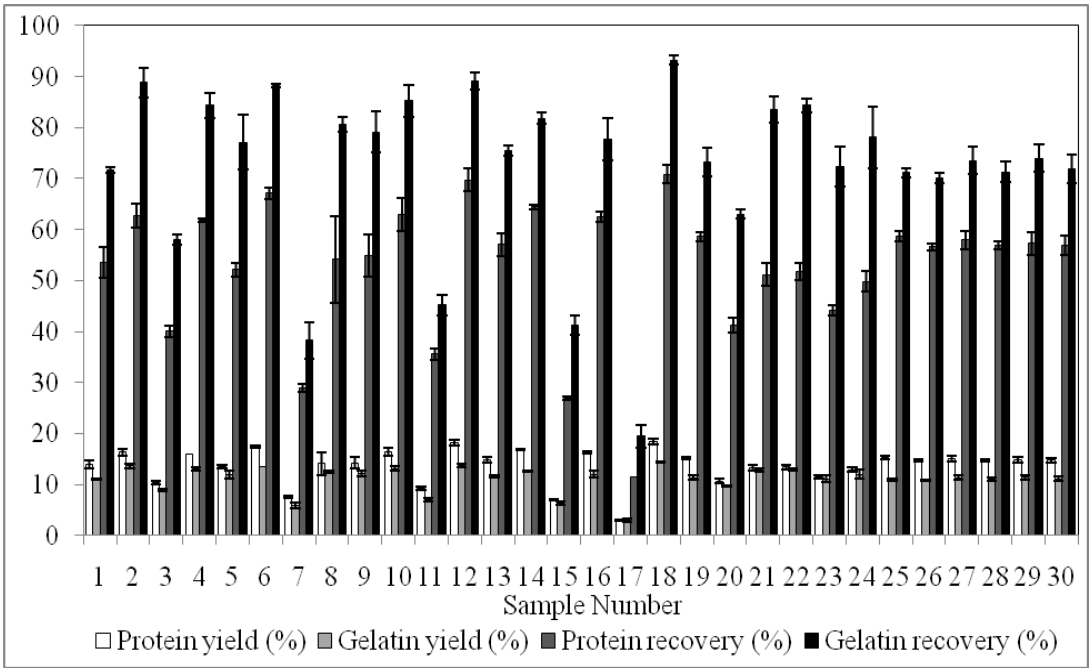

Figure 3.4 PY, GY, PR, and GR values for the optimization samples extracted using different extraction conditions according to the experimental design (the samples are shown in the standard order).

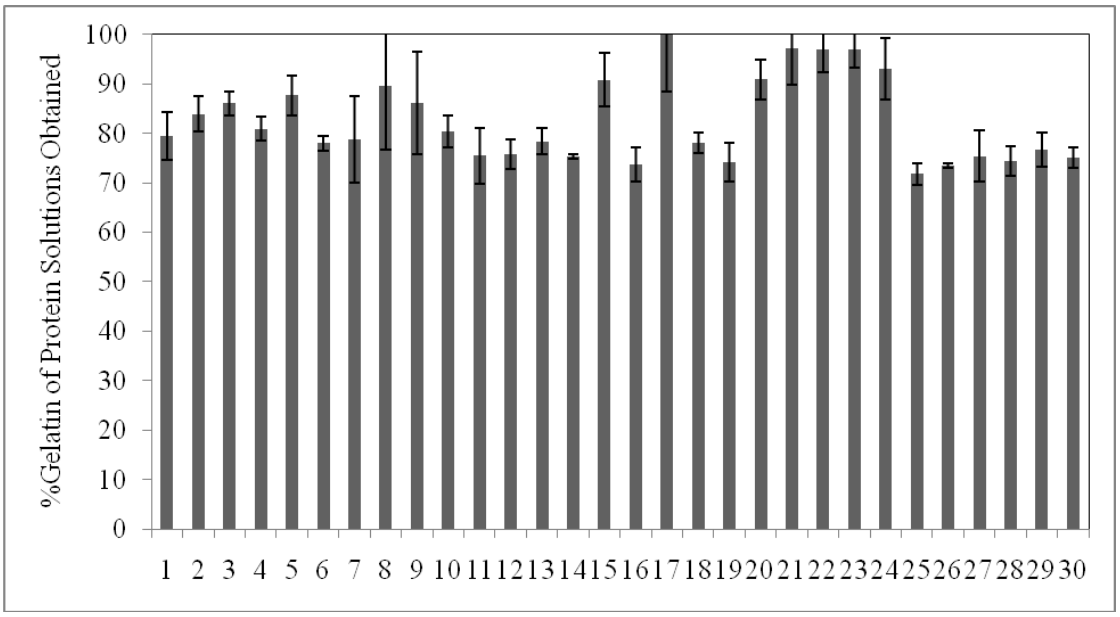

Figure 3.5 Percentage of gelatin in each protein sample extracted using different extraction conditions shown in Table 3.1 and 3.2 (the bars represent plus or minus one standard deviation, obtained from triplicate measurements and the samples are shown in the standard order).

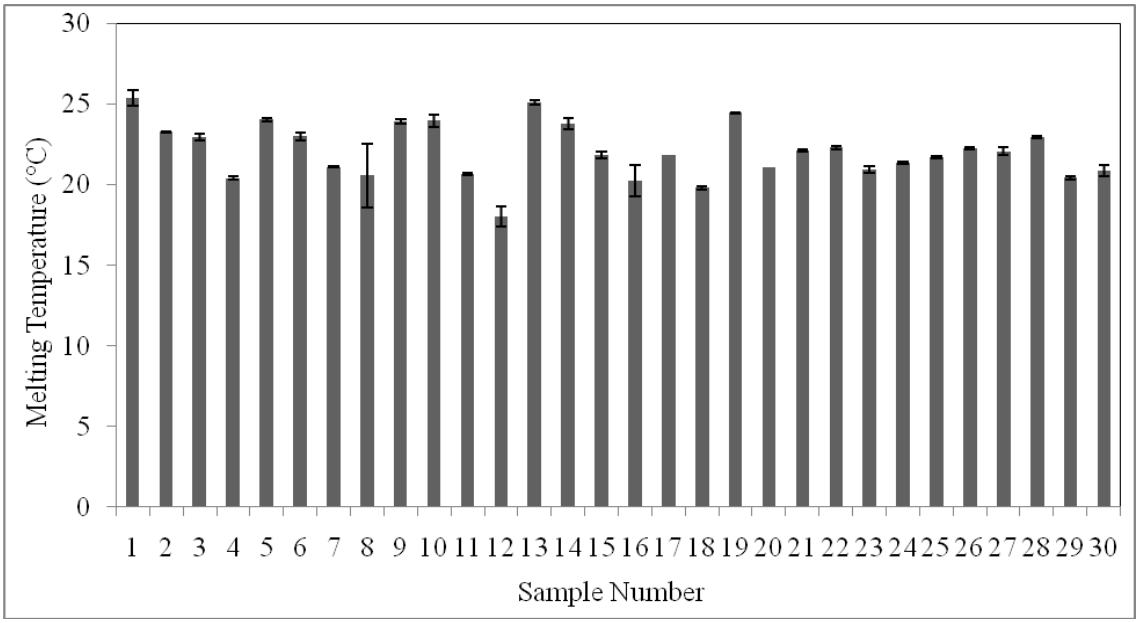

Figure 3.6 Melting temperature of the optimization samples (the bars represent plus or minus one standard deviation, obtained from triplicate measurements and the samples are shown in the standard order).

measured was 25.4 °C among the optimization samples. This was lower than the value of 26.8 °C for grass carp skin gelatin reported by Kasankala and others (2007). They also reported that the melting temperature of porcine and bovine gelatin was 31.5 and 30.0 °C, respectively.

3.4 Conclusions

Silver carp skin might be used as an alternative raw material for high quality gelatin production, giving reasonably high yield, gel strength, and viscosity. According to the model, optimum extraction conditions were found to be 50 °C extraction temperature, 0.1 N the concentration of HCl, 45 min acid pretreatment time, and 4 (v/w) water/skin ratio, giving a predicted set of independent variables with gel strength of 630±74 g, viscosity of 6.3±0.8 cP, and a gelatin recovery of 80.8±8.3%. The results also suggest that the hyp content of gelatin extracted from silver carp skin is higher compared to that of gelatins extracted from various fish species previously studied. GR is proposed as an alternative parameter to be used along with PY and/or GY, as it directly compares the gelatin extracted to the gelatin that was available prior to the extraction, giving a better sense of the efficiency of the process. However, PY and GY still hold significance as indicators of how much product could be obtained from each unit of input material.

REFERENCES

Balian G, Bowes JH. 1977. The structure and properties of collagen. In: The science and technology of gelatin. Ward AG, Courts A, editors. New York: Academic Press. p 1-27.

Barbano DM, Clark JL, Dunham CE, Fleming JR. 1990. Kjeldahl method for determination of total nitrogen content of milk: Collaborative study. J AOAC. 73: 849-59.

Belitz HD, Grosch W, Schieberle P. 2004. Food chemistry. Third revised edition. New York: Springer. p 579-86.

Cho SH, Jahncke ML, Chin KB, Eun JB. 2006. The effect of processing conditions on the properties of gelatin from skate (*Raja kenojei*) skins. Food Hydrocolloid. 20: 810-6.

Cho SM, Gu YS, Kim SB. 2005. Extraction optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins. Food Hydrocolloid. 19: 221-9.

Cho SM, Kwak KS, Park DC, Gu YS, Ji CI, Jang DH, Lee YB, Kim SB. 2004. Processing optimization and functional properties of gelatin form shark (*Isurus oxyrinchus*) cartilage. Food Hydrocolloid. 18: 573-9.

Gornall AG, Bardawill CJ, David MM. 1949. Determination of serum proteins by means of the biuret reaction. J Biol Chem. 177: 751-66.

Gudmundsson M. 2002. Rheological properties of fish gelatin. J Food Sci. 67(6): 2172-6.

Hinterwaldner R. 1977. Technology of gelatin manufacture. In: The science and technology of gelatin. Ward AG, Courts A, editors. New York: Academic Press. p 315-361.

Kasankala LM, Xue Y, Weilong Y, Hong SD, He Q. 2007. Optimization of gelatin extraction from grass carp (*Catenopharyngodon idella*) fish skin by response surface methodology. Bioresource Technol. 98(17): 3338-43.

Muyonga JH, Cole CGB, Duodu KG. 2004. Extraction and physico-chemical characterization of nile perch (*Lates niloticus*) skin and bone gelatin. Food Hydrocolloid. 18: 581-92.

National Institute of Standards and Technology (NIST). 2007. E-handbook of statistical methods. (NIST/SEMATECH). http://www.itl.nist.gov/div898/handbook/.

Stainsby G. 1977. The gelatin gel and the sol-gel transformation. In: The science and technology of gelatin. Ward AG, Courts A, editors. New York: Academic Press. p 179-207.

Woessner JF Jr. 1961. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Arch Biochem Biophys. 93: 440-7.

Yang H, Wang Y, Jiang M, Oh J, Herring J, Zhou P. 2007. 2-step optimization of the extraction and subsequent physical properties of channel catfish (*Ictalurus punctatus*) skin gelatin. J. Food Sci. 72(4): C188-195.

Zhang S, Wang Y, Herring JL, Oh JH. 2007. Characterization of edible film fabricated with channel catfish (*Ictalurus punctatus*) gelatin extract using selected pretreatment methods. J Food Sci. 72(9): C498-503.

Zhou P, Regenstein JM. 2004. Optimization of extraction conditions for pollock skin gelatin. J Food Sci. 69(5): 393-8.

Zhou P, Mulvaney SJ, Regenstein JM. 2006. Properties of Alaska pollock skin gelatin: A comparison with tilapia and pork skin gelatins. J Food Sci. 71(6): 313-21.

CHAPTER FOUR

RHEOLOGICAL PROPERTIES OF GELATIN FROM SILVER CARP SKIN COMPARED TO COMMERCIALLY AVAILABLE GELATINS FROM DIFFERENT SOURCES

Abstract

Gelatin is used as a functional ingredient in many foods, pharmaceuticals, and cosmetics as a stabilizing, thickening and gelling agent. The rheological properties of gelatin gels are important components of gelatin's potential functionality. This study was designed to determine the rheological properties of gelatin extracted from the skin of silver carp (*Hypophthalmichthys molitrix* Valenciennes 1844) and compare it with commercially available gelatins from different sources. The stress strain relationship of silver carp skin gelatin gels remained in the linear region over a broad range of strains and stresses and gave similar elastic moduli at varying frequency, stress, and strain levels. The one exception was a commercial high molecular weight fish skin gelatin that gave a lower elastic modulus indicating that its gel strength was low compared to the other gelatin samples studied. Gel strength varied between 220 and 1230 g while viscosity varied between 4.53 and 6.91 cP among the samples. Melting and gelling temperatures varied between 14.2-32.3 °C and 3.2-25.4 °C, respectively. Texture profile analysis was done at two deformation levels, 25 and 75%, and the results correlated well with gel strength. The correlations between hardness, cohesiveness and gumminess and gel strength were 0.98, 0.82, and 0.99, respectively, at 25% deformation but lower at 75% deformation. The results suggest that rheological measurements might be used to quickly estimate gel strength using less material. In addition, the silver carp skin gelatin seemed to be of equal quality to some of the commercial gelatins.

4.1 Introduction

Gelatin is a term used for all the collagen fractions obtained after heat denaturation that exceed an arbitrary minimum molecular weight of 30 kDa (Eastoe and Leach 1977). The collagen fractions with a lower molecular weight are not considered to be gelatin but are rather considered gelatin hydrolysates, as they are not able to form a gel. Gelatin is obtained by partial hydrolysis of collagen through destruction of crosslinkages between polypeptide chains of collagen along with some level of breakage of polypeptide bonds (Belitz and others 2004). It is the only hydrocolloid widely used in the food, drug, and cosmetic industries that is not a carbohydrate (Nelson and Cox 2005). The chemical composition of gelatin is similar to that of the parent molecule, collagen, and is mainly multiple repetitions of a gly-x-y sequence, where x is often proline (pro), and y is often hydroxyproline (hyp) (Balian and Bowes 1977; Ergel and Bachinger 2005). One of the most important characteristics of gelatin is its low melting point, i.e., below human body temperature, that makes it very favorable for use in the food industry (Choi and Regenstein 2000). Another important characteristic of gelatin is that its gel strength is usually higher than most of the common gelling agents (Badii and Howell 2006).

Gelatin can be obtained from the skins, bones, and scales of various animals, but most importantly from pigskin. Recent studies have shown that fish skin might be an alternative raw material for gelatin production as it provides a relatively good quality gelatin and also eliminates the religious concerns that the Muslim and Jewish communities share. In addition, using fish processing industry by-products for value-added products may help to overcome some disposal and environmental problems and can provide extra profit via complete utilization of fish.

The previous studies done in our laboratory concluded that skin of silver carp (*Hypophthalmichthys molitrix* Valenciennes 1844), an Asian carp species, can be

successfully converted into gelatin with relatively high yield, gel strength and viscosity (Boran and Regenstein 2009). Although gel strength and viscosity are considered to be the most important quality parameters in the gelatin industry, rheological measurements are being used as practical tools for determination of the quality and functional properties of various materials. In this study oscillatory sweep measurements and a creep-recovery test were used to determine the rheological properties of gels prepared with silver carp skin gelatin and commercial gelatins from different sources including pigskin, beef bone, chicken, and other species of fish skin. Some practical aspects must be considered when doing rheology with gelatin gels. First, since gelatin gelation is a kinetic process and continues indefinitely long after the initial setting of the gel, sufficient time must elapse before beginning the experiment so that any further change during the experiment is negligible. Second, the thermal history has a great influence over the properties of gelatin gels. Thus, the temperature and cooling procedure must be carefully controlled. And third, the stress must be chosen so that the strain is measurable but still remains within the linear viscoelastic region (Gilsenan and Ross-Murphy 2001). Therefore, data from preliminary studies were used to determine the test conditions for the rheology measurements and the same sample preparation and test procedures were followed for all the samples studied.

Silver carp skin gelatin obtained under optimum extraction conditions (Boran and Regenstein 2009) was compared to commercial gelatin samples with respect to rheological and textural characteristics of their gels. Stress, strain, time, and frequency sweep measurements were performed along with creep-recovery test, texture profile analysis (TPA) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The results were compared for these samples, while other parameters, including gel strength and viscosity, were also measured.

4.2 Materials and Methods

4.2.1 Silver Carp Skin Gelatin

The frozen skins of silver carp were provided by Schafer Fisheries (Thomson, IL, U.S.A.) with overnight shipping to Ithaca with frozen gel packs included. Upon arriving at the laboratory, frozen skins were immediately washed with running cold tap water. The meat residues and scales, which were around 2/3 of the skin's weight, were removed using a knife. After washing the cleaned skins with running cold tap water, they were frozen once again and kept in a sharp freezer at -20 °C for a maximum period of two months until further processing and use. Frozen skin samples were cut into small pieces (about 2-3 cm squares) while they were still frozen. Then, they were kept in a refrigerator (National Consolidated Industries, Honea Path, SC, U.S.A.) overnight at 4 °C for thawing and then used for extraction. About 500 g of skin was used for gelatin extraction. Skin samples were put in 500 mL Pyrex erlenmeyer flasks and treated with 0.55 N NaOH solution for 67.5 min at ambient temperature and then with 0.1N HCl solution (both 5:1, v/w) for 45 min at ambient temperature. After each alkali and acid treatment, skin samples were washed with distilled water (5:1, v/w) three times at ambient temperature and filtered through four layers of cheesecloth and squeezed dry by hand. After these treatments, water extraction was done in a waterbath (Isotemp Digital, model 205, Fisher Scientific) at 50 °C at 4:1 (v/w) water/skin ratios for 3 h. Prior to extraction, Pyrex erlenmeyer flasks were sealed with two layers of Parafilm (Structure Probe, Inc., West Chester, PA, U.S.A.). After putting the flasks in the waterbath, 15 min was allowed to bring samples to the previously set temperature of the waterbath before starting the timing. After the extraction, gelatin solutions were filtered through four layers of cheesecloth to remove the skin residues. Then, the gelatin solutions were put in aluminum loaf pans (22.9 cm long, 12.7 cm wide, 7.6 cm deep; Pactiv Corp., Lake Forest, IL, U.S.A.)

covered with non-stick aluminum foil (Reynolds Kitchens, Richmond, VA, U.S.A.) to dry in an oven (Sheldon Manufacturing Inc., Cornelius, OR, U.S.A.) at 60 °C until the gelatin sheets were obtained, which usually took about 72 h. Gelatin sheets were carefully separated from the aluminum foil to prevent aluminum contamination and used for the experiments planned. All reagents were analytical grade and obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.), Fisher Scientific (Pittsburgh, PA, U.S.A.), and (Bio-Rad, Hercules, CA, U.S.A.).

4.2.2 Commercial Gelatin Samples

Silver carp skin gelatin was compared to commercial gelatin samples from different sources. These commercial samples were kosher fish gelatin (species not identified) (F1, Food Industry Technology, Miami Beach, FL, U.S.A.), chicken gelatin (C, Food Industry Technology), pigskin edible gelatin (PS, Kind & Knox Gelatine, Inc., Sioux City, IA, U.S.A.), bone gelatin (presumably beef) (B, Kind & Knox Gelatine, Inc.), and high molecular weight fish gelatin (species not identified) (F2, Norland Products Incorporated, Cranbury, NJ, U.S.A.).

4.2.3 Sample Preparations

The gelatin samples were in the form of coarse granules, powders, flakes or sheets. These were used to prepare 6.67% (w/v) gelatin solutions for all the measurements. Gelatin samples were dissolved in distilled water using a waterbath at 60 °C for 30 min with occasional stirring using a spatula. Fifteen mL of gelatin solutions were transferred into small screw-cap plastic jars (36 mm in dia, 16 mm in height, flat bottom) for measurements of both gel strength and TPA. Then, the caps were closed tightly and the samples were refrigerated at 4 °C for 16-18 h for maturation. For rheological measurements, gelatin solutions were dissolved in distilled

water as explained earlier, and then transferred (2.4 mL) into the small plastic jars after applying one drop of mineral oil (Walgreen Co., Deerfield, IL, U.S.A.) to the inner surface of the plastic jars using a small brush to prevent samples from sticking and/or fracturing. After maturation as described, the rheometer was loaded with the gel samples, which were 2 mm thick. The gel samples were glued (Loctite Super Glue, Henkel Consumer Adhesives Inc., Avon, OH, U.S.A.) to the bottom plate of the rheometer and the top plate, which was crosshatched to minimize slippage, was brought into place. The excess sample was trimmed with a sharp knife to fit the size of plate (25 mm in diameter). The exposed outer gel surface was covered with a thin layer of mineral oil using the brush to prevent moisture loss during the measurements.

4.2.4 Gel Strength and Texture Profile Analysis

Matured samples were immediately tested for gel strength at 4 °C while they were still in the plastic jars as described by Boran and Regenstein (2009). For TPA, gel samples were taken out of the jars gently using a spatula right before the measurements. The elapsed time between taking the samples out of the refrigerator and performing the test was less than 30 s in case of gel strength measurements and less than 2 min in case of TPA measurements (less than 1 min for 25% deformation and less than 2 min for 75% deformation). TPA and gel strength measurements were done using a TA-XT2 texture analyzer (Texture Technologies Corp., Scarsdale, NY, U.S.A.). The head penetration speed was 1 mm/s in either case. The force required for the head (cylindrical plastic probe, 12.7 mm diameter, flat bottom) to penetrate 4 mm into the sample was taken as the gel strength in g. The probe used for TPA was 75 mm in diameter (flat bottom). In case of 25% deformation, the head penetrated the sample twice (imitation of the chewing process) for 4 mm with a 5 s interval between the two cycles. In the case of the 75% deformation, the same probe was used and penetration

depth was 12 mm this time causing a 75% deformation as the original height of the samples were 16 mm. Hardness was defined as the height of the force peak (g) on the first compression cycle, cohesiveness was defined as the ratio of the positive force areas under the first and second compressions (dimensionless), and gumminess was defined as hardness multiplied by cohesiveness (g) (Bourne 2002).

4.2.5 Viscosity

Gelatin solutions (6.67%, w/v) were prepared as described and then viscosity was measured by using a calibrated Cannon Fenske routine viscometer (size 200; Cannon Instrument Co., State College, PA, U.S.A.) in a waterbath at 60 °C as described by Boran and Regenstein (2009). The density of the gelatin samples were determined by weighing five mL of gelatin samples in triplicates and the density was found to be 1.108±0.012 g/mL on average. Viscosity of the gelatin samples was calculated in centipoise (cP).

4.2.6 Rheological Measurements

All rheological measurements were done by using an AR-1000N rheometer (TA Instruments, Newcastle, DE, U.S.A.) with parallel plate geometry (25 mm diameter). Gel samples were prepared as described. G' (Pa), G" (Pa), and the phase angle delta (δ, degrees) were recorded. The time sweep tests were done for 30 min at 5 °C at a frequency of 1 Hz and a stress of 200 Pa. The frequency sweep tests were done between 0.01 Hz and 10 Hz at 5 °C and a stress of 3 Pa. The strain sweep tests were done between 0.1 and 10% strain at 5 °C and a frequency of 1 Hz. The stress sweep tests were done between 0.1 and 1000 Pa at 5 °C and a frequency of 1 Hz. The creep-recovery tests were done at 5 °C for 15 min for both creep and recovery at a stress of 200 Pa for the creep cycle. The temperature sweep tests were done from 5 to 35 °C

and from 35 to 5 °C at a scan rate of 1 °C/min, a frequency of 1 Hz and a stress of 3 Pa except for sample F2, where the temperature sweep was from 1 to 25 °C and from 25 to 1 °C for this sample as its melting and gelling temperatures were lower. Melting and gelling temperatures were calculated by interpolation and taken as the cross-over point of G' and G" where tan δ becomes 1 and δ becomes 45°.

4.2.7 SDS-PAGE

Samples of gelatin were dissolved in distilled water at 60 °C for 30 min at an approximate concentration of 5 mg/mL. Then protein samples were resolved by a traditional Laemmli Glycine-SDS-PAGE system (Laemmli 1970) consisting of a 7% acrylamide resolving layer with a 4% acrylamide stacking/loading layer (30% solution of 37.5:1 acrylamide to bisacrylamide solution, Bio-Rad) using a Mini-Protean Tetra Cell system (Bio-Rad). All samples were diluted 2 fold in 2x sample buffer, then heated at 65 °C for 15 min and cooled rapidly on ice. A molecular weight marker offering a range from 6.5 kDa to 200 kDa (Bio-Rad) was diluted according to the manufacturer's instructions then heat treated and cooled in a similar fashion. Four uL of each sample and the standard was loaded onto their respective lane in the gel and resolved at 120 volts for approximately 45 min. Protein bands were visualized after a 60 min wash in a fixing solution (10% glacial acetic acid, 40% methanol), a 60 min wash in a staining solution (10% glacial acetic acid, 0.025% Coomassie Blue) and three 30 min washes in a destaining solution (10% glacial acetic acid). Images were recorded using a digital camera (Nikon D60, Nikon Inc., Melville, NY, U.S.A.).

4.2.8 Statistical Analysis

The gel strength, viscosity, melting-gelling temperature and TPA data obtained were compared statistically by performing ANOVA and Tukey tests to determine

which samples were significantly different from others where the discrimination was set at a significance level of 95%. The correlations between these parameters were also calculated using JMP statistics software (Version 7; SAS Institute, Cary, NC, U.S.A.). MS Office Excel 2007 (Microsoft Corp., Redmond, WA, U.S.A.) was used to draw the relevant graphics to illustrate the data obtained. All experiments were performed in triplicate.

4.3 Results and Discussion

4.3.1 Gel Strength and Viscosity

Gel strength is usually considered as the most important quality characteristic in the gelatin industry and should be reasonably high (Zhou and Regenstein 2004). There are many studies reporting on the gel strength of gelatin extracted from various fish species but, they are not always comparable due to the differences in the procedures for sample preparations, the experimental settings, and the equipments used. Gel strength of the samples in this study varied greatly between 660 and 1230 g while viscosity varied between 4.53 and 6.91 cP (Table 4.1). However, the correlation between gel strength and viscosity was unexpectedly negative and relatively strong (Table 4.2), which might be due to some impurities, i.e., relatively high molecular weight non-collagen protein fractions, in the samples that increase viscosity but not gel strength. The gel strength and viscosity were found to be positively correlated previously (Zhou and Regenstein 2004; Boran and Regenstein 2009). Statistical analysis indicated that all the samples were significantly different from each other in terms of gel strength ($P<0.05$). The average viscosity of gelatin extracted from silver carp skin (SC) was the highest among the samples studied and significantly higher than that of pigskin (PS), chicken (C), bone (B), and fish skin (F1) gelatin, suggesting

Table 4.1 Comparison of some textural properties of silver carp skin gelatin extracted under optimum conditions with commercially available gelatin samples from various sources including chicken, bone, pigskin and fish skin.

	F1	C	PS	B	F2	SC
Gel Strength (g)	770±0^{a}	930±10^{b}	1230±20^{c}	1180±10^{d}	220±10^{e}	660±0^{f}
Viscosity (cP)	4.53±0.21^{c}	4.94±0.53^{c}	5.11±0.41^{bc}	4.71±0.71^{bc}	6.03±0.52^{ab}	6.91±0.33^{a}
Melting temp. (°C)	23.5±0.2^{a}	31.6±0.3^{b}	32.3±0.2^{c}	31.4±0.4^{b}	14.2±0.1^{d}	27.1±0.2^{e}
Gelling temp. (°C)	15.8±0.1^{b}	25.0±0.1^{a}	25.4±0.1^{a}	24.1±0.2^{c}	3.2±0.2^{d}	18.7±0.3^{e}
TPA-25%						
Hardness (g)	790±20^{c}	950±70^{b}	1280±30^{a}	1190±50^{a}	-----	740±70^{c}
Cohesiveness	0.88±0.02^{a}	0.94±0.01^{a}	0.94±0.05^{a}	0.95±0.03^{a}		0.93±0.02^{a}
Gumminess (g)	690±30^{c}	890±60^{b}	1210±20^{a}	1130±40^{a}		680±50^{c}
TPA-75%						
Hardness (g)	14900±1700^{c}	18100±2000^{b}	21900±400^{a}	20400±1600^{b}	-----	14300±1300^{c}
Cohesiveness	0.16±0.05^{c}	0.41±0.06^{b}	0.85±0.02^{a}	0.52±0.07^{b}		0.78±0.03^{a}
Gumminess (g)	2500±900^{a}	7700±600^{b}	18900±400^{c}	10700±1300^{d}		11300±700^{d}

F1: Fish skin gelatin-1; C: chicken gelatin; PS: pigskin gelatin; B: bone gelatin; SC: silver carp skin gelatin; F2: Fish skin gelatin-2. Different superscript letters in the same row indicate significant differences ($P<0.05$).

Table 4.2 Correlations between some of the instrumental measurements.

	GS	V	MT	GT	H25	C25	G25	H75	C75	G75
GS	1.00	-0.52	0.93	0.93	0.98	0.82	0.99	0.95	0.66	0.79
V	-0.52	1.00	-0.32	-0.37	-0.47	-0.31	-0.45	-0.46	0.16	-0.03
MT	0.93	-0.32	1.00	1.00	0.95	0.90	0.96	0.95	0.77	0.80
GT	0.93	-0.37	1.00	1.00	0.96	0.91	0.96	0.97	0.74	0.78
H25	0.98	-0.47	0.95	0.96	1.00	0.90	1.00	0.99	0.73	0.81
C25	0.82	-0.31	0.90	0.91	0.90	1.00	0.88	0.93	0.70	0.65
G25	0.99	-0.45	0.96	0.96	1.00	0.88	1.00	0.99	0.74	0.82
H75	0.95	-0.46	0.95	0.97	0.99	0.93	0.99	1.00	0.73	0.78
C75	0.66	0.16	0.77	0.74	0.73	0.70	0.74	0.73	1.00	0.95
G75	0.79	-0.03	0.80	0.78	0.81	0.65	0.82	0.78	0.95	1.00

GS: Gel strength; V: Viscosity; MT: Melting temperature; GT: Gelling temperature; H25: Hardness for 25% compression; C25: Cohesiveness for 25% compression; G25: Gumminess for 25% compression; H75: Hardness for 75% compression; C75: Cohesiveness for 75% compression; G75: Gumminess for 75% compression.

that silver carp skin might be used as an alternative raw material for production of high viscosity gelatin. However, this higher viscosity might be due to potentially milder extraction conditions being applied in the laboratory as SC was the only sample extracted in the laboratory and all other samples were commercial gelatin samples.

4.3.2 Texture Profile Analysis (TPA)

TPA measurements were done at room temperature after taking the samples out of refrigerator at 4 °C as described. Sample F2 was not included as its melting and gelling temperature was low and the gel was not retained at room temperature needed to do the TPA measurements, which were done at two different deformation levels, 25% and 75%. The measurements at 25% deformation were non-destructive for all samples tested while the measurements at 75% were destructive except for PS, confirming its high gel strength and hardness. At both deformation levels, the highest hardness was observed for the PS and B gels. The highest gumminess was also observed for PS gels at both deformation levels studied but, at 75% deformation, the second highest gumminess was observed for SC gels, pointing out its high elasticity even at large deformations (Table 4.1). Strong correlations were found between gel strength and hardness at both levels of compression, and cohesiveness at 25% compression (Table 4.2). At the 75% compression level, cohesiveness and gumminess had a weaker correlation with gel strength indicating that high compression levels cause greater variations among the gel samples due to a relatively weak viscoelastic structure that may not fracture uniformly under high compression levels.

4.3.3 Melting and Gelling Temperatures

The melting and gelling temperatures of the samples are given in Table 4.1 and the plots of delta (in angular degrees) versus temperature (°C) can be seen in

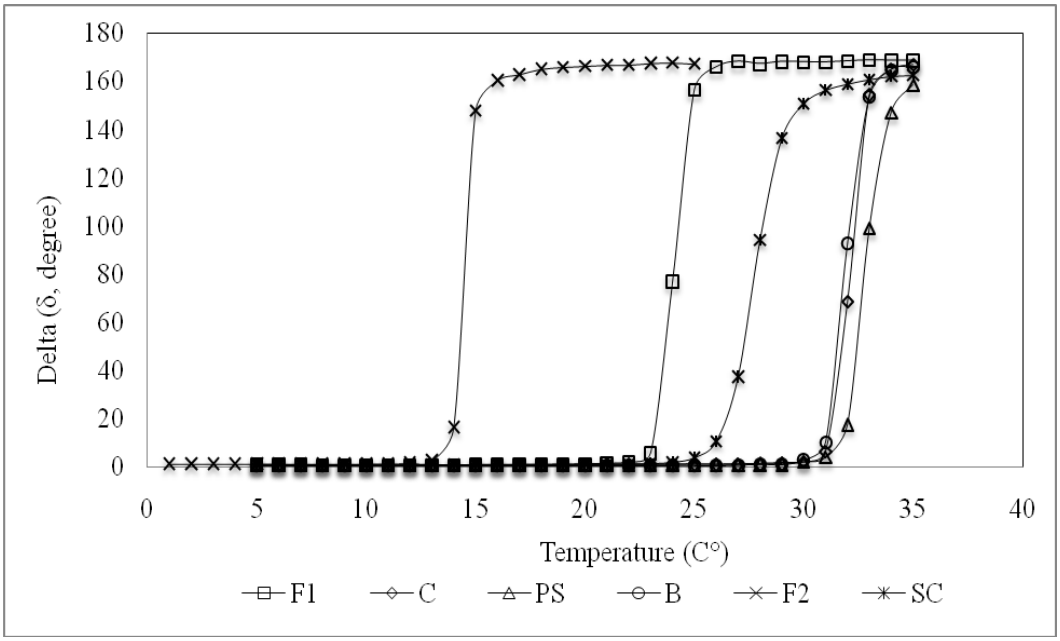

Figure 4.1 Effect of heating (delta versus temperature) on phase angle (delta, δ) for gelatin gels from various sources (temperature ramp from 1 to 25 °C for sample F2 and from 5 to 35 °C for the others at a frequency of 1 Hz and a stress of 3 Pa). F1: Fish skin gelatin-1; C: chicken gelatin; PS: pigskin gelatin; B: bone gelatin; SC: silver carp skin gelatin; F2: Fish skin gelatin-2.

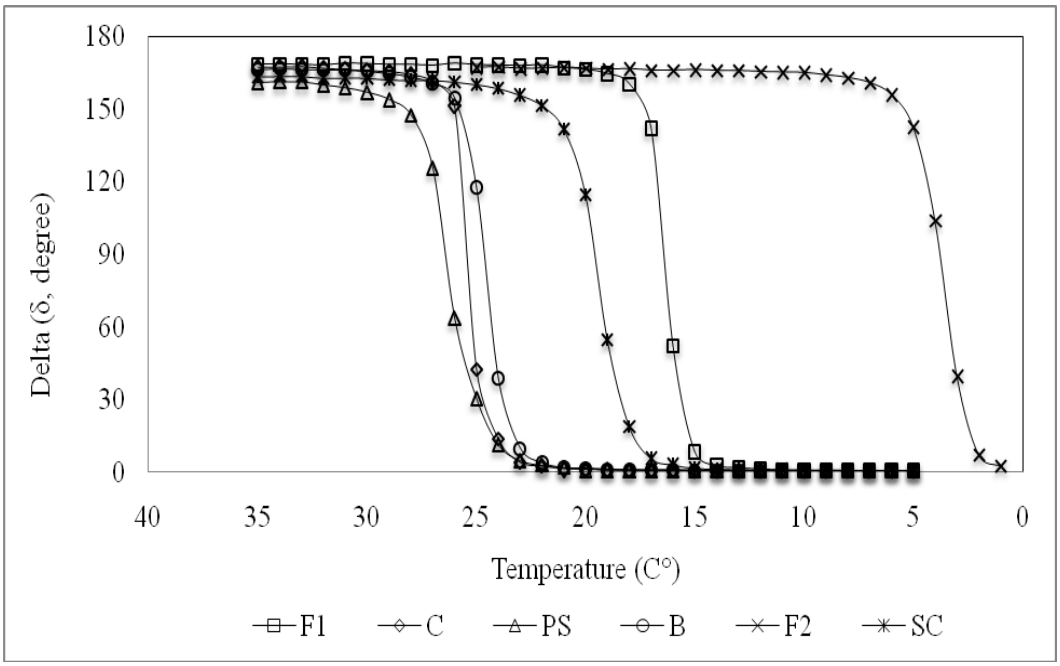

Figure 4.2 Effect of cooling (delta versus temperature) on phase angle (delta, δ) for gelatin gels from various sources (temperature ramp from 25 to 1 °C for sample F2 and from 35 to 5 °C for the others at a frequency of 1 Hz and a stress of 3 Pa). F1: Fish skin gelatin-1; C: chicken gelatin; PS: pigskin gelatin; B: bone gelatin; SC: silver carp skin gelatin; F2: Fish skin gelatin-2.

Figure 4.1 and 4.2, respectively. Melting and gelling temperature of chicken, pigskin, and bone gelatins were close to each other while those for the fish skin gelatins were significantly lower. The samples studied had a relatively sharp increase in delta as the temperature was increased, indicating a rapid transition and phase change for each sample, which suggests a relatively homogeneous molecular structure. The highest melting temperature measured was 32.3 °C for the PS gels and was a little higher than the value of 31.5 °C reported for porcine skin gelatin gels by Kasankala and others (2007). The lowest gelling temperature measured was 3.2 °C for F2, which was consistent with it having the lowest melting temperature. Melting and gelling temperature were found to have a good correlation with gel strength ($r>0.92$), the higher the gel strength the higher the melting and gelling temperatures. Commercial gelatin samples from chicken, pigskin, and bone gave the highest melting temperatures, i.e., over 30 °C and just below human body temperature while gelatin samples from fish skin gave lower melting temperatures. The difference between the melting and gelling temperatures was about 7 °C for chicken, pigskin, and bone gelatins while it was around 10 °C for the fish skin samples (Table 4.1), which might be useful for particular food applications that require a larger gap between the melting and gelling temperatures.

4.3.4 SDS-PAGE

SDS-PAGE was run for all samples and a picture of the gel is shown in Figure 4.3, where the molecular weight distribution of the collagen fractions can be seen. According to the gel; α1, α2 and β-chains were found in all samples with lower quantities in chicken gelatin. In addition, fish gelatins (F1, F2, and SC) also had lower molecular weight fractions and this was especially evident in F1 and SC. Bone gelatin probably has very low amount of low molecular weight fractions, and was mostly

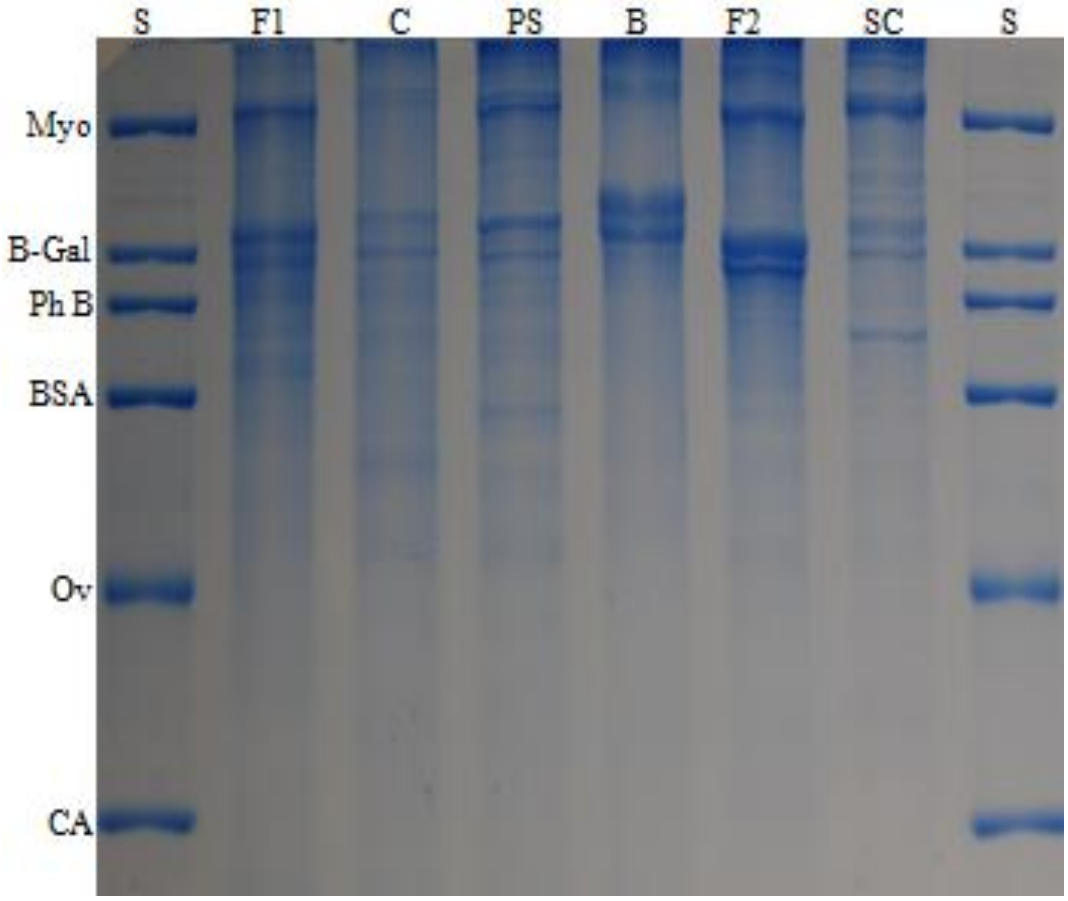

Figure 4.3 SDS-PAGE of gelatins from different sources. S: Standard; Myo: Myosin, 200 kDa; B-gal: Beta-galactosidase, 116.25 kDa; Ph b: Phosphorylase b, 97.4 kDa; BSA: Bovine serum albumin, 66.2 kDa; Ov: Ovalbumin, 45 kDa; CA: Carbonic anhydrase, 31 kDa). F1: Fish skin gelatin-1; C: chicken gelatin; PS: pigskin gelatin; B: bone gelatin; SC: silver carp skin gelatin; F2: Fish skin gelatin-2.

composed of α1, α2 and β-chains while F2 has strong bands for high molecular weight protein fractions, which is inconsistent with its low gel strength, hardness, and melting and gelling temperature. This might be due to the presence of an impurity (i.e., a high molecular weight non-collagen protein) that contributes to the high viscosity but not to gel strength. Along with F2; PS, B, and SC might also include some high molecular weight protein fractions, which might be either minimally hydrolyzed collagen molecules or high molecular weight proteins other than collagen. In addition, chicken gelatin gave the weakest bands for the collagen chains despite having the highest gel strength, melting and gelling temperatures, which suggests that SDS-PAGE alone might not be as useful as other instrumental measurements in evaluation of gelatin quality. As all samples were essentially prepared at the same concentration in terms of the weight of the dry material dissolved in distilled water, the image given might be useful to qualitatively compare the amount of α1, α2 and β-chains among the samples, which varied greatly.

4.3.5 Rheological Measurements

The time sweep tests indicated that some of the samples showed a slightly time dependent response to the applied stress, with the elastic modulus declining slightly with time (Figure 4.4). Under lower stress levels it might be difficult to see this dependency as the stress applied might not be enough to cause a similar change or reorganization in the structure. C and SC gave the highest and almost identical G' values among the samples studied. Similarly; F1, PS, and B gave almost identical G' values during the test while F2 gave the lowest G', which was in good agreement with the other rheological tests and its low gel strength. However, G' was not generally well correlated with gel strength, considering all pairs of correlations among the samples. SC, for example, gave low gel strength while showing the highest elastic

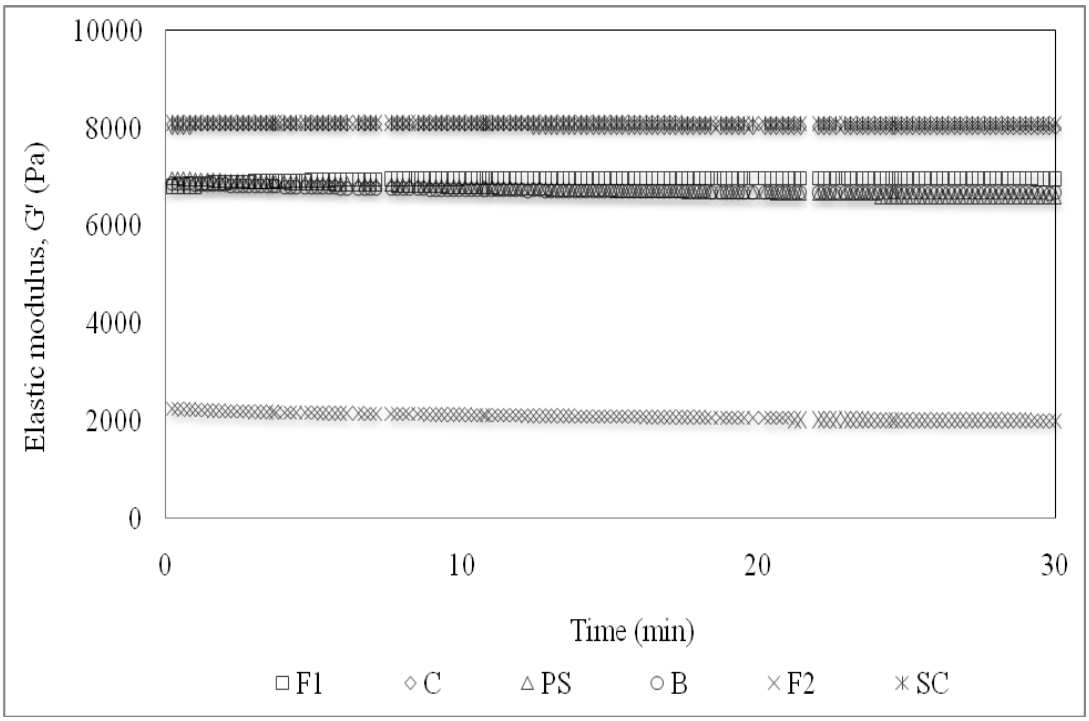

Figure 4.4 Time sweep (elastic modulus versus time) for 30 min at 5 °C, a frequency of 1 Hz and a stress of 200 Pa for gelatin gels from various sources. F1: Fish skin gelatin-1; C: chicken gelatin; PS: pigskin gelatin; B: bone gelatin; SC: silver carp skin gelatin; F2: Fish skin gelatin-2.

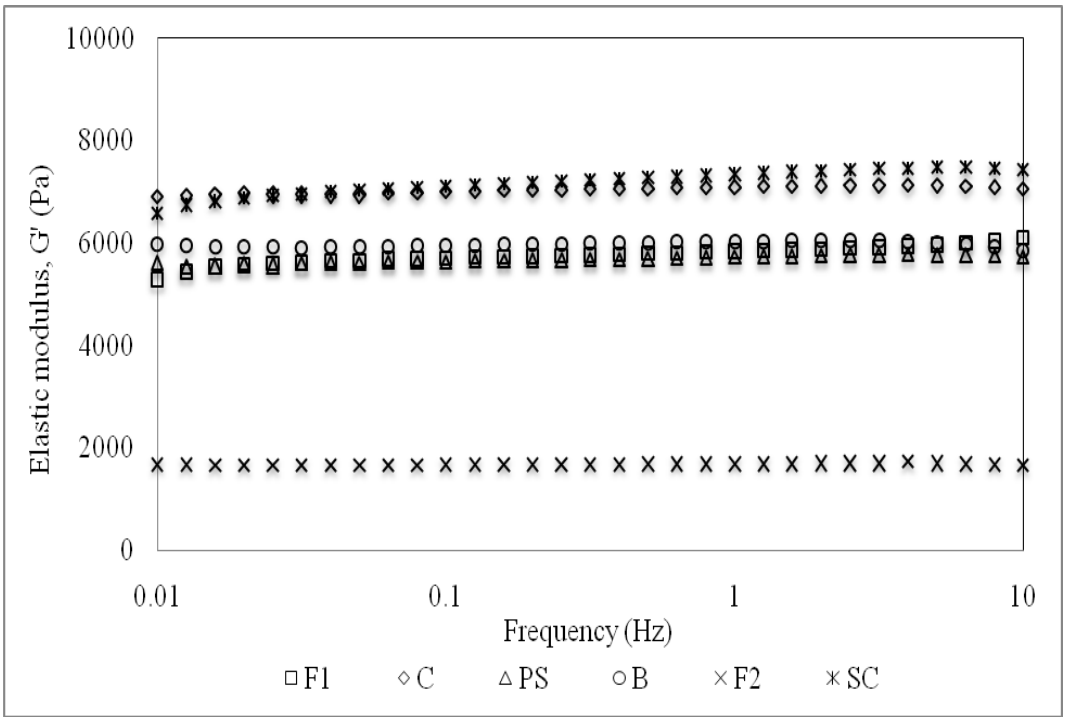

Figure 4.5 Frequency sweep (elastic modulus versus frequency) between 0.01 and 10 Hz at 5 °C and a stress of 3 Pa for gelatin gels from various sources. F1: Fish skin gelatin-1; C: chicken gelatin; PS: pigskin gelatin; B: bone gelatin; SC: silver carp skin gelatin; F2: Fish skin gelatin-2.

response for most of the rheological tests including the time sweep (Figure 4.4). The frequency sweep illustrates that frequency had almost no effect on G' (Figure 4.5), indicating that all the samples were strong gels under these test conditions. As already observed in the time sweep tests, C and SC gave the highest elastic responses but this time their G' values were almost 1000 Pa lower. Similarly; F1, PS, and B gave almost identical elastic response for the time sweep tests and F2 again had the lowest G'. Compared to the time sweep tests, the samples gave almost 1000 Pa lower elastic responses in the frequency sweep tests, probably due to the lower level of stress applied. The strain and stress sweep tests were also performed to determine the linear viscoelastic region of the gelatin gels and the results are given in Figure 4.6 and 4.7, respectively. The strain sweep tests seemed to give the best discrimination of the samples in terms of their elastic response, giving a similar order to that of time and frequency sweep tests; C and SC were the samples giving the highest elastic responses, although SC had an even higher elastic response compared to C. In addition; F1, PS, and B had similar elastic moduli that were lower than that of C and SC while F2 was the lowest among the samples. The samples had the same order for the elastic responses during the stress sweep tests; giving a higher elastic response initially at low stress levels, which was especially evident in all samples except F2. The creep and recovery tests were done for all the samples and the results are shown in Figure 4.8. After the stress was applied, most of the deformation occurred instantaneously followed by a slow creep phase. When the stress was removed, there was an instantaneous elastic response followed by a slower recovery phase. According to the results obtained, F2 had the highest instant deformation at the beginning of the test. None of the samples recovered completely indicating that none of them were ideally elastic but rather viscoelastic, i.e., exhibiting both elastic and viscous characteristics. The highest amount of viscous response was observed for F2

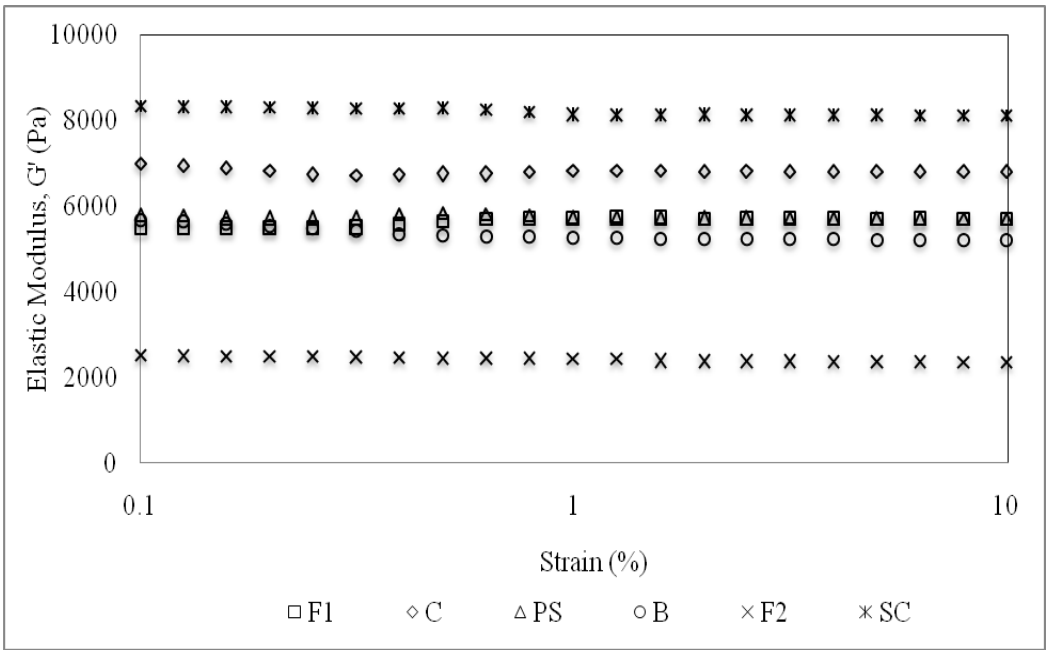

Figure 4.6 Strain sweep (elastic modulus versus strain) between 0.1 and 10% strain at 5 °C and a frequency of 1 Hz for gelatin gels from various sources. F1: Fish skin gelatin-1; C: chicken gelatin; PS: pigskin gelatin; B: bone gelatin; SC: silver carp skin gelatin; F2: Fish skin gelatin-2.

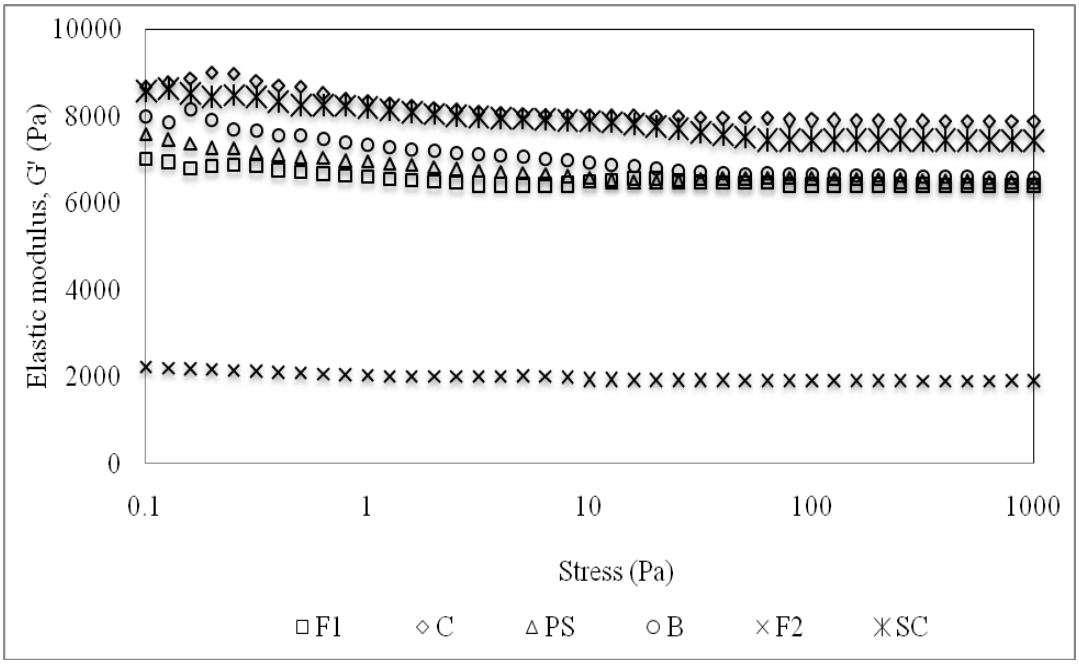

Figure 4.7 Stress sweep (elastic modulus versus stress) between 0.1 and 1000 Pa at 5 °C and a frequency of 1 Hz for gelatin gels from various sources. F1: Fish skin gelatin-1; C: chicken gelatin; PS: pigskin gelatin; B: bone gelatin; SC: silver carp skin gelatin; F2: Fish skin gelatin-2.

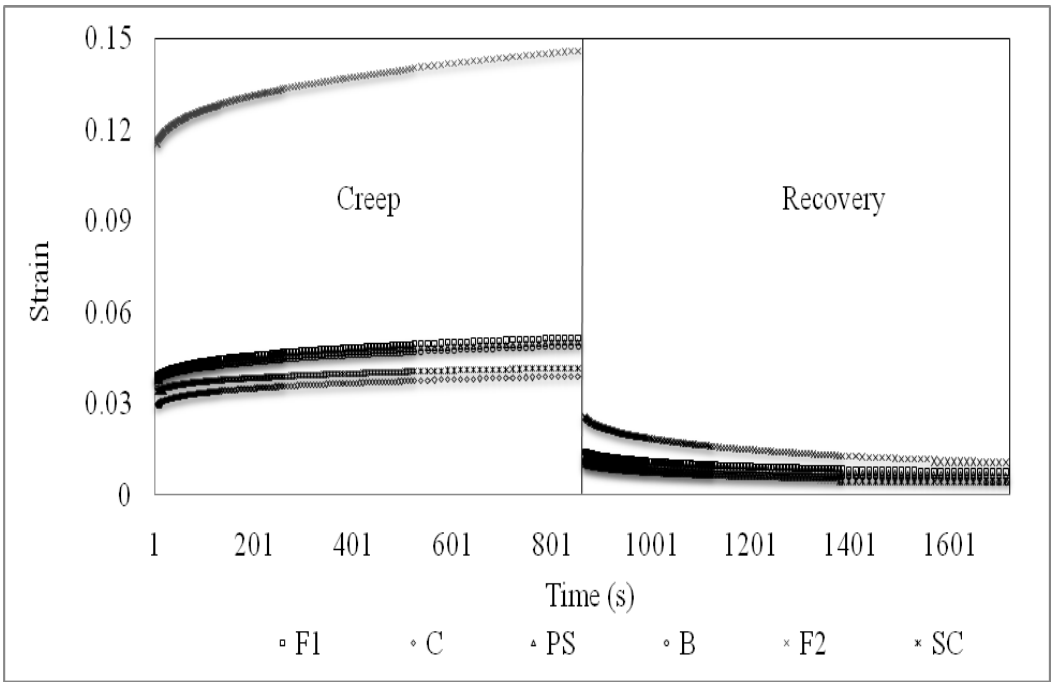

Figure 4.8 Creep-recovery (strain versus time, 15 min for creep and recovery, at 5 °C and a creep stress of 200 Pa) for gelatin gels from various sources. F1: Fish skin gelatin-1; C: chicken gelatin; PS: pigskin gelatin; B: bone gelatin; SC: silver carp skin gelatin; F2: Fish skin gelatin-2.

consistent with its relatively low gel strength and melting temperature compared to the other samples. The order of the samples during the creep cycle was completely opposite from the other rheological tests discussed above, indicating a good correlation between the sweep tests and the creep-recovery tests in the discrimination of differences in the gelatin gels. However, the strain sweep tests seemed to be better than the other tests in discriminating among the samples, suggesting that the strain sweep test might be a useful test to determine the quality of gelatin. The preliminary results indicated that the samples studied had a broad linear region for several combinations of test conditions applied (data not shown). However, G' varied significantly among the samples indicating that the viscoelastic characteristics of the samples varied widely. The gels of SC extracted under optimum conditions (Boran and Regenstein 2009) gave even higher elastic response compared to that of PS, indicating that silver carp skin gelatin might be as good as pigskin gelatin in quality.

4.4 Conclusions

The results obtained indicate that the most of the quality characteristics of silver carp skin gelatin were similar to those of chicken, bone, and pigskin gelatin so that silver carp skin might be successfully used for gelatin production to provide a substitute giving relatively high gel strength, viscosity, melting and gelling temperatures. Rheological tests might be standardized so they could be used in the gelatin industry to determine the quality aspects of the gelatin in addition to the simple traditional methods such as viscosity and gel strength measurements.

REFERENCES

Badii F, Howell NK. 2006. Fish gelatin: Structure, gelling properties and interaction with egg albumen proteins. Food Hydrocolloid. 20: 630-40.

Balian G, Bowes JH. 1977. The structure and properties of collagen. In: The science and technology of gelatin. Ward AG, Courts A, editors. New York: Academic Press. p 1-27.

Belitz HD, Grosch W, Schieberle P. 2004. Food chemistry. Third revised edition. New York: Springer. p 579-86.

Boran G, Regenstein JM. 2009. Optimization of gelatin extraction from silver carp skin. J Food Sci. (accepted).

Bourne M. 2002. Food texture and viscosity: Concept and measurement. New York: Academic Press. p 183-4.

Choi SS, Regenstein JM. 2000. Physicochemical and sensory characteristics of fish gelatin. J Food Sci. 65(2): 194-9.

Eastoe JE, Leach AA. 1977. Chemical constitution of gelatin. In: The science and technology of gelatin. Ward AG, Courts A, editors. New York: Academic Press. p 73-105.

Ergel J, Bachinger HP. 2005. Structure, stability and folding of the collagen triple helix. In: Collagen: Primer in structure, processing and assembly. Brinckmann J, Notbohm H, Muller PK, editors. New York: Springer. p 23-4.

Gilsenan PM, Ross-Murphy SB. 2001. Shear creep of gelatin gels from mammalian and piscine collagens. Int J Biol Macromol. 29: 53-61.

Kasankala LM, Xue Y, Weilong Y, Hong SD, He Q. 2007. Optimization of gelatin extraction from grass carp (*Catenopharyngodon idella*) fish skin by response surface methodology. Bioresource Technol. 98(17): 3338-43.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227: 680-5.

Nelson DL, Cox MM. 2005. Lehninger's principles of biochemistry. Fourth edition. New York: WH Freeman and Company. p 127-9.

Zhou P, Regenstein JM. 2004. Optimization of extraction conditions for pollock skin gelatin. J Food Sci. 69(5): 393-8.

CHAPTER FIVE

THE EFFECTS OF EXTRACTION CONDITIONS ON THE SENSORY AND INSTRUMENTAL CHARACTERISTICS OF FISH GELATIN GELS

Abstract

Fish skin gelatin has recently been of interest as a product that eliminates religious concerns shared by the Jewish and Muslim communities and as an alternative value-added product obtained from the waste generated by the fish processing industry. Recent work has shown that the extraction conditions greatly affect the quality of gelatin. In this study, the gelatin samples obtained from Asian silver carp skin and extracted under different conditions have been studied for their sensory properties using descriptive analysis and time intensity testing to determine how extraction conditions affect sensory properties of gelatin. Three pairs of gelatin samples were selected based on their gel strength, viscosity, and melting temperature. Some functionality measurements were also done to determine how sensory measurements correlate with instrumental measurements. The gel strength varied between 60±10 and 590±30 g while the viscosity varied between 1.9±0.0 and 7.4±0.2 cP. The hardness, melting and gelling temperature of the samples were well correlated with the gel strength ($r > 0.90$). The results indicated that the strongest correlation among all the sensory attributes was between firmness and melting temperature, which was a negative correlation (-0.75) suggesting that the firmer the gel samples the slower they melt. The viscosity was found to be very discriminative between samples in terms of sensory properties. The functional measurements were found to be strongly correlated within themselves while the sensory measurements were not, mostly due to the lack of the precision among the panelists. The firmness, melting rate, and aftertaste were those sensory attributes most successfully discriminated by the panelists.

5.1 Introduction

Gelatin is a structural animal protein, conventionally obtained from animal tissues, specifically, the skins and bones of cattle and pigs. Skins and bones are rich sources of collagen, gelatin's parent molecule. Gelatin is the collagen fractions after heat hydrolysis that exceeds an arbitrary minimum molecular weight of 30 kDa (Eastoe and Leach 1977). The molecular weight of the collagen molecule is around 330 kDa (Ergel and Bachinger 2005). Gelatin is obtained by partial hydrolysis of collagen through destruction of crosslinkages between polypeptide chains of collagen along with some breakage of polypeptide bonds (Belitz and others 2004). Gelatin has many extraordinary properties as the only hydrocolloid that is a protein (e.g., melting reversibly below human body temperature) and is widely used in the food, drug, and cosmetics industries (Nelson and Cox 2005). The chemical composition of gelatin is mainly multiple repetitions of a gly-x-y sequence, where x is often proline (pro), and y is often hydroxyproline (hyp) (Balian and Bowes 1977; Ergel and Bachinger 2005). The presence of hyp is almost exclusively unique to collagen and can be used to determine the amount of collagen or gelatin (Nelson and Cox 2005).

The lower the melting temperature the greater the flavor release, which can be important to the food industry (Choi and Regenstein 2000). In addition, the gel strength of gelatin is relatively higher than most of the common gelling agents, which are usually carbohydrates obtained from vegetable sources (Badii and Howell 2006). However, as almost all gelatin comes from pork skins or non-religiously slaughtered beef bones and hides, Jewish and Muslim communities have objections to its use in food products. However, recent studies showed that by-products such as bones, skins, and scales from chicken (having the same slaughter issues for the religious communities) and fish can be successfully used to produce gelatin and the fish gelatin, in particular, might provide great opportunities for gelatin marketing (Boran and

Regenstein 2009). In addition, the idea of using these processing by-products for value-added products has attracted substantial attention from researchers. Many fish species have been investigated as raw materials for gelatin extraction and the properties of gelatin obtained from these sources have also been examined (Boran and Regenstein 2009; Cho and others 2004; Cho and others 2005; Kasankala and others 2007; Muyonga and others 2004; Yang and others 2007; Zhou and Regenstein 2004).

The recent studies showed that the extraction conditions greatly affect the quality of the gelatin obtained (Boran and Regenstein 2009; Zhou and Regenstein 2004). Most of the research studied instrumentally determined quality characteristics of gelatin gels but only limited sensory work has been done to date as training and upkeep of a descriptive analysis sensory panel is time consuming and expensive (Lawless and Heymann 1998). A few sensory studies have been done to evaluate the quality of gelatin gels or to attempt to discriminate between gelatins from different sources (Choi and Regenstein 2000). Instrumental methods have much higher reproducibility and do not require dealing with the physiological and psychological variations associated with human subjects. However, the correlation of sensory and instrumental measurements is still important for quality control and sensory analysis is a very useful tool in the direct evaluation of food products. Furthermore, the instruments cannot always predict the human responses sufficiently. Thus, this study aimed to investigate the sensory characteristics of gelatin gels and how they correlate with instrumental measurements.

Asian silver carp (*Hypophthalmichthys molitrix* Valenciennes 1844) skin was used as the raw material for gelatin extraction under different conditions. From previous work (Boran and Regenstein 2009), three pairs of gelatin samples were selected; one pair giving different gel strengths (high (H) and low (L) but similar viscosity and melting temperature (GSH and GSL), one pair giving different viscosity

but similar gel strength and melting temperature (VSH and VSL), and one pair giving different melting temperature but similar gel strength and viscosity (MTH and MTL) from among over 40 screening and optimization samples (Boran and Regenstein 2009). In addition to the instrumental and sensory measurements, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was run to determine the molecular weight distribution of collagen fractions for all the samples.

5.2 Materials and Methods

5.2.1 Silver Carp Skin Gelatin

The frozen skins of silver carp were provided by Schafer Fisheries (Thomson, IL, U.S.A.) with overnight shipping to Ithaca, NY with frozen gel packs included. Upon arriving at the laboratory, frozen skins were immediately processed as described by Boran and Regenstein (2009). About 500 g of skin was used to obtain each gelatin sample. Skin samples were put in 500 mL Pyrex erlenmeyer flasks and treated with NaOH solution and then with HCl solution (5:1, v/w) both at varying concentrations and temperatures for varying periods of time (Table 5.1). After each alkali and acid treatment, skin samples were washed with distilled water (5:1, v/w) three times at ambient temperature and filtered through four layers of cheesecloth and squeezed dry by hand. After these treatments, water extraction was done in a waterbath (Isotemp Digital, model 205, Fisher Scientific) at varying temperatures and for varying periods of time at varying (v/w) water/skin ratios (Table 5.1). Prior to extraction, the Pyrex erlenmeyer flasks were sealed with two layers of Parafilm (Structure Probe, Inc., West Chester, PA, U.S.A.). After putting the flasks in the waterbath, 15 min was allowed to bring samples to the previously set temperature of the waterbath before starting the timing. After the extraction, gelatin solutions were treated as described by Boran and Regenstein (2009) to obtain dried gelatin sheets. All reagents were analytical grade

Table 5.1 Extraction conditions for 3 pairs of gelatin samples extracted from silver carp skin (Boran and Regenstein 2009).

	GSH	GSL	VSH	VSL	MTH	MTL
Alkali Treatment	0.55 N NaOH for 67.5 min at 24 °C	0.55 N NaOH for 67.5 min at 24 °C	1 N NaOH for 45 min at 4 °C	0.55 N NaOH for 67.5 min at 24 °C	0.55 N NaOH for 67.5 min at 24 °C	0.1 N NaOH for 45 min at 4 °C
Acid Treatment	0.1 N HCl for 45 min at 24 °C	0.1 N HCl for 90 min at 24 °C	0.1 N HCl for 45 min at 24 °C	1 N HCl for 45 min at 24 °C	1 N HCl for 45 min at 24 °C	1 N HCl for 90 min at 4 °C
Water Extraction	Extraction at 60 °C for 3 h at 4 (v/w) water/ skin	Extraction at 60 °C for 3 h at 4 (v/w) water/ skin	Extraction at 60 °C for 4 h at 4 (v/w) water/ skin	Extraction at 40 °C for 3 h at 6 (v/w) water/ skin	Extraction at 60 °C for 3 h at 6 (v/w) water/ skin	Extraction at 60 °C for 4 h at 4 (v/w) water/ skin

GSH: High gel strength sample; GSL: Low gel strength sample; VSH: High viscosity sample; VSL: Low viscosity sample; MTH: High melting temperature sample; MTL: Low melting temperature sample.

and obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.), Fisher Scientific (Pittsburgh, PA, U.S.A.), or Bio-Rad (Hercules, CA, U.S.A.).

5.2.2 Sample Preparations

For instrumental measurements, gelatin samples were dissolved in distilled water at a concentration of 6.67% (w/v) in a waterbath at 60 °C for 30 min with occasional stirring using a spatula. Fifteen mL of gelatin solutions were transferred into small screw-cap plastic jars (36 mm in dia, 16 mm in height, flat bottom) for measurements of both gel strength and texture profile analysis (TPA). Then, the caps were closed tightly and the samples were matured in a refrigerator (National Consolidated Industries, Honea Path, SC, U.S.A.) at 4 °C for 16-18 h. For rheological measurements (melting and gelling temperatures), the same plastic jars were used but this time, 2.4 mL of gelatin solutions were transferred into the jars after applying one drop of mineral oil (Walgreen Co., Deerfield, IL, U.S.A.) to the inner surface of the jars using a small brush to prevent samples from sticking and/or fracturing.

5.2.3 Gel Strength and Texture Profile Analysis

Matured samples were immediately tested for gel strength at 4 °C while they were still in the plastic jars as described by Boran and Regenstein (2009). For TPA measurements, separate gel samples were taken out of the jars gently using a spatula right before the measurements. The elapsed time between taking the samples out of the refrigerator and performing the test was less than 30 s in case of gel strength measurements and less than 2 min in case of TPA measurements (less than 1 min for 25% deformation and less than 2 min for 75% deformation). TPA and gel strength measurements were done using a TA-XT2 texture analyzer (Texture Technologies Corp., Scarsdale, NY, U.S.A.). The head penetration speed was 1 mm/s for both

measurements. The force required for the head (cylindrical plastic probe, 12.7 mm diameter, flat bottom) to penetrate 4 mm into the sample was taken as the gel strength in g. The probe used for TPA was 75 mm in diameter (flat bottom). In the case of 25% deformation, the head was set to penetrate 4 mm into the sample twice (imitation of chewing process) with a 5 s interval between the two cycles. In the case of 75% deformation, the same probe was used and penetration depth was 12 mm this time. Hardness was defined as the height of the force peak (g) on the first compression cycle, cohesiveness was defined as the ratio of the positive force areas under the first and second compressions (dimensionless), and gumminess was defined as hardness multiplied by cohesiveness (g) (Bourne 2002).

5.2.4 Viscosity

Gelatin solutions (6.67%, w/v) were prepared as described and then viscosity was measured using a calibrated Cannon Fenske routine viscometer (size 200; Cannon Instrument Co., State College, PA, U.S.A.) in a waterbath at 60 °C as described by Boran and Regenstein (2009). The density of the gelatin samples were determined by weighing five mL of gelatin samples in triplicate and the density was found to be 1.131±0.015 g/mL on average. Viscosity of the gelatin samples was calculated in centipoise (cP).

5.2.5 Rheological Measurements

Melting and gelling temperature of samples were measured using an AR-1000N rheometer (TA Instruments, Newcastle, DE, U.S.A.) with parallel plate geometry (25 mm diameter). Gel samples were prepared and matured as described. Then, the rheometer was loaded with the gel samples, which were 2 mm thick. The gel samples were glued (Loctite Super Glue, Henkel Consumer Adhesives Inc., Avon,

OH, U.S.A.) to the bottom plate of the rheometer and the top plate was crosshatched to minimize slippage. The excess sample was trimmed with a sharp knife to fit the size of plate (25 mm in diameter). The exposed outer gel surface was covered with a thin layer of mineral oil to prevent moisture loss during the measurements. Storage (elastic) modulus (G', Pa), loss (viscous) modulus (G", Pa), and delta (δ, degrees) were recorded (Rao 2007). The temperature sweep tests were performed from 5 to 35 °C and from 35 to 5 °C at a scan rate of 1 °C/min, frequency of 1 Hz and oscillating stress of 3 Pa. Melting and gelling temperatures were calculated by interpolation and taken as the cross-over point of G' and G" where tan δ becomes 1 and δ becomes 45° (Kasankala and others 2007).

5.2.6 SDS-PAGE

Gelatin samples were dissolved in distilled water at 60 °C for 30 min at an approximate concentration of 5 mg/mL. Then protein samples were resolved by a traditional Laemmli Glycine-SDS-PAGE system (Laemmli 1970) consisting of a 7% acrylamide resolving layer with a 4% acrylamide stacking/loading layer (30% solution of 37.5:1 acrylamide to bisacrylamide solution, Bio-Rad) using a Mini-Protean Tetra Cell system (Bio-Rad). All samples were diluted 2 fold in 2x sample buffer, then heated at 65 °C for 15 min and cooled rapidly on ice. A molecular weight marker offering a range from 6.5 kDa to 200 kDa (Bio-Rad) was diluted according to the manufacturer's instructions then heat treated and cooled in a similar fashion. Four uL of each sample or standard was loaded onto the gel and resolved at 120 volts for approximately 45 min. Protein bands were visualized after a 60 min wash in a fixing solution (10% glacial acetic acid, 40% methanol), a 60 min wash in a staining solution (10% glacial acetic acid, 0.025% Coomassie Blue) and three 30 min washes in a

destaining solution (10% glacial acetic acid). Images were recorded using a digital camera (Nikon D60, Nikon Inc., Melville, NY, U.S.A.).

5.2.7 Sensory Analysis

Cranberry juice cocktail (Food Club, Abingdon, VA, U.S.A.) was used to prepare gelatin gels. Gelatin samples were dissolved in the cranberry juice cocktail (Food Club) at a concentration of 6.67% (v/w) at 60 °C for 30 min with occasional stirring using a spatula (Choi and Regenstein 2000). Then, the samples (5 mL for each) were matured at 4 °C for 16-18 h in half sphere shaped 6-7 mL capacity plastic candy molds (Make'n Mold, Inc., Buffalo, NY, U.S.A.). Nine panelists (3 males and 6 females, between 20 and 60 years old) were trained in the technique of descriptive analysis at three sessions on different days. Most of the panelists were regular participants of ongoing sensory studies so they are considered experienced. Panelists were trained to analyze the appearance, aroma, flavor, texture, and residual of fruit flavored gelatin gels using a 12 cm line scale with word anchors, as well as time intensity measurements for two attributes, overall flavor intensity and sensory firmness, where they graded these two attributes for a min at 10 s intervals. All panelists signed an informed consent document, as required by the Institutional Review Board of Cornell University. Judges were also asked to complete a short questionnaire with information about their age, consumption of gelatin containing food products, and possible health problems was collected and the panelists were selected based on the information obtained. The panelists were served with two reference food samples containing gelatin and asked to write down all the sensory attributes they perceive in the order that they occurred. Then the panelists discussed any point of difference between the samples, any redundancies, and which terms to eliminate. After preparing a draft questionnaire based on the information collected, the

panelists used the draft questionnaire with two different food products on the second day. A discussion followed to rank the order of the attributes, to select the anchor terms for each attribute, and to determine if there was any term that requires a modification and then the questionnaire was modified accordingly. On the third day of training, the panel evaluated two different pairs of samples using the modified questionnaire and a discussion followed to determine if any modification was still needed and then the questionnaire was finalized to be used in the actual taste tests. Sensory attributes and anchor terms for the attributes were determined by panel consensus. The major appearance term was transparency (cloudy/clear) while texture terms included firmness (soft/firm), melting rate (slow/fast), and elasticity (low/high). Flavor and aroma terms were overall aroma intensity and overall flavor intensity (weak/strong). The residual term was aftertaste (short-weak/long-strong). Thus, 7 questions in total were included in the questionnaire. Panelists analyzed all samples in duplicate, tasting 6 samples per session per test, i.e., descriptive analysis and time intensity. Time intensity measurements of the fruit flavored gelatin samples were analyzed by placing the sample between the tongue and the palate and grading the two attributes, overall flavor intensity (weak/strong) and sensory firmness (soft/firm), simultaneously, at every 10 s intervals for one min. The panelists controlled the time using an analog clock on the computer screen, starting at the beginning of a min by putting the sample into the mouth and grading two sensory attributes every 10 s interval thereafter. The same 12 cm line scale was used for the time intensity tests including two attributes and anchor terms on the same sheet and a separate line for each attribute for each 10 s interval.

5.2.8 Statistical Analysis

Gel strength, viscosity, melting-gelling temperatures, and TPA data were compared statistically by performing ANOVA and Tukey tests to determine which samples were significantly different. Sensory and instrumental measurements were analyzed for correlations separately using JMP statistics software (Version 7; SAS Institute, Cary, NC, U.S.A.). MS Office Excel 2007 (Microsoft Corp., Redmond, WA, U.S.A.) was used to draw the relevant graphics to illustrate the results obtained. All the instrumental measurements were done in triplicate while the sensory measurements were done in duplicate.

5.3 Results and Discussion

5.3.1 Gel Strength and Texture Profile Analysis

All 3 pairs of samples extracted under different conditions (Table 5.1) were selected from screening and optimization samples based on the results (Table 5.2) obtained in a previous study (Boran and Regenstein 2009). After the samples were extracted in sufficient amounts, the instrumental measurements were carried out again and the results are summarized in Table 5.3. All 3 pairs of samples gave similar gel strength values to those previously reported for the same extraction conditions (Boran and Regenstein 2009). The pair of GSH and GSL was found to be significantly different in gel strength as expected. Their gel strength was also significantly higher compared to the other samples studied. The pair of VSH and VSL gave similar gel strength values as expected. The pair of MTH and MTL was significantly different in gel strength although this pair was supposed to have similar gel strength. However, although statistically different they were sufficiently similar so that in the bigger picture they could still be considered as giving similar gel strength and both were at the lower end of gel strength. The sample of MTH was not included in the TPA

Table 5.2 Gel strength, viscosity, and melting temperature of 3 pairs of gelatin samples selected (The data is from a previous study, Boran and Regenstein 2009).

	GSH	GSL	VSH	VSL	MTH	MTL
Gel strength (g)	600 ± 0^{a}	400 ± 10^{b}	310 ± 0^{c}	300 ± 0^{c}	90 ± 10^{d}	100 ± 10^{d}
Viscosity (cP)	4.8 ± 0^{b}	4.7 ± 0^{b}	7.0 ± 0.1^{a}	2.7 ± 0^{c}	2.2 ± 0^{d}	2.6 ± 0^{c}
Melting temperature (°C)	23.3 ± 0.1^{a}	23.0 ± 0.2^{a}	20.6 ± 0.4^{b}	20.7 ± 0.1^{b}	18.0 ± 0.6^{d}	15.3 ± 0.2^{c}

Different superscript letters in the same row indicate significant difference ($P<0.05$). GSH: High gel strength sample; GSL: Low gel strength sample; VSH: High viscosity sample; VSL: Low viscosity sample; MTH: High melting temperature sample; MTL: Low melting temperature sample.

Table 5.3 Comparison of rheological and textural properties of gels of gelatin extracted from silver carp skin under different conditions.

	GSH	GSL	VSH	VSL	MTH	MTL
Gel Strength (g)	590 ± 30^{a}	380 ± 20^{b}	310 ± 10^{c}	290 ± 10^{c}	60 ± 10^{e}	110 ± 10^{d}
Viscosity (cP)	5.1 ± 0.1^{b}	4.9 ± 0.1^{b}	7.4 ± 0.2^{a}	2.3 ± 0.1^{c}	1.9 ± 0.0^{d}	2.0 ± 0.1^{cd}
Melting temp. (°C)	24.4 ± 0.2^{c}	23.8 ± 0.2^{d}	20.5 ± 0.3^{a}	19.3 ± 0.2^{e}	17.2 ± 0.2^{f}	14.9 ± 0.1^{b}
Gelling temp. (°C)	14.4 ± 0.3^{c}	13.7 ± 0.2^{d}	10.3 ± 0.2^{a}	8.9 ± 0.1^{b}	5.8 ± 0.1^{e}	9.3 ± 0.1^{b}
TPA-25%						
Hardness (g)	640 ± 30^{c}	470 ± 40^{d}	370 ± 10^{a}	350 ± 10^{a}	---	140 ± 10^{b}
Cohesiveness	0.93 ± 0.01^{c}	0.92 ± 0.01^{c}	0.46 ± 0.02^{a}	0.38 ± 0.04^{d}		0.82 ± 0.02^{b}
Gumminess (g)	610 ± 40^{a}	420 ± 30^{b}	140 ± 20^{c}	60 ± 30^{d}		120 ± 20^{cd}
TPA-75%						
Hardness (g)	11080 ± 1820^{a}	8060 ± 1170^{b}	3480 ± 230^{c}	2160 ± 680^{cd}	---	920 ± 70^{d}
Cohesiveness	0.18 ± 0.03^{a}	0.18 ± 0.02^{a}	0.12 ± 0.02^{ab}	0.06 ± 0.03^{b}		0.10 ± 0.02^{b}
Gumminess (g)	2170 ± 210^{a}	1430 ± 180^{b}	460 ± 120^{c}	180 ± 50^{cd}		110 ± 20^{d}

Different superscript letters in the same row indicate significant difference ($P<0.05$). GSH: High gel strength sample; GSL: Low gel strength sample; VSH: High viscosity sample; VSL: Low viscosity sample; MTH: High melting temperature sample; MTL: Low melting temperature sample.

measurements as its gelling temperature was too low and the gel could not be maintained at room temperature for the TPA measurements. The measurements at 25% deformation were non-destructive for all the samples, while all measurements at 75% were destructive. The pair of GSH and GSL gave similar cohesiveness but their hardness was significantly different at both deformation levels. The GSL sample gave lower hardness and gumminess compared to that of GSH, which was strongly correlated with their gel strength. The pair of VSH and VSL gave similar TPA measurements at both compression levels although their cohesiveness and gumminess were significantly different at 25% compression. The sample of MTL gave the lowest hardness at both compression levels as expected, which was in good agreement with its low gel strength. The cohesiveness of this sample, however, was higher compared to that of VSH and VSL at 25% compression. The correlations (r) between gel strength and the hardness at both compression levels were found to be very strong among the samples, which were 0.97 and 0.93 for 25% compression and 75% compression, respectively (Table 5.4). In addition, the correlation of gel strength with melting temperature, gelling temperature, and gumminess at both compression levels were found to be strong and around 0.90. The TPA parameters varied greatly with increasing compression level. Also, the correlation between gel strength and hardness decreased with increasing compression as the variation also increased as also reported by Zhou and Regenstein (2007). The results confirmed that the extraction conditions greatly affect the quality characteristics of gelatin.

5.3.2 Viscosity

The viscosity is considered to be an important quality characteristic in the gelatin industry, therefore; it was used to determine if different viscosities caused any significant difference in the perception of the sensory attributes of food products with

Table 5.4 Correlations of the instrumental measurements.

	GS	V	MT	GT	H25	C25	G25	H75	C75	G75
GS	1.00	0.62	0.91	0.89	0.97	0.63	0.88	0.93	0.78	0.90
V	0.62	1.00	0.66	0.60	0.64	0.33	0.48	0.56	0.62	0.50
MT	0.91	0.66	1.00	0.83	0.89	0.48	0.83	0.90	0.72	0.88
GT	0.89	0.60	0.83	1.00	0.92	0.88	0.94	0.93	0.94	0.91
H25	0.97	0.64	0.89	0.92	1.00	0.69	0.85	0.90	0.82	0.87
C25	0.63	0.33	0.48	0.88	0.69	1.00	0.79	0.72	0.88	0.71
G25	0.88	0.48	0.83	0.94	0.85	0.79	1.00	0.96	0.86	0.99
H75	0.93	0.56	0.90	0.93	0.90	0.72	0.96	1.00	0.83	0.97
C75	0.78	0.62	0.72	0.94	0.82	0.88	0.86	0.83	1.00	0.84
G75	0.90	0.50	0.88	0.91	0.87	0.71	0.99	0.97	0.84	1.00

GS: Gel strength; V: Viscosity; MT: Melting temperature; GT: Gelling temperature; H25: Hardness for 25% compression; C25: Cohesiveness for 25% compression; G25: Gumminess for 25% compression; H75: Hardness for 75% compression; C75: Cohesiveness for 75% compression; G75: Gumminess for 75% compression.

gelatin. Samples GSH, GSL, and VSH had a higher viscosity, while the samples VSL, MTL, and MTH had a lower viscosity compared to those values previously determined (Table 5.2 and 5.3). The viscosity of GSH and GSL was similar and higher than that of all the other samples except VSH as expected. The viscosity of VSH and VSL was significantly different from each other while VSH had the highest viscosity among the samples studied. The pair of MTH and MTL had similar viscosities and their viscosity was low compared to other samples. The correlation of viscosity with other instrumental measurements was relatively low compared to the correlations between other instrumental measurements (Table 5.4).

5.3.3 Rheological Measurements

The melting and gelling temperatures of the samples are given in Table 5.3. The melting temperatures of GSH and GSL were a little higher than those values previously reported while the melting temperatures of the other samples were slightly lower (Table 5.2 and 5.3). The melting temperature of GSH and GSL were found to be significantly different although they were very close. Similarly, the pair of VSH and VSL was also significantly different in melting temperature although the difference was small. As rheological measurements of melting and gelling temperatures are very precise, even very small differences might be statistically significant. As the melting temperature of these two pairs was significantly different but very similar to each other within the pairs, they could still be considered similar in melting temperature. The melting temperature of MTH and MTL was also significantly different and the difference was dramatically larger compared to the differences between the other two pairs of samples. In addition, the melting temperature of MTH and MTL was the lowest among the samples studied as well as their gelling temperature. In general, there was about 10 °C difference between melting and gelling temperatures of the

samples except for MTL. For this sample, the difference between melting and gelling temperatures was about 5°C.

5.3.4 SDS-PAGE

SDS-PAGE was run for all the samples and a picture of the gel is shown in Figure 5.1 where the molecular weight distribution of the collagen fractions can be seen. According to the gel, α1, α2 and β-chains were only found in VSH, GSH, and GSL. The other samples did not show a clear band for those protein fractions. These three samples showed the highest gel strength, viscosity, hardness (at both compression levels), melting and gelling temperatures, which is in agreement with SDS-PAGE results. Samples VSL, MTL, and MTH had lower molecular weight fractions and this was especially evident in the samples of VSL and MTH. GSH and GSL both had strong bands of high molecular weight collagen fractions, which was in good agreement with the sensory results as there were few differences between these samples in terms of sensory measurements. Similarly, MTH and MTL both had a similar pattern in the SDS-PAGE, which was again in good agreement with the sensory results obtained. VSH and VSL had very different patterns in the SDS-PAGE, which was confirmed by the differences determined by the panelists, indicating that SDS-PAGE and sensory measurements were related. As all samples were essentially prepared at the same concentration in terms of the weight of the dry material dissolved in distilled water, the image given might be useful to qualitatively compare the amount of α1, α2 and β-chains among the samples, which varied greatly.

5.3.5 Sensory Analysis

The sensory results of the 3 pairs of samples are shown in Figure 5.2, 5.3, and 5.4. According to the results obtained, the only significant difference for the pair of GSH

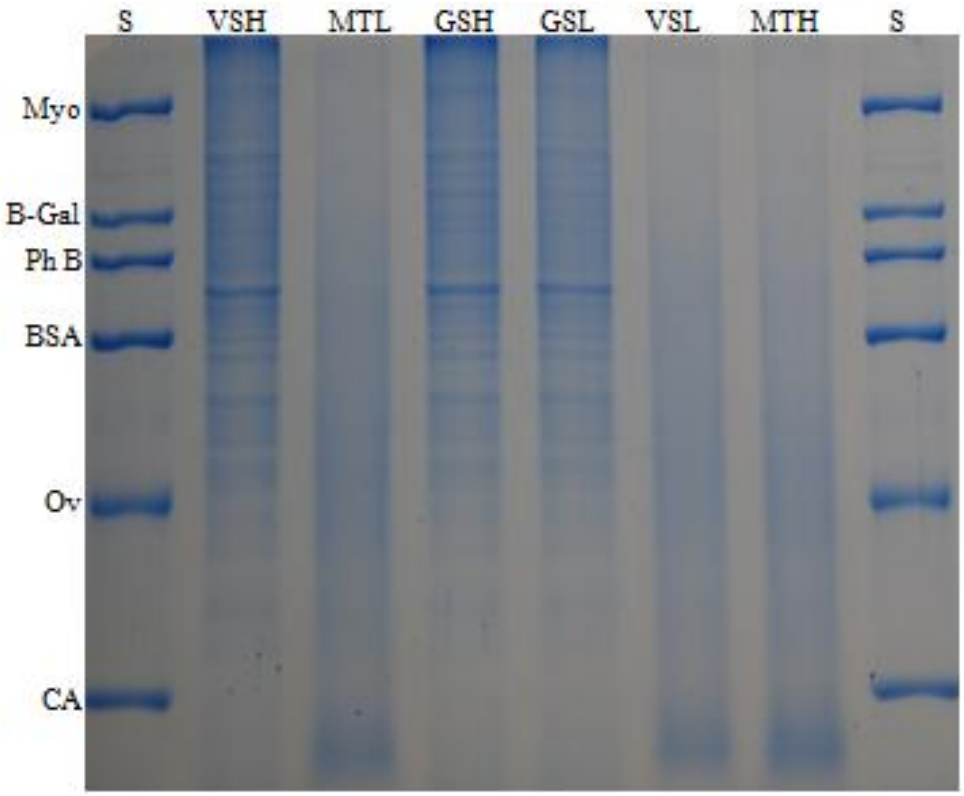

Figure 5.1 SDS-PAGE of gelatin samples extracted from silver carp skin under different conditions. S: Standard; Myo: Myosin, 200 kDa; B-gal: Beta-galactosidase, 116.25 kDa; Ph b: Phosphorylase b, 97.4 kDa; BSA: Bovine serum albumin, 66.2 kDa; Ov: Ovalbumin, 45 kDa; CA: Carbonic anhydrase, 31 kDa. GSH: High gel strength sample; GSL: Low gel strength sample; VSH: High viscosity sample; VSL: Low viscosity sample; MTH: High melting temperature sample; MTL: Low melting temperature sample.

and GSL was in transparency ($P<0.05$) while there was no significant difference in other sensory attributes for this pair and GSH was clearer compared to GSL probably due to a better structured gel network giving higher strength. On the contrary, the pair of VSH and VSL varied greatly in several sensory attributes studied including overall flavor intensity, firmness, elasticity, melting rate, and aftertaste ($P<0.05$), which was also evident in their SDS-PAGE patterns. The transparency and overall aroma intensity were not significantly different for this pair. The sensory firmness and sensory elasticity of VSH was higher than that of VSL, while VSL was higher in overall flavor intensity, aftertaste, and sensory melting rate compared to VSH, suggesting that firmness and elasticity were negatively correlated with flavor release and melting rate, which was also confirmed by the time-intensity tests. The pair of MTH and MTL was found to be significantly different in firmness alone ($P<0.05$) with a higher firmness in MTL unexpectedly as a negative correlation was found overall between sensory firmness and melting rate, i.e., the higher the firmness the slower the melting rate. Similarly, there was a positive correlation between gel strength and melting temperature. Therefore, the sample with the lower melting temperature (MTL) would have been expected to give lower firmness. The other sensory attributes were not found to be significantly different in this pair of samples.

The time intensity testing results are shown in Figure 5.5 and 5.6. All samples showed the highest firmness at the beginning of the test and then they showed gradual declines during the rest of the test. For overall flavor intensity, however, the samples varied greatly and some of them have shown a peak around 30 s while the others gave the highest values at the beginning similar to the firmness. The firmness of VSH, GSH, and GSL was found to be significantly different from that of VSL, MTL, and MTH at every 10 s intervals except at 60 s where GSL and MTL were found to be not significantly different. MTL was also found to be significantly different from VSL and

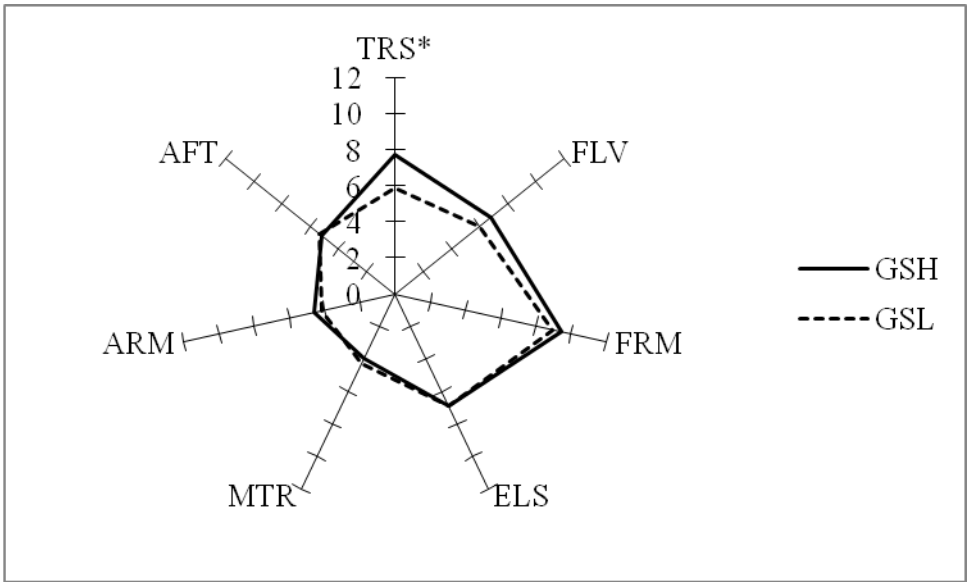

Figure 5.2 Qantitative descriptive analysis of 7 sensory attributes for samples GSH and GSL differing in gel strength ('*' indicates significant difference between two samples at a level of $P<0.05$. TRS: Transparency; FLV: Flavor; FRM: Firmness; ELS: Elasticity; MTR: Melting rate; ARM: Aroma; and AFT: Aftertaste).

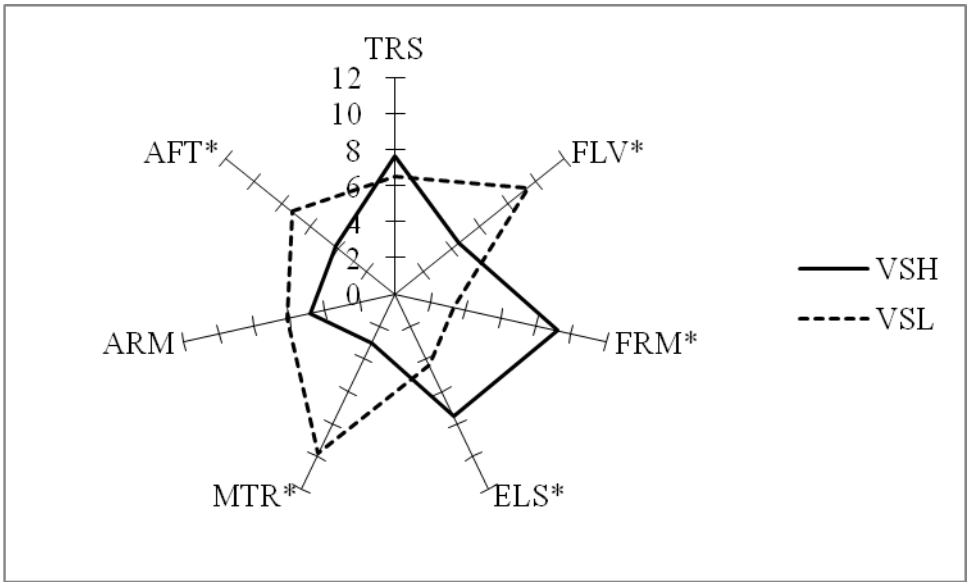

Figure 5.3 Quantitative descriptive analysis of 7 sensory attributes for samples VSH and VSL differing in viscosity (‘*’ indicates significant difference between two samples at a level of $P<0.05$. TRS: Transparency; FLV: Flavor; FRM: Firmness; ELS: Elasticity; MTR: Melting rate; ARM: Aroma; and AFT: Aftertaste).

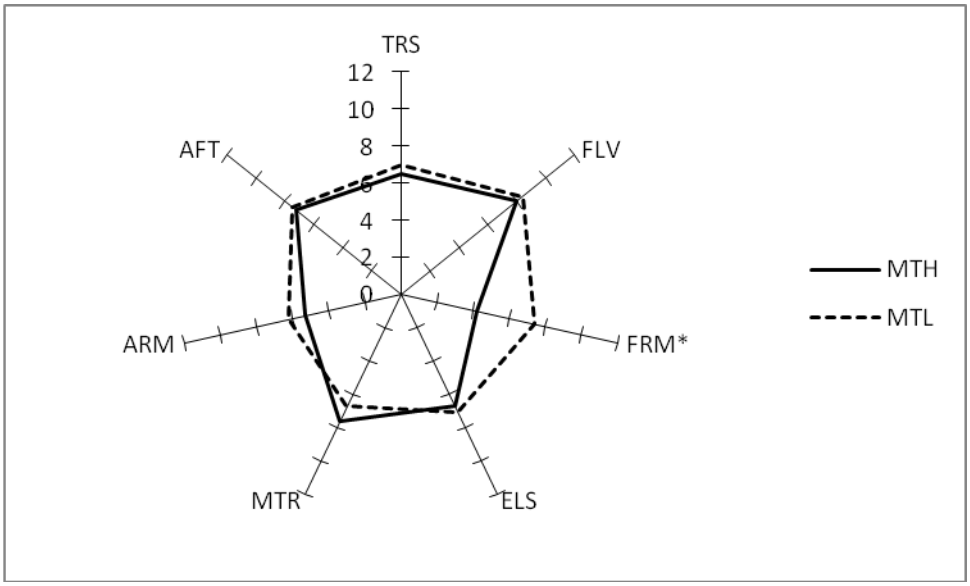

Figure 5.4 Quantitative descriptive analysis of 7 sensory attributes for samples MTH and MTL differing in melting temperature (‘*’ indicates significant difference between two samples at a level of $P<0.05$. TRS: Transparency; FLV: Flavor; FRM: Firmness; ELS: Elasticity; MTR: Melting rate; ARM: Aroma; and AFT: Aftertaste).

MTH initially for the first 20 s of the test but then there was no difference for the rest of the test. Similarly, the overall flavor intensity of VSH, GSH, and GSL was found to be significantly different from that of VSL, MTL, and MTH initially for the first 20 s but then they were found to be not different for the rest of the test. At the 20 s interval, however, GSL and MTL were not different and all the samples were the same in terms of overall flavor intensity after 20 s. The results are in good agreement with the high gel strength, viscosity, hardness, melting and gelling temperature of VSH, GSH, and GSL and indicate that these samples gave higher firmness and consequently lower initial flavor release compared to the other samples.

The correlations between sensory attributes are given in Table 5.5. The results indicate that the strongest correlation among all the sensory attributes studied was between sensory firmness and sensory melting rate. This was a negative correlation (minus 0.75) suggesting that the firmer the gel samples the slower they melt. There were also relatively strong positive correlations between flavor and aftertaste; flavor and melting rate; and aroma and aftertaste, suggesting that the melting rate plays an important role in aroma and flavor release. Compared to the instrumental measurements, the correlations between the sensory measurements were lower due to high variation among the panelists.

The results obtained suggest that using a high acid concentration in gelatin extraction might lead to higher yields but it also causes dramatically low gel strength and viscosity. The samples of VSH, GSH, and GSL were all extracted with 0.1 N HCl while the remaining samples were extracted with 1 N HCl and gave lower gel strength, viscosity, hardness, and melting and gelling temperatures (Table 5.1). On the other hand, the acid concentration is very critical in getting high yield as well as colorless and odorless gelatin; therefore, its level must be well balanced to give a sufficient yield with good functional properties (Zhou and Regenstein 2007). Among the pairs,

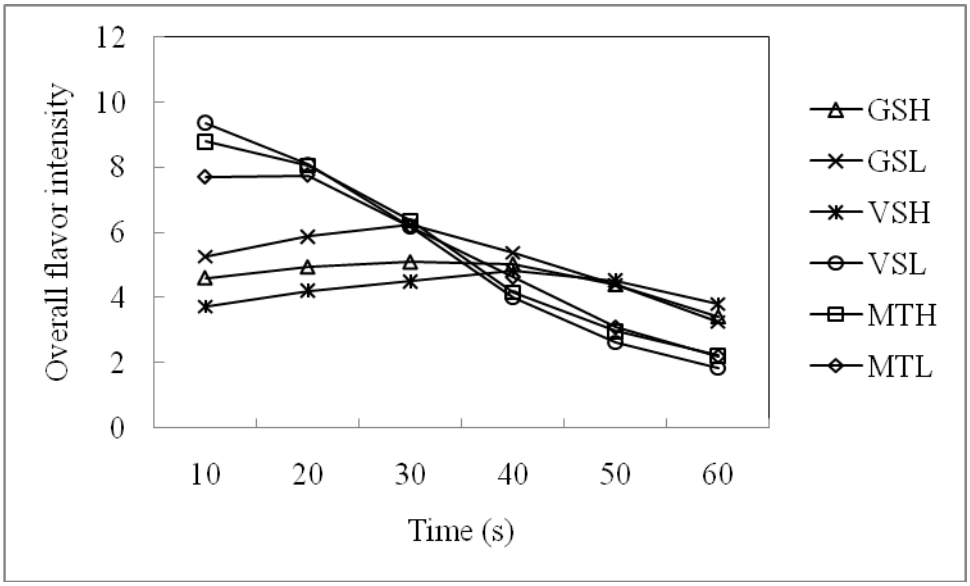

Figure 5.5 The changes of the overall flavor intensity among the samples studied. GSH: High gel strength sample; GSL: Low gel strength sample; VSH: High viscosity sample; VSL: Low viscosity sample; MTH: High melting temperature sample; MTL: Low melting temperature sample.

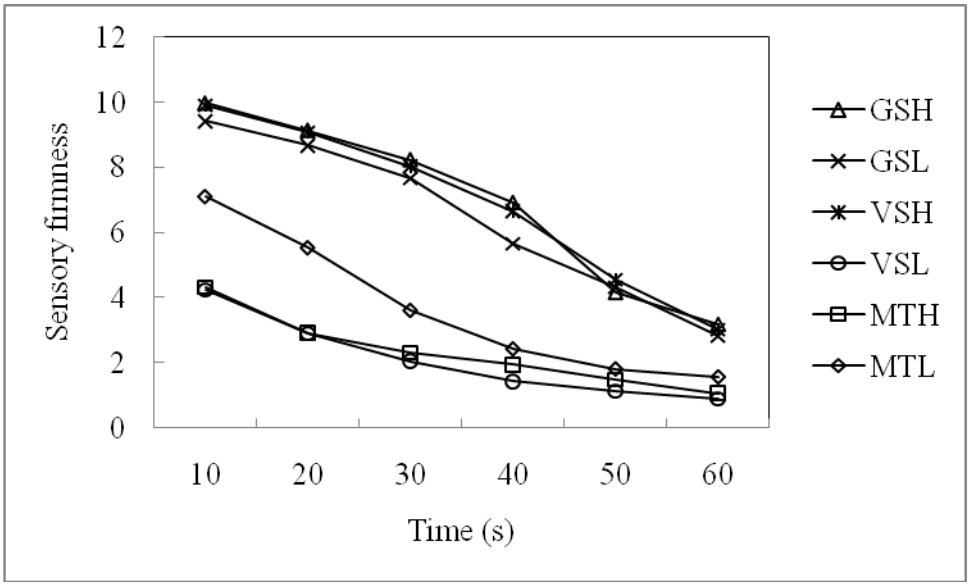

Figure 5.6 The changes of the sensory firmness among the samples studied. GSH: High gel strength sample; GSL: Low gel strength sample; VSH: High viscosity sample; VSL: Low viscosity sample; MTH: High melting temperature sample; MTL: Low melting temperature sample.

Table 5.5 Correlations of the sensory attributes studied from the quantitative descriptive analysis.

	TRS	FLV	FRM	ELS	MTR	ARM	AFT
TRS	1.00	0.20	0.10	0.08	-0.12	0.06	-0.09
FLV	0.20	1.00	-0.38	-0.10	0.47	0.36	0.50
FRM	0.10	-0.38	1.00	0.27	-0.75	-0.24	-0.30
ELS	0.08	-0.10	0.27	1.00	-0.18	-0.01	0.14
MTR	-0.12	0.47	-0.75	-0.18	1.00	0.24	0.40
ARM	0.06	0.36	-0.24	-0.01	0.24	1.00	0.46
AFT	-0.09	0.50	-0.30	0.14	0.40	0.46	1.00

TRS: Transparency; FLV: Flavor; FRM: Firmness; ELS: Elasticity; MTR: Melting rate; ARM: Aroma; and AFT: Aftertaste.

the pair of VSH and VSL gave significant differences for almost all the sensory attributes studied, suggesting that the viscosity is a very critical parameter for food products when gelatin is used as an ingredient. The other pairs that were different in gel strength and melting temperature did not give much significant difference in terms of sensory measurements partly because, the difference in gel strength and melting temperature for those pairs were not sufficiently large. The GSH and GSL having the highest gel strength among the samples clearly showed significant differences in the time intensity tests for overall flavor intensity and firmness compared to the other samples, suggesting that if the gel strength was large enough, then it would be possible to discriminate among these samples in the sensory attributes studied. Similarly, MTH and MTL were different in melting temperature; however, the difference probably was not large enough for sensorial discrimination as that is not an easy task.

5.4 Conclusions

The results obtained suggest that sensory analysis is useful in the discrimination of gelatin gels as the sensory results correlates generally well with the instrumental measurements. The extraction conditions affect the perception of sensory attributes of gelatin gels. The acid concentration is confirmed to be the one of the most important extraction parameters that needs to be optimized as it plays a very important role in quality of gelatin in several aspects.

REFERENCES

Badii F, Howell NK. 2006. Fish gelatin: Structure, gelling properties and interaction with egg albumen proteins. Food Hydrocolloid. 20: 630-40.

Balian G, Bowes JH. 1977. The structure and properties of collagen. In: The science and technology of gelatin. Ward AG, Courts A, editors. New York: Academic Press. p 1-27.

Belitz HD, Grosch W, Schieberle P. 2004. Food chemistry. Third revised edition. New York: Springer. p 579-86.

Boran G, Regenstein JM. 2009. Optimization of gelatin extraction from silver carp skin. J Food Sci (accepted).

Bourne M. 2002. Food texture and viscosity: Concept and measurement. New York: Academic Press. p 183-4.

Cho SM, Gu YS, Kim SB. 2005. Extraction optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins. Food Hydrocolloid. 19: 221-9.

Cho SM, Kwak KS, Park DC, Gu YS, Ji CI, Jang DH, Lee YB, Kim SB. 2004. Processing optimization and functional properties of gelatin form shark (*Isurus oxyrinchus*) cartilage. Food Hydrocolloid. 18: 573-9.

Choi SS, Regenstein JM. 2000. Physicochemical and sensory characteristics of fish gelatin. J Food Sci. 65(2): 194-9.

Eastoe JE, Leach AA. 1977. Chemical constitution of gelatin. In: The science and technology of gelatin. Ward AG, Courts A, editors. New York: Academic Press. p 73-105.

Ergel J, Bachinger HP. 2005. Structure, stability and folding of the collagen triple helix. In: Collagen: Primer in structure, processing and assembly. Brinckmann J, Notbohm H, Muller PK, editors. New York: Springer. p 23-4.

Kasankala LM, Xue Y, Weilong Y, Hong SD, He Q. 2007. Optimization of gelatin extraction from grass carp (*Catenopharyngodon idella*) fish skin by response surface methodology. Bioresource Technol. 98(17): 3338-43.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227: 680-5.

Lawless HT, Heymann H. 1998. Sensory evaluation of food: Principles and practices. New York: Springer. p 351-6.

Muyonga JH, Cole CGB, Duodu KG. 2004. Extraction and physico-chemical characterization of nile perch (*Lates niloticus*) skin and bone gelatin. Food Hydrocolloid. 18: 581-92.

Nelson DL, Cox MM. 2005. Lehninger's principles of biochemistry. Fourth edition. New York: WH Freeman and Company. p 127-9.

Rao MA. 2007. Rheology of fluid and semisolid foods. New York: Springer. p 104-7.

Yang H, Wang Y, Jiang M, Oh J, Herring J, Zhou P. 2007. 2-step optimization of the extraction and subsequent physical properties of channel catfish (*Ictalurus punctatus*) skin gelatin. J. Food Sci. 72(4): C188-195.

Zhou P, Regenstein JM. 2004. Optimization of extraction conditions for pollock skin gelatin. J Food Sci. 69(5): 393-8.

Zhou P, Regenstein JM. 2007. Comparison of water gel desserts from fish skin and pork gelatins using instrumental measurements. J Food Sci. 72(4): C196-201.

CHAPTER SIX

OVERALL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDIES

The results obtained suggest that silver carp skins might be successfully used in gelatin production, giving relatively high protein yields, viscosity and gel strength compared to commercially available gelatin products from different sources. In addition the hydroxyproline content of gelatin extracted from silver carp skin is found to be unusually high compared to that of gelatins obtained from other fish species. Gelatin recovery as a new parameter is proposed in place of protein yield to express the efficiency of the extraction process and other parameters including protein yield, gelatin yield, and protein recovery are defined and discriminated to hopefully prevent future confusion. The optimization study showed that the extraction temperature is one of the most significant factors affecting the quality of gelatin although other processing factors such as acid concentration and acid pretreatment time are also important.

According to the model generated, optimum extraction conditions are found to be 50 °C extraction temperature, 0.1 N the concentration of HCl, 45 min acid pretreatment time, and 4 (v/w) water/skin ratio, giving a predicted set of independent variables with gel strength of 630±74 g, viscosity of 6.3±0.8 cP, and a gelatin recovery of 80.8±8.3%. Rheological tests are successfully used to discriminate gelatin gels from different sources including pork skin, fish skin, chicken and bones, suggesting that rheological test might be standardized so they could be used in the gelatin industry to determine the quality aspects of the gelatin in addition to the simple traditional methods such as viscosity and gel strength measurements. The results obtained in the sensory study suggest that sensory analysis is useful in the discrimination of gelatin

gels as the sensory results correlates generally well with the instrumental measurements. The extraction conditions are found to be affecting the perception of sensory attributes of gelatin gels extracted under different conditions. The acid concentration is also confirmed to be the one of the most important extraction parameters that needs to be optimized as it plays a very important role in several aspects of gelatin quality.

Such optimization studies should be designed for other sources that might have a potential use in gelatin production. The results accumulated to date should be used in scaled-up projects so the more useful data might be obtained from an industry perspective. Rheological test should be further used to determine gelatin quality and a comparison of different sources in terms of the quality of the resulting gelatin as these tests provide precise data and successful discrimination. Conventional cost analysis should be done to show how gelatin extraction from by-products from the animal processing industries might create additional value for processors while increasing product variety while eliminating waste and turning it into value added products which could improve overall profitability of the enterprise.