The Influence of Processing Conditions on Extraction of Gelatines from Chicken Deboner Residues

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2. In the practical part, study the influence of selected parameters of processing of chicken deboner residue on gelatine yield.
3. Characterise prepared gelatines according to standard testing methods.
4. Work out the results using the statistical software, into tables, graphs, analyse them and compare/contrast them with the results of similar studies.
5. Evaluate the importance of the results for the practice and for further research.

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Erge, A.; Zorba, Ö. Optimization of gelatin extraction from chicken mechanically deboned meat residue using alkaline pre-treatment. *LWT-Food Sci. Technol.* 2018, *97*, 205–212.
Rafieian, F.; Keramat, J.; Kadivar, M. Optimization of gelatin extraction from chicken deboner residue using RSM method. *J. Food Sci. Technol.* 2011, *50*, 374–380.

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ABSTRAKT

V posledních desetiletích se celosvětově výrazně zvyšuje množství odpadu v potravinářském průmyslu. Potravinářské odpady lze rozdělit na dva druhy: potravinářské odpady z lidské spotřeby a vedlejší průmyslové nejedlé potravinářské produkty. Jedna kategorie vedlejších potravinářských produktů se nazývá vedlejší produkty živočišného původu. Naše výzkumná studie se zaměřuje na snížení množství těchto vedlejších produktů živočišného původu využitím dosud hodnotných částí zvířat pro další prospěšnou výrobu jako surovin pro extrakci želatiny. Během našeho výzkumu jsme optimalizovali podmínky extrakce želatiny ze zbytků kuřecích debonerů a získali fyzikálně-chemické a reologické vlastnosti želatiny. Kontrolovanými nezávislými faktory byly teplota a doba extrakce, které byly analyzovány pomocí Taguchi plánu experimentu. Výsledkem bylo zjištění, že všechny naše želatiny vykazují vysokou pevnost gelu (mezi 196 a 353 Bloom) a viskozitu (mezi 3,2 a 7,6 mPa·s), s nejvyššími hodnotami při maximální nastavené teplotě nebo maximální nastavené době extrakce. Naše výsledky naznačují, že díky vysoké gelové pevnosti a viskozitě mohou být naše želatiny dobře využity v potravinářském průmyslu jako želírující látky v želé cukrovinkách.

Klíčová slova: vedlejší produkty živočišného původu, želatina, pevnost gelu, viskozita, povrchové vlastnosti, mechanicky vykostěné zbytky kuřecího masa, vícestupňová extrakce

ABSTRACT

In the last decades the waste in the food industry has been significantly increasing worldwide. The food wastes can be divided into two types: food wastes from human consumption and industrial non-edible food by-products. One category of the food by-products is called animal by-products. The study focuses on the reduction of these animal by-products by using the still valuable animal parts for further beneficial manufacturing as raw materials of gelatine extraction. During the research we optimized the gelatine extraction conditions from mechanically chicken deboner meet residues and gained the gelatine's physicochemical and rheological characteristics. The controlled independent factors were temperature and extraction time which were analysed by Taguchi experimental design. As a result, we acquired that all of our gelatines perform high gel strength (between 196 and 353 Bloom) and viscosity (between 3.2 and 7.6 mPa·s), with the highest values at the maximum set temperature or maximum set extraction time. Our results indicate that hence of our high gel strength and viscosity our gelatines can be well utilized in the food industry as gelling agents in the jelly confectioneries.

Keywords: animal by-products, gelatine, gel strength, viscosity, surface properties, mechanically deboned chicken meat residue, multi-stage extraction

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INTRODUCTION

Two of the current biggest issues in the developed countries are the produced high amount of food waste and the increased animal husbandry, which have a terrible bad effect on the environment. Finding a sustainable solution for these two problems is crucial, both for the planet and for the people. In this thesis an alternative solution is presented for these two problems by showing a potential in the animal by-products, which mostly generated in the slaughterhouses during meat production, for further processing into high-quality and protein-rich products.

In this study we have dealed with gelatine extractions from chicken deboner residues. Gelatine is one of the most versatile biopolymers, due to its properties and wide usage at several industries. They are used in the cosmetic industry as a gelling agent in bath salts, shampoos, sunscreens, body lotions, hair spray and facial cream, in food industry as a gelling, foaming, clearing, and stabilising agent in canned meat products, in the brewing of wine and beer, in confectionery products such as fruit salads, ice cream, foam and cottage cheese. Due to its film-forming capability, gelatine can be also utilized as coating material or edible film. In the medical and pharmaceutical industry, gelatine is used as the shell of the soft gelatine and hard gelatine capsules, hydrogel, nanomicrosphere containers, nanofibers, absorbable sponge, pharmaceutical additives, matrix for intravenous infusions, injection drug delivery microspheres, implants and cell transplantation carriers. There are some newly tested utilizations of gelatine in the medical industry, namely as ink for 3D/4D-printing, tissue engineering and gelatine-based 3D scaffolds. In the photographic industry as adhesive additive to the silver salts. In addition, gelatine is applied in the forensic sciences as a gelatine-lifter in the shoe print lifting, fabric imprints and fingerprints.[1,2]

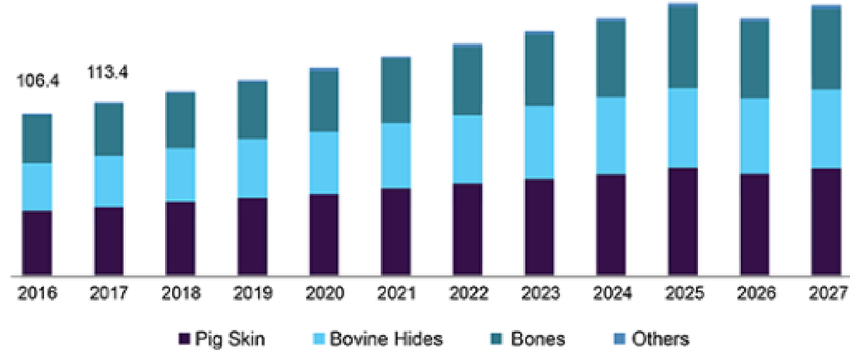


Figure 1. Gelatine demand in the US market between 2016 and 2027.[2]

Gelatine has a large-scale production worldwide, 620,6 kilotons of gelatine was processed just in 2019 and by the end of 2027 it is predicted to be expanded by 5.9%. (As a result of the COVID-19 pandemic the demand for gelatine decreased a bit, but it was just a temporary change.) For this big increasement in the gelatine processing, the beef and pork tissues will be insufficient in the future, therefore finding new suitable alternative raw materials are essential. In addition, these alternative collagen raw materials have a huge advantage, that they can fulfil the different religious intolerances (Muslim and Jewish people do not consume the pork gelatines, while Hindu believers avoid the gelatines from bovines), therefore the market of gelatine can be also extended. Instead of porcine and bovine, the fish (mostly in aquatic countries) and poultry gelatine can be used. (In some cases some other, more exotic raw materials are used as bugs, camels, salamander, frog.) Worldwide the poultry livestock, mainly chicken, is significant and their slaughter is increasing annually. (According to the FAOSTAT data from 2020, in Hungary 30,874 pieces, in Europe 2,347,505 pieces and in the world 33,097,116 pieces of chicken are in the stocks.[3]) In 2018, the chicken processing in the world was approximately 111.3 million tons, which showed a 28.4% increasement in the slaughtered chickens from 2008. Due to this increased chicken meat processing, the amount of the chicken by-products grew as well, which utilization would be a sustainable raw material for gelatine extraction.[1,2,4]

The aim of this master thesis is to optimize the gelatine extraction from enzymatically (Protamex®) pre-treated chicken deboner residues according to the earlier found effecting factors, which are the extraction time and temperature. Furthermore, the characterisation of the properties of the gained gelatines and their utilizations are also discussed.

I. THEORY

1 FOOD WASTE AND BY-PRODUCTS IN THE FOOD INDUSTRY AND IN THE HOUSEHOLDS

The food waste and by-products in the food industry has several categories. They can be selected by their material quality as solid (in Hungary in 2017 51.03%) or liquid (in Hungary in 2017 49.97%), by their origin as plant or animal, by their type as can be avoided food waste (these are the totally good products, just because of their wrong preservation or personal mood they are not consumed), potentially can be avoided food waste (this is the waste, which is not consumed cause of individual allergies, preferences and other health problems), and can not be avoided food waste (these are usually the animal by-products like bones, nails, and etc.).[5]

The international list which is accepted by the Food and Agriculture Organization of the United Nations (FAO) the food waste can be categorized into the following 16 main types[6]:

1) Dairy products
2) Fats and oils, oil-based products
3) Ice cream, sorbets etc.
4) Fruits and vegetables, including nuts and seeds
5) Confectionery
6) Cereals and cereal products
7) Bakery wares
8) Meat and meat products, including game
9) Fish and fish products, including molluscs and crustaceans
10) Eggs and egg products
11) Sweeteners, including honey
12) Salt, spices, soups etc.
13) Food stuff
14) Beverages, excluding dairy products
15) Ready to eat food
16) Composite food not possible to include in other groups

It should be also mentioned that fortunately not all of the food waste ends in a dustbin. There are several households where some of the waste are recycled by home composting or animal feeding.[5]

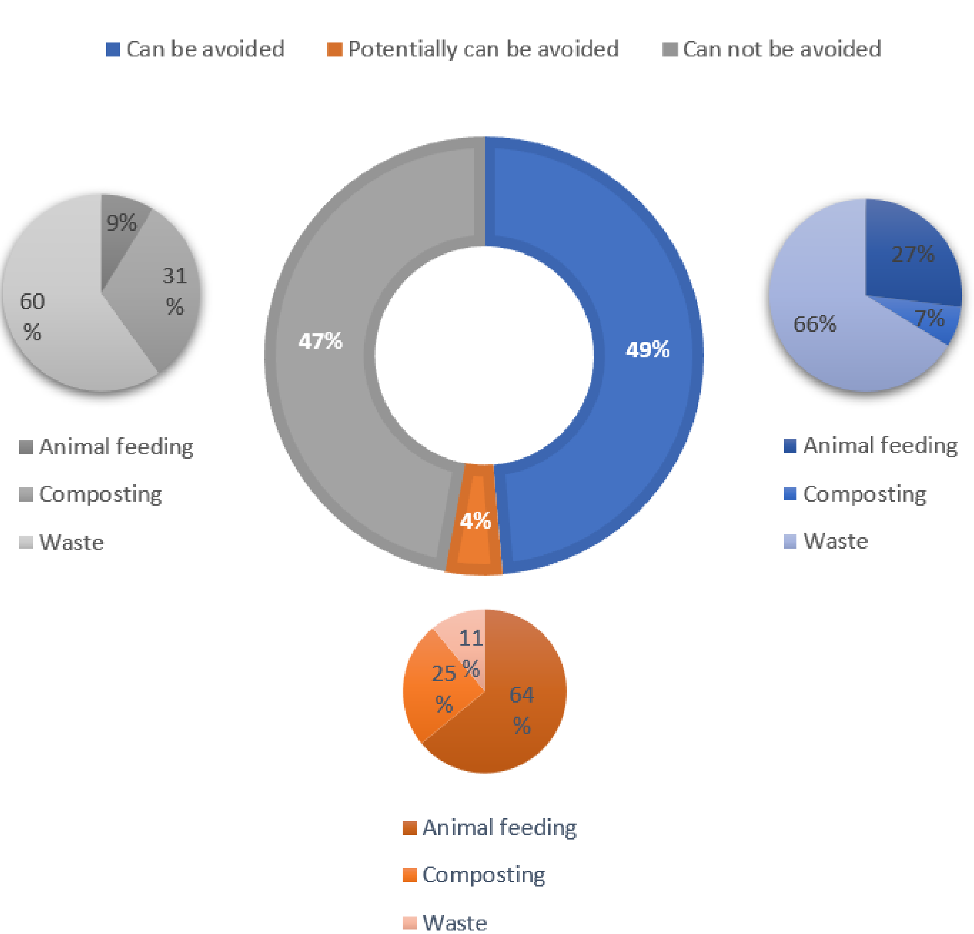


Figure 2. Percentage distribution of the type of food waste in the households and their final stage percentage distribution in Hungary (data from 2017).[5]

1.1 The food waste from human consumption

The improvement of our lifestyle, with the help of different kind of diets, better quality foods and eating habits, is prioritized nowadays. Beside the healthy lifestyle, a similarly frequently discussed topic is the sustainable agriculture. According to earlier published results in this topic, it can be said that higher quality diets often go with lower greenhouse gas emissions, eutrophication, water and cropland use.[7]

These issues are often negotiated in the context of reducing the environmental impact caused by humans. But from the globally point of view on decreasing the human's carbon footprint on this planet, the food waste also has to be considered, which is usually excluded in this type of researches/discussions. The food waste is a hidden issue, that people usually do not

care about. Therefore, in the environmentally sustainable lifestyle, the improvement of our quality diet and the reduction of our food waste should be also discussed Currently in the European Union (EU), almost one third of the food, which was grown for human consumption, ends in a dustbin like waste annually. It means around 88 million tonnes food waste yearly, which is 173 kg/capita/year waste in 2021. In contrast, the average food waste in Africa was 86 kg/capita/year, in Latin America and the Caribbean 62 kg/capita/year, in Asia and the Pacific 63 kg/capita/year, in West Asia 105 kg/capita/year, in North America 123 kg/capita/year, and in Australia 124 kg/capita/year. More detailed results can be found in Appendix I-VI.[8]

The highest amount of food waste is vegetable and fruit (39%), but high percentage of meat (14%) and dairy product (17%) are also thrown away. More detailed data on the can be avoided food waste in the EU is shown in Table 1. Another interesting data is, that in the EU approximately 45% of the total municipal solid waste is food waste, which is a huge number. At developing countries this percentage is an even higher number, reaches the 55%.[7,9]

Food or dish item	Mean (kg/person/year)	Percent (in %)
Fruits and vegetables and mixed fruit and vegetable dishes	59.8	38.9
Dairy products	26.4	17.1
Meat and mixed meat dishes	20.7	13.5
Grains and mixed grain dishes	18.5	12.1
Candy, soft drinks and other beverages	8.9	5.8
Salty snacks	5.7	3.7
Soup	4.3	2.8
Potatoes and mixed potato dishes	3.1	2.0
Nuts and seeds	2.1	1.4
Mexican dishes	2.0	1.3
Eggs and mixed egg dishes	1.0	0.7
Table oils and salad dressings	0.8	0.5
Total	153.8	100

Table 1. Can be avoided food waste percentage and kg/person/year amount in the EU.[7]

In Table 2. the Hungarian can be avoided food waste distribution is shown which was made in 2017.

Food or dish item	Mean (kg/person/year)	Percent (in %)
Ready to eat food	13.28	40.08
Bakery wares	6.5	19.63
Vegetables	3.02	9.1
Dairy products	2.91	8.79
Fruits	2.59	7.81
Beverages (coffee, syrups, juices)	1.91	5.76
Processed meat and meat-mixed products	0.75	2.25
Cans and pickles	0.7	2.12
Raw meat	0.28	0.84
Oil based dressings and other type of sauces	0.28	0.83
Grains	0.25	0.77
Nuts, seeds, dried products, cereals	0.24	0.71
Jams	0.13	0.4
Candies and salty snacks	0.09	0.28
Eggs and egg products	0.08	0.24
Fats and oil	0.06	0.18
Frozen products	0.05	0.16
Spices, salt	0.02	0.05
Total	33.14	100

Table 2. Can be avoided food waste percentage and kg/person/year amount in Hungary.[1]

1.2 Animal by-products (ABPs)

Beside the food waste, which is harvested and grown for human consumptions, there are several other kinds of waste, which are not edible for humans. In this thesis our main focus will be on this type of food waste, which one category is so-called animal by-products (ABPs). The Commission of the European Communities Regulation (EC) No. 1096/2009 defines ABPs as, whole body or parts from the body of an animal or products derived from animals which are not meant for human consumption. Among the ABPs, the following non-

edible products can be found as skin/ hide, bone, wool, digestive materials, horns, hair and edible products as blood, internal organs, connective tissues. Important to add here, that in purpose of making ABPs edible during the animal husbandry and production strict EU legislations and regulations must be followed. In the EU legislations six fields/stations of the ABPs formation are listed, where the meat is controlled[10]:

1) Production of ABPs;
2) Collection of ABPs;
3) Transportation of ABPs;
4) Storage of ABPs;
5) Usage of ABPs,
6) Disposal of ABPs.

In the PORCIÓ-ÉK Élelmiszeripari és Kereskedelmi Korlátolt Felelősségű Társaság (PORCIÓ-ÉK Kft.) slaughterhouse, which I have visited in Hungary (2730 Hungary, Albertirsa, Homokrész 1.), they separated the ABPs, according to their disposal/further utilization, into the next categories:

1) Thrown out automatically:
   the claw and the hair of the pig which are from the scalding-knocking machine;

2) The ABPs which are given to research centres and universities for researches:
   eyes, internal organs, gut and meninges;

3) Parts which are sold to another company for further processing:
   skinless and skiny pork offcuts, fat, gut fat, beef suet, the skin of the pig, colon/large intestine and the small intestine.

Worthy mentioning, that there are several companies for purchasing different ABPs, but every slaughterhouse has to make their own connections, which is very time-consuming task, therefore many slaughterhouses do not pay attention on finding partners for further procession of the ABPs and just get rid of them with the other unreusable ABPs waste.

In Table 3. the approximately ABPs amount and their percentage are listed in case of bovine, porcine and sheep.[11]

	Porcine	Bovine				Sheep
		Yearling	Steers	Cows	Bulls	
	Weight (kg) / Percentage (%)	*Weight (kg) / Percentage (%)*	*Weight (kg) / Percentage (%)*	*Weight (kg) / Percentage (%)*	*Weight (kg) / Percentage (%)*	*Weight (kg) / Percentage (%)*
Animal	60-75 / 100	200-300 / 100	300-500 / 100	300-600 / 100	400-450 / 100	55-65 / 100
ΣABPs	17.56-23.335 / 28.52-31.96	86.35-120.53 / 39.9-43.43	128.82-201.41/ 39.51-43.65	135.02-254.59/ 41.2-46.11	164.73-190.66/ 40.8-42.83	26.2-36.3 / 47.11-56.22
Hide/skin	3.6-4.5 / 6	14-21 / 7	21-35 / 7	21-42 / 7	28-32 / 7-7.1	7.5-8.5 / 13.1-13.6
Bones	3.5-5.6 / 5.8-7.5	30-42 / 14-15	40-55 / 11-13.3	40-65 / 10.8-13.3	45-52 / 11.25-11.56	4-6 / 7.3-9.2
Head	3.6-4.5 / 6	16-24 / 8	24-40 / 8	24-48 / 8	32-35 / 7.8-8	5.5-6.5 / 10
Feet	1.0-1.5 / 1.7-2	4-6 / 2	6-10 / 2	6-12 / 2	8-9 / 2	1.1-1.3 / 2
Blood (l)	3.5-3.8 / 5.1-5.8	14-16 / 5.3-7	18-25 / 5-6	18-36 / 6	24-26 / 5.8-6	1.5-1.8 / 2.7
Heart	0.18-0.245 / 0.3-0.33	1.275-1.47 / 0.49-0.64	1.2-2 / 0.4	1.8-2.4 / 0.4-0.6	1.687-2.062 / 0.42-0.46	0.3-1 / 0.55-1.5
Kidney	0.13-0.22 / 0.22-0.29	0.635-0.94 / 0.32	0.6-1.2 / 0.2-0.24	0.58-1.6 / 0.19-0.27	0.8-1.2 / 0.2-0.27	0.3-0.6 / 0.55-0.9
Liver	1.15-1.66 / 1.9-2.21	2.7-4.8 / 1.35-1.6	3.5-6.2 / 1.2-1.24	3-8.6 / 1-1.4	5.18-6.4 / 1.3-1.4	0.9-2.2 / 1.6-3.4
Lungs& Trachea	0.75-1.1 / 1.25-1.5	2.24-2.57 / 0.86-1.12	3.98-6.64 / 1.3	6-8.6 / 1.4-2	3.48-6.71 / 0.87-1.5	0.7-2 / 1.3-3.1
Tongue	0.15-0.21 / 0.25-0.28	1.5-1.75 / 0.58-0.75	1.4-1.88 / 0.38-0.47	1.38-1.49 / 0.25-0.46	1.57-1.94 / 0.4-0.43	0.5-0.6 / 0.91-0.92
Rumen& Reticulum			6.34-10.6 / 2.1-2.12	6-15.5 / 2-2.6	8.47-10.35 / 2.1-2.3	2.9-4.6 / 5.3-7.1
Omasum			1.8-4.86 / 0.6-0.97	5.12-8.7 / 1.45-1.7	4.31-5.27 / 1.1-1.2	1-1.2 / 1.8
Abomasum			1-3.03 / 0.33-0.61	2.14-4.7 / 0.71-0.78	2.23-2.73 / 0.56-0.61	

Table 3. Expected weight of co-products based on animal type and weight and their percentage distribution comapring to the total animal weight.[11]

In EU, the ABPs amount reaches the 20 million tons annually, from which an enormous quantity is generated in slaughterhouses and during other meat processing activities in the food industry.[11] Our research aim is to minimalize the volume of this type of waste by utilizing them for further high-value products. Hence, the reduction of the ABPs in the food industry, the environmental impact of the food products can be also cut. Furthermore, the meat consumption (which increased in the last 50 years to its three times[12]), which well-known to have a strong bad effect on the environment, can be also decreased by producing animal proteins from the ABPs.

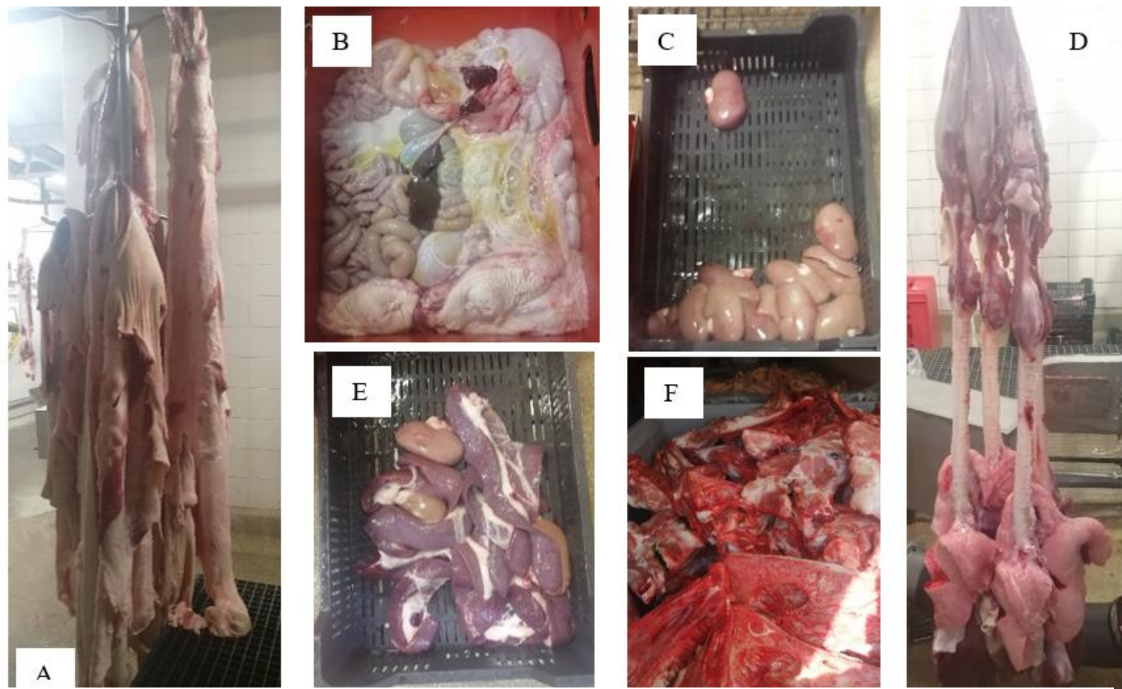


Figure 3. Eatable ABPs of the pig. **A** picture shows the cut pig skin, **B** picture shows the whole small intestinal, **C** shows the kidney, **D** shows the heart, **E** shows the spleen and **F** shows the scalp of the pig. The pictures were taken in the PORCIÓ-ÉK Kft. by myself.

1.2.1 Types of ABPs

The ABPs can be categorized into three main groups by the risk of the animal waste.
The first category of ABPs with low health risk – it means that this category of ABPs is not intended for human consumption, but can be used for organic fertilizers, petfood and animal feed – are the followings[13,14]:

- carcasses or body parts for humans to eat, at a slaughterhouse
- products or animal origin foods which are meant for human consumption but they became waste
- domestic catering waste

- shells from shellfish with soft tissue
- eggs, egg by-products, hatchery by-products and eggshells
- aquatic animals, aquatic and terrestrial invertebrates
- hides and skins from slaughterhouses
- animal hides, skins, hooves, feathers, wool, horns, and hair that had no signs of infectious disease at death
- processed animal proteins like casein and gelatine

The second category of ABPs with moderate health risk – it means these ABPs are not intended for animal consumption, but can be used as landfill or safe technical uses – are the followings[13,14]:

- animals rejected from abattoirs due to having infectious diseases
- carcasses containing residues from authorised treatments
- unhatched poultry that has died in its shell
- carcasses of animals killed for disease control purposes
- carcasses of dead livestock
- manure
- digestive tract content

And the third category with a high health risk for humans – it means these ABPs are just for disposal as for incineration and fuel for approved combustion plant – contains the followings[13,14]:

- carcasses and all body parts of animals suspected of being infected with TSE (transmissible spongiform encephalopathy)
- carcasses of wild animals suspected of being infected with a disease that humans or animals could contract
- carcasses of animals used in experiments
- parts of animals that are contaminated due to illegal treatments
- international catering waste
- carcasses and body parts from zoo and circus animals or pets
- specified risk material (body parts that pose a particular disease risk, cows' spinal cords)

Usage field of the chicken by-products	EU classification of the by-products
Livestock feed	Category 1.
Pet food	Category 1.
Aqua feed	Category 1.
Cosmetic products	Category 1.
Compost	Category 1. and 2.
Production of biogas	Category 1. and 2.
Production of thermal and electrical energy	Category 1., 2. and 3.
Production of biofuel	Category 1., 2. and 3.

Table 4. The utilising ways of chicken by-products and their corresponding categories according to the EU legislation.[15]

1.2.2 EU legislation on food products

Important to always keep in mind, that on food productions and food products there are very strict EU regulations and legislations, which should be followed. Due to these regulations, the following criteria must be kept in mind during our research[16]:

1) regulatory framework (specifically for each livestock category and food supply chain),
2) market and specific economic situation,
3) environmental issues,
4) social issues,
5) hygiene,
6) life cycle assessment.

In the EU Food Safety website, some low-value utilization of the ABPs is noted, which are the followings (only from low health risk ABPs):[13,17]

1) Animal feed – animal proteins
2) Organic fertilisers and soil improvers
3) Technical products – like, leather producing from the animals' hide, wool, blood for diagnostic tools, fuels, cosmetic products

Those ABPs, which are not able or forbidden to use like a low-value product, should be disposed. Their disposal can happen in incinerator or in co-incinerator or by sending them to authorised landfills or/and by burying them into the authorised landfills (in the EU it is forbidden).[18] But in all of these cases, the companies have to spend money on disposing the waste, which has a high cost. However, in Hungary if the low health risk ABPs waste does not exceed the 20 kg per week amount, the waste can be treated like the normal communal waste and can be sent to a regular landfill without any additional cost.[13]

1.2.3 Food safety (HACCP)

HACCP is a protocol which helps to produce food products, which are safe for the consumers. The main focus in the HACCP is on the prevention of possible chemical, physical and biological danger(s). H stands for Hazard, which means that the product is hazard to human health, A stands for Analysis which means that the product is investigated to hazard factors. CCP stands for Critical Control Points, which mean the points, where the controls on the products/processes can be applied and the prevention of hazard factors or the reduction of these effects can go below a critical value. The HACCP is very important to the Good Manufacturing Practise (GMP) which maintain to produce a good quality food product to the costumers. However, in the porcine and poultry industries, the Chain Quality Control (CQC) is also very important, where the whole process from the feeding of the animals and their conditions through the production of the meat products till the consumer's satisfaction is supervised.[19]

The food safety is important cause of the following reasons[19]:

1) to stagnate the sale of the product(s)
2) avoid and decrease the complaining of the customers – it is important also because of the financial point of view
3) increase the satisfaction of customers
4) to fulfil the increased food safety standards of the EU legislations

2 POTENTIAL OF THE ANIMAL BY-PRODUCTS CONTAINING COLLAGEN

The ABPs are categorized into edible and non-edible ABPs. Naturally these categories can differ a little bit according to countries, due to each country culinary custom.

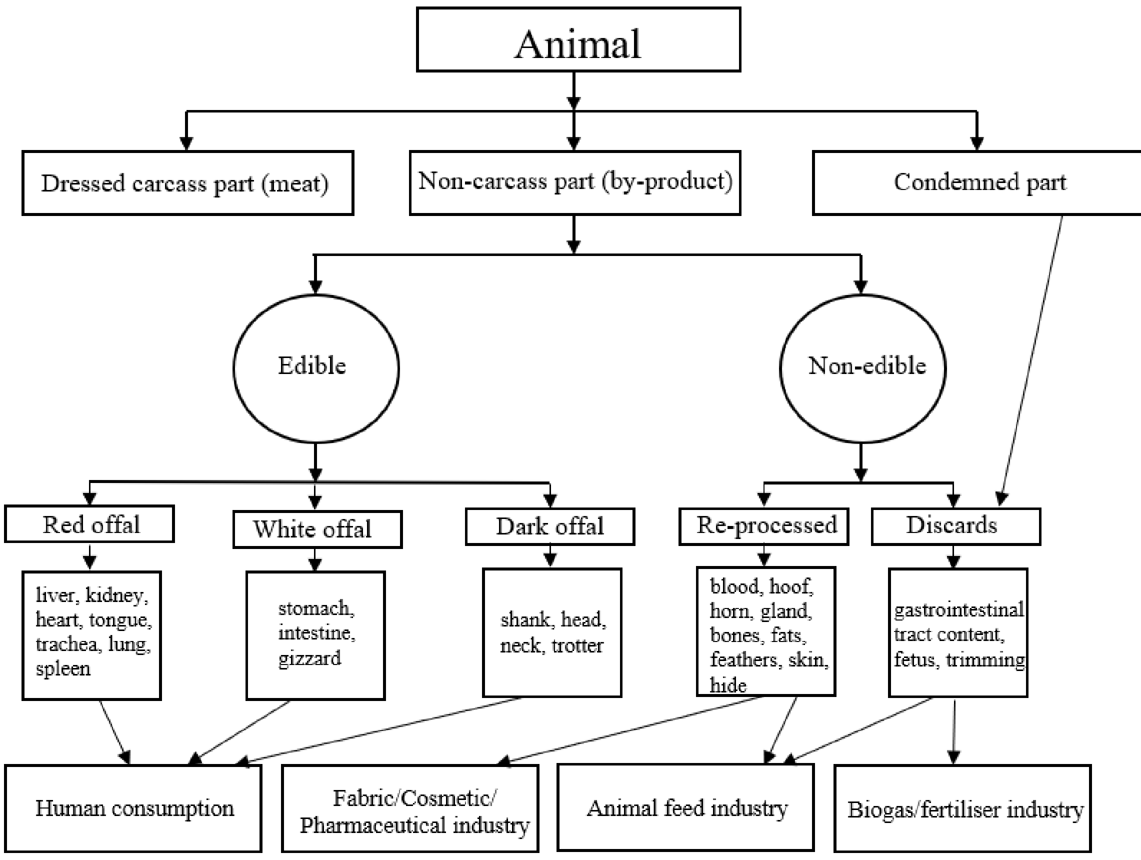


Figure 4. Classification of ABPs.[20]

2.1 Utilization of ABPs

As it was written in the earlier chapter, the reusage of the ABPs promotes the reduction of food waste and provides new potential for the production of high-quality gelatine products. Beside these reasons the porcine and bovine gelatine products are not allowed or has limited permission in Jewish and Muslim areas, while the poultry (chicken), fish, frog and insect origin gelatines can be used without complications worldwide.[21]

Animal By-Products	Reprocessed Products	Major Uses
Hides and Skin	Cured hides & skin. Leather & Textiles	Leather clothes, belts, car and household upholsteries, bags, footwear, drums, luggage, wallets, sports goods, gelatine etc.
Hoof and horns	Hoof & horn meal Gelatin and keratin extraction	Combs, buttons, plates, souvenirs, Fertilizer, Collagen, glue, gelled food products, foaming in fire extinguishers
Bone	Extraction of collagen Bone meal	Cutlery handles, Shortening, bone gelatine, bone meal, Collagen
Blood	Pharmaceutical products Blood meal	Catgut, tennis strips, blood sausages or pudding, fertilisers, animal feeds, emulsifier and stabilizer
Intestine	Sausage casings Surgical sutures Musical instruments	Sports guts, musical strings, prosthetic materials, collagen sheets, burn dressing, strings for musical instruments, sausage casings, human food, pet food, meat meal, tallow, casings
Organs & Glands	Pharmaceuticals Medicinal Xenotransplantation	Heart stimulant, heparin, corticotrophins, enzymes, steroids, oestrogen, progesterone, insulin, trypsin, parathyroid hormone
Hair/Wool	Textiles Extraction of keratin	Cloths or woven fabrics, mattress, keratin, carpets, knitted apparels, insulators

Table 5. Common usage of different animal by-products which are inedible for humans.[20]

There are several ABPs, which are reused in the different nation's culinary and handicraft industries as heart, liver, stomach, spleen, neck, blood, lung, cerebrum, tongue, gizzard, thymus, calf gland, eyes, testis, kidney and fat.[20,22,23] The utilization of ABPs varies between countries, cause the different nation's culinary highly depends on the people's acceptance and rejection. The rejection can originate from the fear of new, unknown or unfamiliar foods, which can contain toxins and pathogens. (The fear of people is much higher in case of ABPs than plant by-products cause of the bigger animal pathogenic thread.) On the other hand, the acceptance towards the new meat products can be explained by the seeking of new, healthier, higher nutritional content and curiosity. This quandary name is "omnivore's dilemma". [24] The edible ABPs contain several essential nutrients such as vitamins (B1, B2, B6, and folic acid), proteins, minerals and fat, with important poly-unsaturated fatty and amino acids.[20]

In Hungary, there are several meals which are made out of ABPs, which are not that regularly used worldwide. For example, these meals are the fried blood, kakasherepörkölt (rooster testicle stew), aspic (made out of ear, skin and claw), körömpörkölt (claw stew) and pacalpörkölt (stew made out of pig stomach).[25]

2.2 Chicken deboner residue (CDR)

The preparation of gelatine out of CDR is not a widely researched area, only few researches have been done in this topic so far. Therefore, our research is pioneer on this field. Prior to dig deeper in this topic, it should be specified what CDRs are. The CDRs are chicken parts which are derived from chicken waste by mechanical deboning operations. During this mechanical deboning operation, pressure is applied for separating the chicken meat from the slurry of ground meat and bones in a mechanical deboner. After this mechanical processing, the waste material will be the CDR which has a high content of bone, skin and connective tissues (its composition highly depends on the input raw material). Usually, the 20% of the CDR is protein, and out of this approximately 30-40% is collagen.[26]

2.3 Fish ABPs

As a result of the approximately 70% water covering of the Earth, the marine environment provides an enormous resource of sustainable natural ingredients. The processing of fish in aquaculture, generates a lot of fish-by-products (FBPs) – after filleting, the FBPs can be up to 70%, which means annually approximately 9.1 tons globally – which utilization is still unexploited despite of their high-nutritional values. The demand of these FBPs utilization is getting higher due to the fact, that it reduces the energy consumptions during product processing, cuts processing costs and moderates the environmental impacts of the new products. In contrast to the reutilization, the incinerating and discarding of the FBPs increase the energy consumption, cause extra financial costs and have significant environmental impacts (e.g. air pollution). So far, the FBPs are usually applied as additives into animal feeding or biofuels. However, their high-nutritional composition would indicate a wider application field in the nutraceutical, pharmaceutical, and cosmeceutical industries. The FBPs have great protein, hydrolysate, peptide and fatty acid compositions and in addition, their peptides have an antioxidant, antimicrobial, photo-protective and anti-aging activities. The fish collagen, which gained from FBPs, has also various applications and can be used as a precious additive in cosmeceuticals (e.g., moisturizing agent, skin regenerating agent), in functional foods, in tissue engineering (e.g., scaffold for replacing skin lost, healing skin wounds) and in anti-diabetic medications. The processes of isolation fish collagen are shown in Figure 5. The two processing methods have the same technological steps with only one difference. This difference is in the third step, where in the (A) method only 0.5 M acetic

acid is used, while in the (B) method beside the 0.5 M acetic acid also 10% $\frac{w}{v}$ pepsin is added to the system.[27,28]

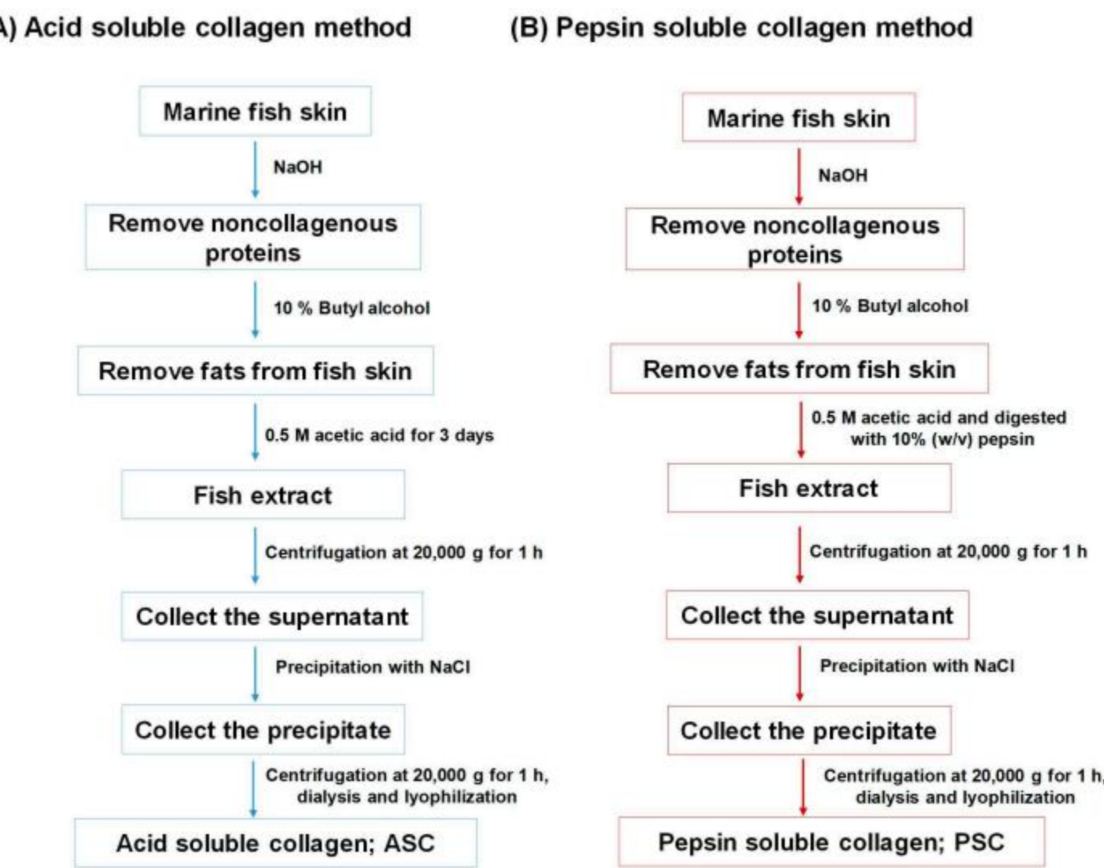


Figure 5. A flowchart about the isolation of collagen from marine fish skin. **(A)** method is the acid-soluble collagen method and **(B)** method is the pepsin-soluble collagen method.[27]

FBPs nutritional composition is very vary between the fish species, but generally it can be said that the head, intestines and bones of the fish are good lipid sources, the skin is a great protein source and the trimmings and bones are high in calcium. The most abundant fatty acids in FBPs are the oleic acids (monounsaturated ω-9 fatty acid), palmitic acids (saturated fatty acid), linoleic acids (polyunsaturated ω-6 fatty acid), and eicosenoic acids (monounsaturated ω-9 fatty acid). The most plentiful proteins in FBPs are the adenosine triphosphate (ATP) synthase subunit epsilon, mitochondrial nicotinamide adenine dinucleotide (NADH) dehydrogenase and mitochondrial cytochrome b-c1 complex subunit 8. The nutrient composition and mineral content of the fish-by-products are collected in Appendix VII.[29]

2.4 Processing of collagen from ABPs

The processing of collagen comprises sequence of technological steps like chemical, thermal, physical and mechanical techniques. The different technological treatments have effects on the properties of the nascent collagen (as solubility, physical stability, DNA content and colony forming units). In Figure 6. the collagen processing steps are highlighted with their effects on the main properties of collagen.[30]

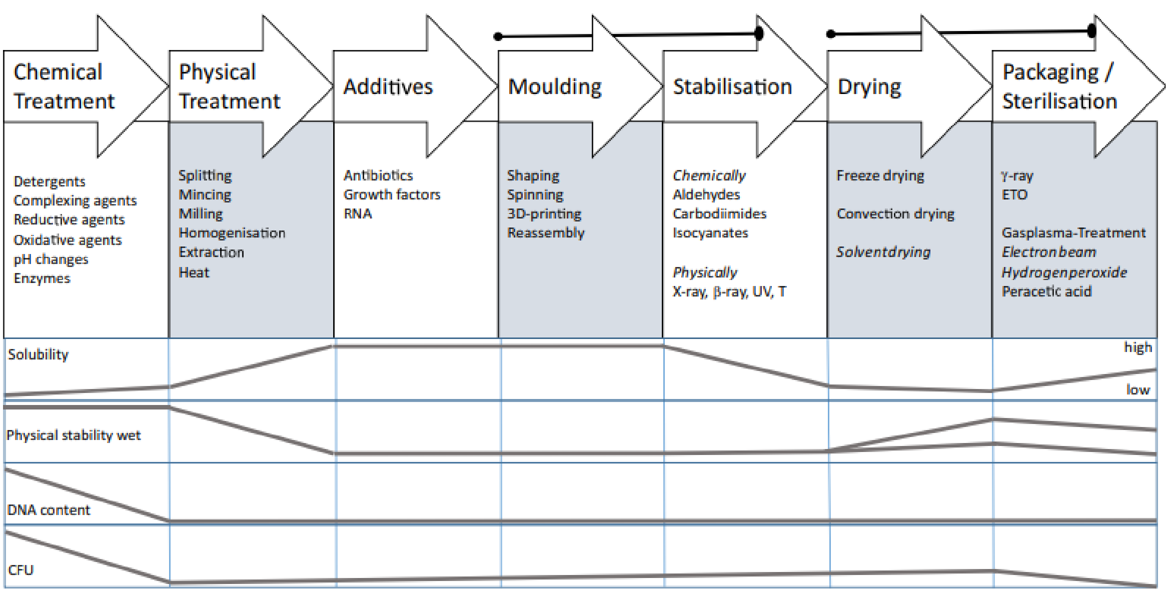


Figure 6. Collagen processing technologies, their processing steps and their effect on the parameters as solubility, physical stability, DNA content and colony forming units.[30]

2.4.1 Chemical processing

The aim of the chemical treatment in the collagen processing is to purify the collagen from bound ions, carbohydrates, globular proteins, lipids, nucleic acids and endotoxines from the tissue.

Unwanted adsorptive	Used agent	Removal mechanism
Monovalent cations	Acids (H+)	Ion exchange
Divalent cations	Acids (H+)	Ion exchange
	Chelating agents	Sequestration
Monovalent anions	Alkali, chloride	Ion exchange
Saccharides	Alkaline treatment	Degradation
	Enzymes	Degradation

Proteins	Proteases	Degradation
	Solvent	Exchange
	Surfactants (Triton X 100; CHAPS)	Exchange and dissolving
Lipophilic components	Surfactants (SDS; Triton X 100)	Dissolving
	Solvent	Dissolving
Nucleic acids	Nucleases	Degradation
Endotoxines	Peroxide, alkali, acid	Unknown

Table 6. To sum up the remnants and impurities of tissues, the used agents and the chemical reactions which are used during the purifying method.[30]

2.4.1.1 Purifying the collagen by acids and alkalis

To purify the collagen from ions, the used agents:

1) acids in the case of cations,
2) alkalis in the case of anions.

By adding alkalis to the collagen, the isoelectric point can be decreased, while by adding acid the isoelectric point is increased. The swelling of the collagen highly depends on the pH. Due to the swelling of the collagen, the molecules can be isolated from each other which can cause transparency and glassy look to it.[30]

2.4.1.2 Purifying the collagen by organic solvents and detergents

For clearing the cells from different cell components like lipids and endotoxins, the most usually used organic solvents are methanol, ethanol, acetone and tributylphosphate. The added alcohol to collagen leads to unswelling and an increasement in the denaturation temperature (DT) of the tissue. In case of flammable solvent, it is important to count on the several drawbacks it can may cause. The used solvent after the purification, should be completely removed for fire prevention reasons. Therefore, the usage of solvents are rare and mostly water-based systems are applied. But sometimes in water-based systems crosslinking reactions can occur which is also unlikely. Lipids also can be removed by detergents like sodium dodecylsulfate (SDS) or sodium dodecylbenzene (SDB).[30]

2.4.1.3 Purifying the collagen by chelating agents

With the help of the chelating agents the minerals can be extracted from the collagen. Chelating agents (for example: ethyleneaminotetraacetate (EDTA)) bind to polyvalent metal

ions (like cobalt, calcium and iron). Though, the chelating agents do not change the amino acid order or the structure of the collagen, they have to be cleared away before further processing, because during the hydroxylated treatment the chelating agents would bind the Fe^{2+} as well, which is unlikely.[30]

2.4.1.4 Purifying the collagen by enzymes

The collagen triple helical structure is very strongly resistant against degrading enzymes. In vivo experiments demonstrated that the collagen structure can be only digested by matrix metallo proteinases (MMPs) due to their pexin side, which is able to acknowledge the cleavage sites and unwind the collagen triple helical structure. In bone and cartilage, the cleavage of the collagen can be also done by cathepsin K. In comparison with the non-collagen specified proteases, cathepsin K and MMPs can cause isolated triple helical molecules in the telopeptide part of the collagen. They can be used also to boost the yield during soluble collagen production. Beside the proteases, nucleases are used to remove the remnants of DNA and RNA from the collagen and lipases are used to cleave ester bonds of triglycerides and cholesterolesters. After the utilization of enzymes, it is always crucial to completely remove them from the sample.[30]

2.4.2 Thermal processing

This processing technique is used on fibrous collagen materials, which can not be transferred into a powder with spherical particles form, only into a wadding-like material. In case of processing a thermoplastic collagen (TC), firstly the skin/hide should be unhaired and decellularized. The unhairing treatment of the hide/skin starts by the hide/skin soaking and liming with $Ca(OH)_2$, and sodium sulfide. Then the resulting pelt is delimed with $(NH_4)_2SO_4$ and the pH is set to 8.5. The final pH must be between 6.5 and 7.5, which is reached by formic acid neutralization. The last preparation step before denaturation is the bleeching of the material with 0.5% H_2O_2. After the preparation of the hide/skin, the partly thermal denaturation of the collagen can be done in three different ways[31]:

A) The first way of the denaturation is done by heating in excess water. In this case the TC is heated in an 80°C hot water for 20 minutes. After the heating process, the wet material is placed into a frame and let it dry in air under ambient conditions for 48 hours.
B) Second way is done by extrusion at hot temperature (115°C).

C) The last way is done by microwave treatment. In this case, the material is exposed to microwave radiation and then the hide/skin is dried in hot oven.

Due to these treatments, the triple helix of the collagen is partly denatured, which destroys the regular fibrous form of it, and allows to be formatted in a thermoplastic machine. After the curing, the TC is dried and extruded into a powder form, which shows some of the properties of the original collagen, like gel-forming attitude in water, swelling water and the degradability by proteases. On the other hand, it loses some of its original properties, like solubility in hot water.[30] After getting the powder form of TC, the thermoplastic processing can be initiated. The first step is mixing the TC powder with different amount of water or glycerol (if it is needed) and then homogenized it by laboratory mixer for getting a proper TC-mixture. On the following day of the preparation step the extrusion can be done by twin-screw extruder. For the production of TC blowing film, a ring-shaped die should be used.[31]

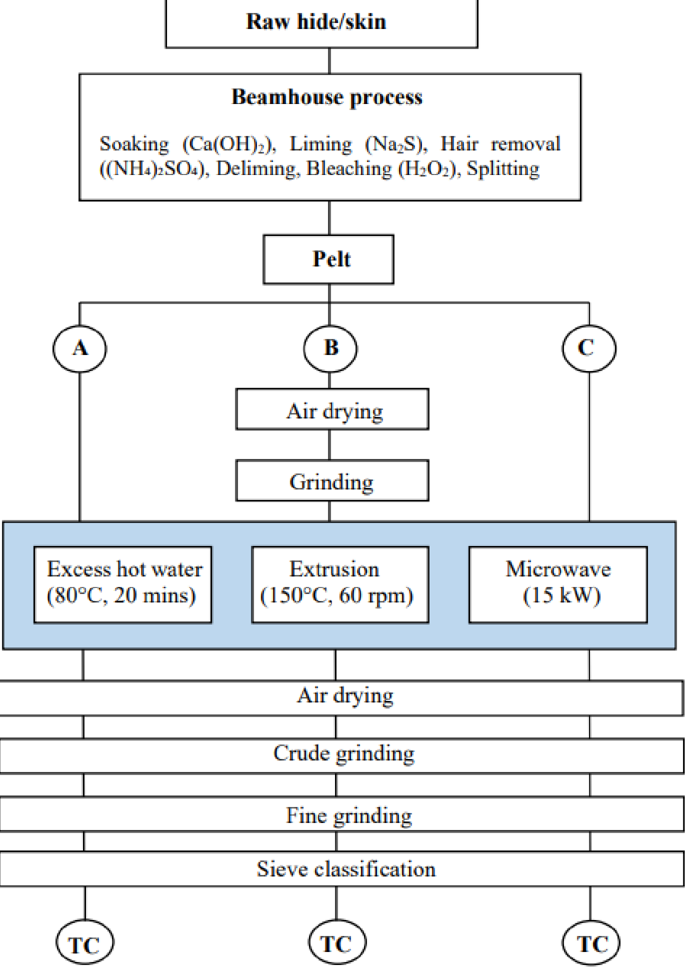


Figure 7. Production of the thermoplastic collagen (TC) with the different denaturation methods.[30]

2.4.3 Mechanical processing

In order to purify the hide/skin, mechanical processes are also applied. Fleshing and splitting are treatments for getting rid of the unwanted parts of the hide/skin. Splitting is also used to maintain a constant thickness of the hide/skin, which can be important during further processes. Splitting and fleshing can be performed in both manually and automatic way. Flashing is made by a rotating knife cylinder, while splitting is by a rotating band knife.

In case of the preparation of fibrous collagen suspension out of collagenous tissues, the key mechanical steps are the following: mincing, milling and homogenizing. During mincing the starting material is chopped into small pieces, then in the milling step a top-down manufacturing technique is executed, while in the homogenizing step the homogenization of the suspension happens. The homogenization can be done in two different ways: wet and dry process. In the wet process the homogenization is gained with colloid mills, whilst in the dry process it is achieved by using series of punched discs at high pressure (several bars).

In contrast with the thermal treatment, during the mechanical process the aim is to save the triple helical structure of the collagen. Therefore, during the mechanical treatment the prevention of heat (climb above the swelling temperature of the collagenous tissue) at any production step is crucial and very important.[30]

2.4.4 Physical processing

This processing technique covers the extraction, temperature treatment and radiation effects.[30]

2.4.4.1 Extraction

Collagen appears in several tissues of the animals, but there are two types of it. There is soluble and insoluble collagen. During extraction only the soluble collagen can be extracted from the collagenous tissues by added organic acids. The employed organic acid can be weak or strong acid also, but in case of strong acids, the yield of the collagen is higher. The solubility of the collagen highly depends on the age, type and part of the animal. In younger animals the extraction is easier than in older animals. Similarly, from the calf hide the extraction is better than from pig skin, but the best raw material for extraction is the fish skin.

To increase the obtained collagen amount, there are several methods how to solubilize the insoluble collagens as well. The most often applied two methods are:

1) deamidation, which is a chemical modification,

2) pepsin treatment, usually thiols and cysteamine are used for this purpose.[30,32]

2.4.4.2 Temperature treatment

Temperature treatment of the collagen is performed when the collagen is exposed to different temperatures. The different temperatures can be the followings:

1) lower than freezing temperature of water or the buffer,

(2) between melting and DT,

(3) above DT.

When the water/dry matter is above $35\frac{m}{m}\%$, then the water bounds to the collagen and decrease its freezing temperature below -60°C. It means in this case, the freezing of the collagen does not have a strong effect on the collagen's mechanical behaviour. During the freeze-drying method it should be kept in mind that freezing can cause unrepairable damages on the cells by the grown ice crystals (e.g., cell membrane disruption).

The best temperature range is between the melting and the DT. It keeps the triple helical structure of the collagen saved, and it makes easy to process the collagen. The earlier mentioned mechanical processes are always made in this temperature window.

Above the DT the triple helical structure is opened/ruptured. Due to the rupture of the triple helical structure, the soluble collagens are fallen into single proteins, which take their most preferable position, the coiled structure. The denatured collagen is highly sensitive to mechanical effects and enzymatic reactions. However, during the production of TC these advantages are used.[30]

2.4.4.3 Radiation effects

In this section the γ-, β-, and UV radiations effects are monitored. These radiations are propagating the number of physical crosslinks in the collagen, but they can also cause chain scissions in the collagen backbone.[30] The different effects only depend on the condition of the sample. If the sample is wet the physical crosslinks are more often observed, while the sample is dry the chain scissions occur more frequently.[33] The presence of oxygen radicals during γ-and β-irradiation accelerates the formation of superoxide radicals, which preferably cause chain scissions in the collagen backbone. While in the absence of oxygen, hydroxyl radicals occur, which evoke the polymerization of the soluble collagen.[30]

3 GELATIN TESTING AND ITS APPLICATIONS

Gelatine is a water-soluble, odourless, transparent and high molecular weight polypeptide, derived from collagen by partial hydrolysis. Collagen is a natural structural protein, which is mostly found in the connective tissues of animals/humans. Approximately the 30% of the total animal's proteins is collagen – this protein is the most abundant one in the animal body. The gelatine's parent molecule is collagen, which explains the similarity between their chemical composition. However, the gelatine is composed of the combination of many collagen fractions and peptide chains. The fractions are diverse in size and weight, which mainly responsible of the low melting temperature (below 35 °C) of the gelatines. Although the collagens are indigestible for humans, the partly hydrolysed collagen – gelatine – has a better digestibility. The best digestibility belongs to collagen hydrolysates, but on the other hand these collagen hydrolysates unfortunately do not have the same gelling/adhesive properties like the gelatine.[34,35,36]

	Digestibility	**Solubility in water**	**Main property**	**Form**
Natural collagen	Indigestible	Insoluble	Medical material, collagen casings	
Gelatine	Slow, partly digestible	Soluble just in warm water – medium solubility	Adhesive, gelling	
Collagen hydrolysate	Digestible	Soluble	Functional food	

Table 7. Comparison of natural collagen, gelatine and collagen hydrolysate according to their digestibility, solubility in water and main property.[36]

The gelatine's adhesive properties have already used from the ancient times like a natural glue. In addition, its medical effects known from the Middle Ages. In written sources from the 12th century, a soup recipe was found, which was made from cattle's feet and it was recommended to the treatment of joint pains.[34] However, the discovery of gelatine is connected to a French scientist, Denis Papin (b. 1647, d. 1712), in 1682.[37] The name of

gelatine originates from the Latin '*gelatus'*, which means firm or frozen. The usage of this name dates back to the beginning of the 18th century.[34]

The raw material of gelatine is mostly pigskin, cattle hide and bones. Nowadays because of the increasing awareness of animal's rights, religious concerns, sustainable aspects and increasing demands, the fish, poultry, insects and bugs as alternative raw materials for gelatine, are getting more and more popular. Beside the earlier mentioned reasons, the fish gelatine is also preferred due to its wide range of melting and gelling temperature while it still have a high gel strength (GS) and viscosity. However, in my thesis I will focus on the gelatines' properties and the processing conditions, which are obtained from CDRs. Additionally, relied on previous literatures and other fellow colleagues work at Tomas Bata University, I will highlight and contrast the different properties of different type of gelatines.[34,38]

3.1 Preparation of the gelatine

The raw materials for producing gelatines are usually from the slaughterhouses, where the leftover of the meat production is frozen at –15 °C to preserve them till they can go to the gelatine producing factories. In the factories, firstly the raw materials should be unfrozen, cleaned, degreased, dried, sorted and chopped/cut into smaller pieces, approximately 0.5-3 mm diameter pieces. The chopping is followed by rinsing the small pieces in water to decrease their fat content. After the washing step, acid or alkaline or heat-pressure treatment is applied to release the collagen from the raw material (breaking up crosslinks) and demineralize it.

In case of A type gelatines, the raw material, which area mostly pigskin, is treated in acidic environment, around pH 1.5-2 for 18-30 hours. In term of alkaline production method, which is called as B type gelatines, the raw materials, which are mostly cattle hides and bones, are treated in alkaline environment, around pH 12 for several weeks or months. In case of alternative raw materials, a similar treatment to A treatment is applied. The chopped raw fish/poultry materials are placed into acidic environment, around pH 4 for 12-48 hours or in case of fishbone for 9-12 days, at low temperature (5-10°C) or room temperature. The third type of treatment is heat-pressure treatment. In this treatment the raw material, which is usually ossein/bone, is placed into a high-pressure tank with boiled water for around 5 hours.[39]

From the listed raw material treatments, the most widely used ones are the acid and alkaline treatments due to their higher quality of gelatine. Important to mention, that the quality of high-pressure treatment gelatine is much lower than in the other treatments but the preparation time in this case is much shorter and in this treatment there is no need of chemicals during the production.[40]

After the acid (A type) or alkaline (B type) treatments, most of the crosslinks within the collagen are cleaved. (The treatments should be executed carefully, to not treat the raw material with too much alkalis or acids because then the gelatine can be extracted even in cold water which is against of the production desire.) Following the treatments, the gelatine can be “melt out” into the hot water. This gelatine extraction takes place in an extraction tank, which can be three types: batch process with stirrers, continuous counter-current process and semi-continuous process with circulation. The extraction is made in several steps (3-6 typically) and in each steps the temperature of the water is increased (the beginning temperature is around 50-60°C and the subsequent extractions are made with 5-10°C temperature increasement).

Afterwards, the filtration step comes to separate the divided mixture. The top layer of the mixture is fat, which can be further processed in other factories to soap and biofuel. The middle layer is the aqueous gelatine layer, which consists of gelatine, water and the leftover of minerals. The bottom layer is the undissolved solid layer of the raw material.

Then the gelatine solution must be deionized. In spite of the several washing steps, usually the gelatine solution still consists of 2,5%-4% wt of mineral salts inside. The level of ash (mineral salt) is acceptable in food, at 2% wt, and in pharmaceutical applications at 3% wt. In contrast of it, the photographic gelatines almost have to be “mineral salt-free gelatines”. This purification step can be made in two different ways:

1) the diluted gelatine solution is poured into an ion exchanger where anions and cations are removed;
2) by nanofiltration.

After the deionization, the gelatine solution must be filtrated again. The next step is to reach the final concentration of the gelatine solution, which means that the maximum water concentration inside must be around 10-12% instead of the original 90%. This step is important because of the long shelf life (to gain gelatines without expire date) and done in a vacuum evaporation system by gently increased temperature.

It is followed by a sterilization step, to make a microbiologically safe product. The sterilization can be performed by plate heat exchanger and indirect steam sterilizer.

Figure 8. Extrusion of gelatine noodles.[41]

The rest of the production steps are needed in term of obtaining transferable gelatine product. For achieving this, firstly the gelatine must be cooled down to set-point, then extruded into gel noodles (in Figure 8.), dried in drying chamber and milled into the required shape, and lastly packed. During the drying process direct hot air can not be used and the temperature should be increased by step-by-step. In opposite way, the gelatine can easily be hardened or melted, and none of them is desired. The flowchart of the gelatine production is shown in Figure 9.[39]

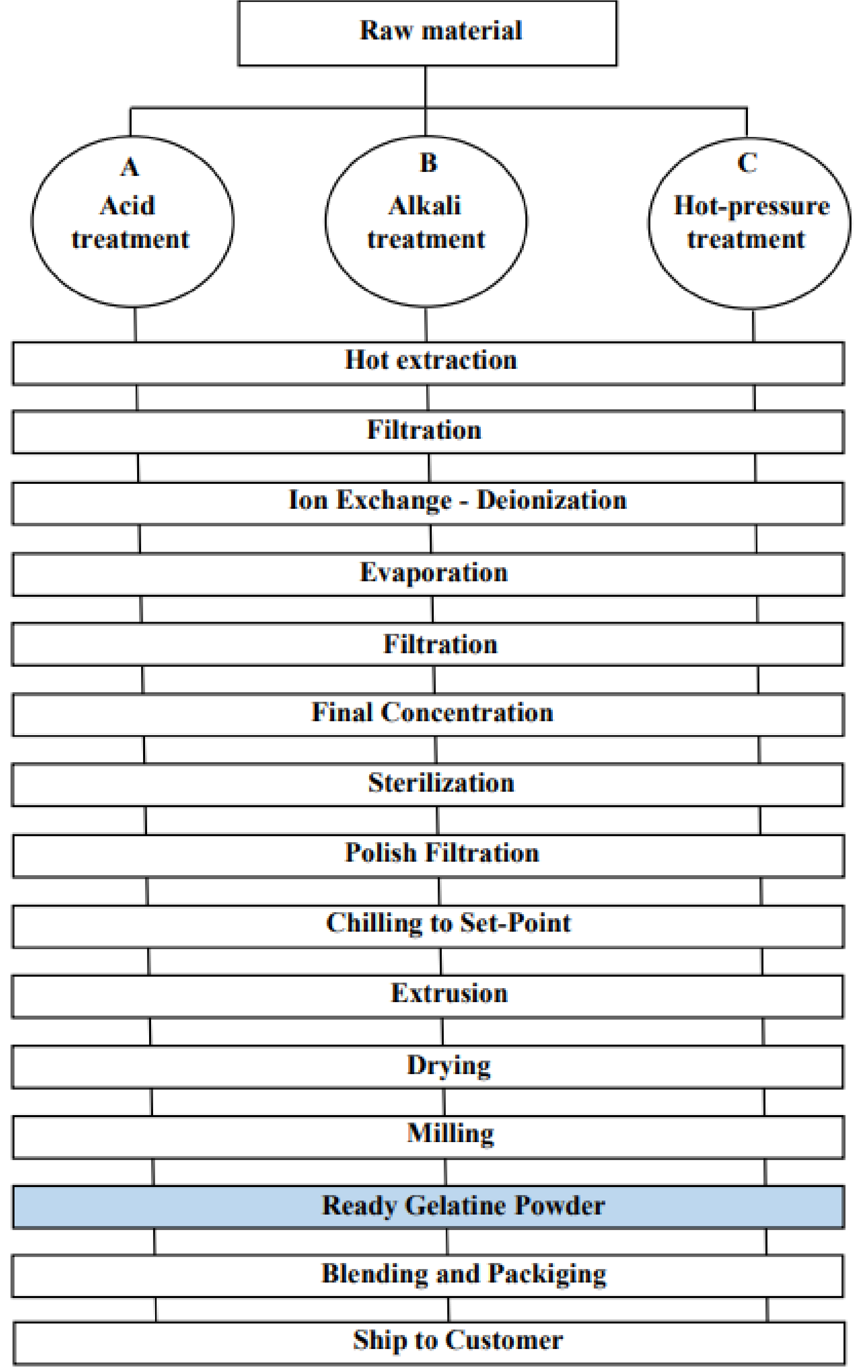


Figure 9. Flow chart of gelatine production.[39]

3.2 Applications of the gelatine

Since the discovery of gelatine, it has become a widely used material in many areas of our life. Gelatine can be found at the food industry in the e.g., gummy bears, cakes and yoghurts as stabilizer, adhesive, thickener, gel-forming and foam forming additive. Gelatine is also utilized in the breweries, where they can be applied as a clarifying agent for the beers. In the pharmaceutical industry, it is used for the shells of soft gelatine and

hard gelatine capsules. In the photographic industry, during the film making, gelatine is employed as adhesive additive to silver salts for producing photosensitive layer. In the medical industry there are some well-known usages of it as foam powders, absorbable sponges, implants, but there are some newly tested utilizations also like potential ink for 3D/4D-printing, tissue engineering, gelatine-based 3D microgels, organ on-chip scaffold, drug delivery, regenerative medicine applications, bendable osteoinductive tape.[42]

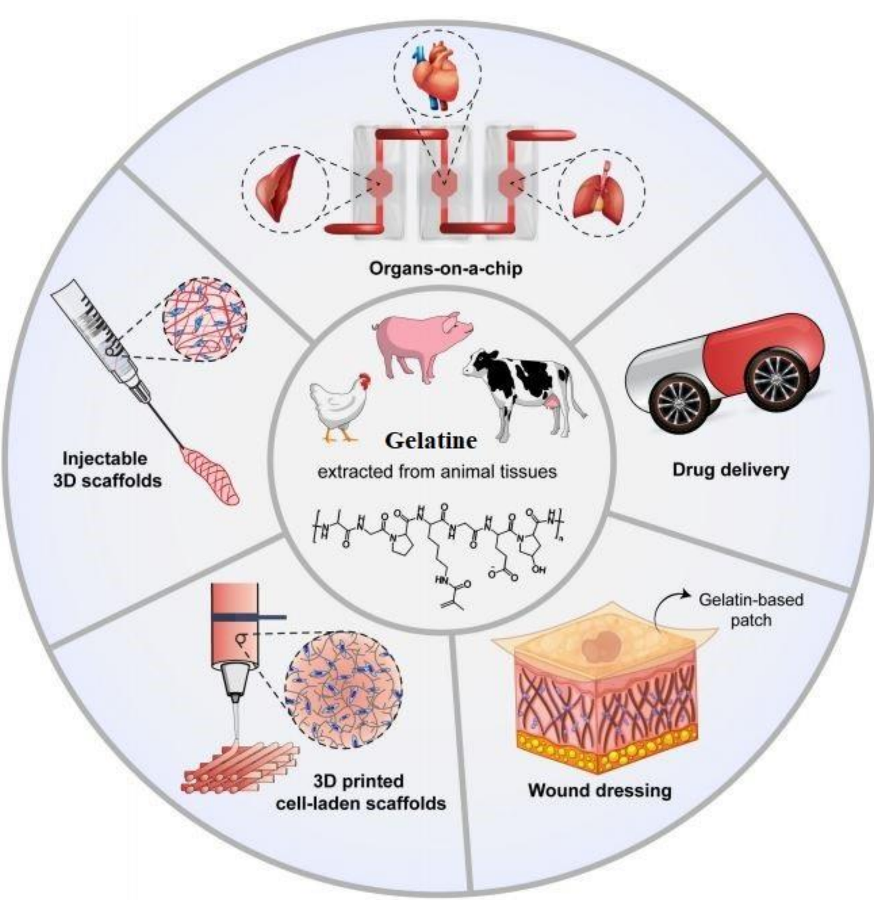


Figure 10. Recent advances in gelatine-based therapeutics.[42]

The blended bioink consisted of sodium alginate, gelatine methacrylate and 4-arm poly (ethylene glycol)-tetra-acrylate. The organ on-chip scaffolds are multi-channel 3D microfluidic cell culture chips and with the help of them, the functionality of tissues and organs can be tested and screened in a wide range of drugs. Gelatine is a good potential drug delivery material for therapeutic transdermal substances, cause thanks to the gelatine-based microneedles the substances can go through the skin in a safe and cost-efficient way. The gelatine has a wide field of usage due to its good water-solubility, creation polyionic complexes with charged therapeutic compounds, high bioactivity, biocompatibility,

biodegradability and easy fabricability. At drug delivery utilization the bioavailability, controllability, biocompatibility, the ability of targeted release of the bioactive compound and biodegradability is necessary.[42] In the 3D scaffold utilization, a network is made by the enzymatic crosslinking of gelatine systems. A photocrosslinked 3D scaffold is made by gelatine methacrylamid. For this application a bovine, type B gelatine is utilized which iso-electric point is approximately 5 and Bloom strength is around 257. During the hydrogel preparation, the gelatine solution is dissolved in phosphate buffer at pH 7.8, and then it was reacted with methacrylic anhydride. With the help of the gelatine methacrylamide, the scaffold can maintain a high viability of the cells, therefore it is a good device for tissue engineering.[43] Gelatine is also a great wound healing compound because it is tolerated by the human body (biocompatibility) and also has a good ability of accelerating the wound healing processes. A good example of this gelatine usage is the research of Wang L. et al. where they combined gelatine with poly(ε-caprolactone) for producing a scar-inhibiting electrospun fibrous scaffold.[44] At tissue engineering nowadays there are more and more attempts to replace the burnt or lost skin with an artificial one. Gozde Eke et al. made a try on the combination of methacrylated gelatine and methacrylated hyaluronic acid into a hydrogel to increase the vascularization of the target tissues and provide them elasticity and flexibility.[45] In the Appendix VIII. more biomedical application is shown.

Gelatine is also a very often used additive in the cosmetic industry and in the technical industry in gels, face masks, body lotions and glues due to their stabilizer, adhesive, emulsifier, thickener, gel-forming, foam-forming and film-forming properties.[39]

3.3 Gelatine testing

3.3.1 Main properties of gelatine and their importance

The gelatine quality is industrially determined by the following properties:

- ***colour:*** It mostly depends on the raw material type and the concentration of the gelatine solution. Porcine gelatine is lighter comparing with ossein or cattle gelatines. But the colour of the gelatine does not have any effect on the quality of the gelatine.[39,46]
- ***gel strength:*** Apart from the basic physicochemical properties (structure, solubility, transparency, colour, odour and taste), the gelatine's main attribute, which tells the most about the quality of gelatine, is the GS. The commercial gelatine GS is between

60 and 300 Bloom. For measuring the GS the Bloom test is utilized. During Bloom test, a 6.67% gelatine solution is prepared at 10°C and 17 hours of maturation time. [47,49] Under this condition the Bloom value (in gram) can be measured by depressing the surface of the gel with 4 mm without breaking it.

Figure 11. Machine for measuring the Bloom value in our laboratory.

If the Bloom value is higher, the melting point of the gelatine is also higher and the gelling time is shorter. The viscosity of gelatine is also higher at higher Bloom value (at constant gelatine concentration).

In short, the higher GS of the gelatine provides a higher firmness to gelatine, makes the setting time shorter and even decrease the needed amount of it. Therefore, these gelatines have much better gel-forming quality, which makes them a good choice for confectionery products as gummy bears, marshmallows, gelatine desserts. These gelatines can also be well-applied in frozen or/and dairy products and pastries, where this property is essential. The gelatine which has worse GS can be used for clarifying agents in beer, wine, juice and soft gelatine capsules and lozenges.[39,49]

The GS mostly depends from:

- the amino acid composition – species-specific (see in Figure 12.);
- the molecular weight distribution (MWD) – it depends mainly on the processing conditions. [39,46]

	Type A (Porkskin)		Type B (Calf Skin)		Type B (Bone)	
Alanine	8.6	10.7	9.3	11.0	10.1	14.2
Arginine	8.3	9.1	8.55	8.8	5.0	9.0
Aspartic Acid	6.2	6.7	6.6	6.9	4.6	6.7
Cystine	0.1		Trace		Trace	
Glutamic Acid	11.3	11.7	11.1	11.4	8.5	11.6
Glycine	26.4	30.5	26.9	27.5	24.5	28.8
Histidine	0.9	1.0	0.74	0.8	0.4	0.7
Hydroxylysine	1.0		0.91	1.2	0.7	0.9
Hydroxyproline	13.5		14.0	14.5	11.9	13.4
Isoleucine	1.4		1.7	1.8	1.3	1.5
Leucine	3.1	3.3	3.1	3.4	2.8	3.5
Lysine	4.1	5.2	4.5	4.6	2.1	4.4
Methionine	0.8	0.9	0.8	0.9	0.0	0.6
Phenylalanine	2.1	2.6	2.2	2.5	1.3	2.5
Proline	16.2	18.0	14.8	16.4	13.5	15.5
Serine	2.9	4.1	3.2	4.2	3.4	3.8
Threonine	2.2		2.2		2.0	2.4
Tyrosine	0.4	0.9	0.2	1.0	0.0	0.2
Valine	2.5	2.8	2.6	3.4	2.4	3.0

Figure 12. Amino acid composition of pigskin, cattle hide and ossein (bone). The data is given in grams per 100 grams of dry gelatine.[39]

- ***viscosity:*** The viscosity of the gelatine depends mainly on the MWD, molecular weight (MW), the used raw material, concentration of gelatine, GS of the gelatine and the temperature of the system. The viscosity of the commercial gelatine is between 1.5 to 7.5 mPa·s. If the temperature is higher, the viscosity decreasing, however, if the MW is higher, the viscosity is higher as well. Among gelatine types the amount of viscosity can have a big variety. For example, the gelatine made out of the giant catfish skin has a higher viscosity than other fish gelatines, which cause that this type of gelatine has a better foam capacity than the others.
  The flow behaviour of the different gelatines is important due to their processing. The gelatines with higher viscosity do not flow that easily which is useful e.g., at hard gelatine capsules and photographic applications. The low viscosity is used e.g., at soft gelatine capsules and tablet production. [46,48,49]
- ***melting or gelling temperatures:*** it strongly depends on the Bloom value. As it has been already written at the GS property, if the Bloom value is higher, the gelling temperature is also higher.
- ***the water content:*** It is an important parameter due to the long shelf-life. Normally gelatine contains 8-13% of moisture. At higher water content the gelatine would be an excellent growth medium for bacteria.
- ***microbiological safety:*** The growth of bacteria in the gelatine solution or the degradation of the gelatine can be prevented by adding preservatives to it. The chosen

preservatives highly depend on the gelatine product application. The gelatine gels need higher concentration of preservatives than the diluted gelatine solutions.[39,46]

3.3.2 Analysing the gelatine and the by-product occurring during gelatine production

During the gelatine preparation many by-products as dry matter, fat and protein arise. All of the by-products and the ready gelatine can be analysed by conventional food methods.[38] In case of gelatine, the dry matter content, GS, viscosity, pH, foaming capacity (FC) and stability (FS), gelling point (GP), melting point (MP), ash content (AC), digestibility, water holding capacity (WHC), fat binding capacity (FBC), emulsification capacity (EC) and stability (ES) can be measured. Define these properties of the gelatine is important to find their best application. In all analysing method, the Official Procedure of the Gelatine Manufacturers Institute of America protocols must be followed. Most of the analysing methods of the gelatine properties will be discussed in the analysing part of the thesis, here just the most important attributes (dry matter, GS, dynamic viscosity (DV), AC, yield and pH) will be discussed.

The dry matter can be determined by indirect method. It means that the sample weight should be measured and then placed into oven at given temperature (at 103± 2.0 °C) for 18 hours for drying. Then the weight of the sample should be measured again and the weight difference is given the water content of the gelatine sample.

The generated fat can be determined by Soxhlet extraction.[50]

For calculating the protein by-product content of the gelatine, first the nitrogen content of it should be determined by Kjeldahl method. After the nitrogen content is calculated, the protein content can be acquired easily with the help of a conversion factor, which is 6.25.[51]

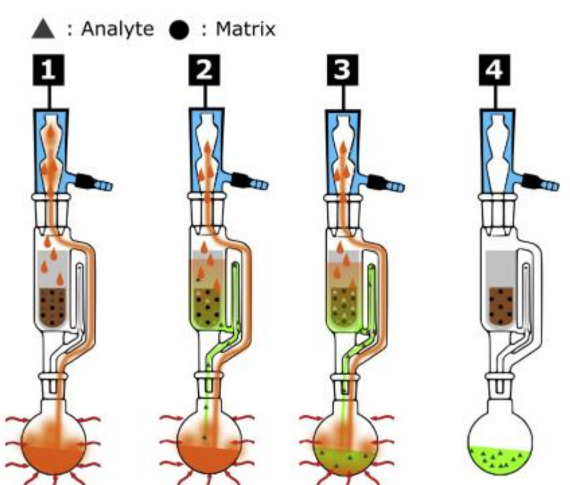


Figure 13. Schematic illustration of the workflow of Soxhlet extraction:
1) Solid matrix is placed into Soxhlet thimble. Solvent is heated under reflux.
2) Condensation and extraction with “fresh” solvent. Solutes are transferred from the extraction chamber into the reservoir.
3) Continuous repetition of the extraction.
4) Exhaustive extraction is complete.[50]

For analysis the gelatine's main properties the following methods and equations are used:

- **Gel strength:** it can be determined by making a 6.67% gelatine solution at specified condition and the force (in grams) is measured which is needed to depress the prescribed area of the surface for 4 mm deep.[38]
- **Dynamic viscosity:** the same solution what was used in the earlier measurement can be used here as well. Firstly, the solution must warm up till 60°C and then the flow time of 100 ml solution can be measured through a standard pipette. The proper value is calculated from the following equation[38]:

$$\eta = (A*\tau - B/\tau)\ d$$

Where

η - gelatine shear viscosity (mPa·s)

A, B – pipette constant

τ - efflux time (s)

d – solution density (6.67% gelatine solution at 60°C) (g/cm^3)

- **Gelatine yield:** it is an important parameter for gelatine manufacturers. It is calculated by using the following equation:

$$GY = m_2 / m_0 \times 100$$

Where

GY – gelatine yield (%)

m_2 – weight of gelatine (g)

m_0 – weight of defatted raw material (g)

- **pH of the gelatine:** a 1-2% gelatine solution should be made and then the pH can be determined by pH paper or pH meter at 25.6°C. [46]
- **Ash content:** it is done gravimetrically after burning and annealing the sample.[52]

II. ANALYSIS

4 THE AIMS AND HYPOTHESIS OF THE WORK

The ascending demand for convenience and functional food and beverage products as pasta, salads, yogurts, ice-creams, jams and jellies, alongside with the rising utilization of biopolymers in the pharmaceutical industry is anticipated to drive the market demand for gelatine during the next five years. Instead of the mainly used bovine and porcine raw materials for gelatines (due to their quick and low-cost production and their rapid breeding), new sources should be searched. According to earlier published results, there are several alternative raw materials, including poultry (chicken, turkey, duck), fish, frog, horse skin, camel and salamander for the gelatine production. However, these different raw materials have distinct protein content (mainly lower), structure of amino acids, rheological and thermostability properties, therefore the replacement of the porcine and bovine gelatines is restricted. Thus far, the gelatine production based on alternative raw materials is getting more known and searched, but their production cost is still more expensive, therefore it remains difficult to fully replace the mammalian-based gelatines. For enhancing the processing of the alternative gelatines, a good way is to reduce the cost of the raw material of it, by using ABPs as a secondary raw material. Gelatines can be derived from the following ABPs: connective tissue, bones, intestines of the animal and the skin via a partial hydrolysis process.

In this thesis the CDR-based gelatine is investigated, which is a not often used alternative secondary raw material for gelatine production. For the efficiency and utility of this type of gelatine several properties are checked. The most important physicochemical property of the gelatine is the GS, which mainly determines what the gelatine can be used for. The secondary most important properties are the DV, AC, GP and MP. Not often measured properties of the gelatines are the WHC, FBC, FC, FS, EC and ES. Due to the wide overview of the gelatine properties, the following information can be determined: temperature interval of the product for utilization (by the gelling and melting temperature), texture, spread ability and sensory properties of the product. During the industry utilization, a very important property is the yield of the gelatine. The gelatine yield is essential for effectiveness of commercial production and financial feasibility.[76]

By this study, we want to contribute to a pioneer study field by enlarging the knowledge about CDR-based gelatines and assuming that the prepared gelatines will have similar

properties as gelatines from traditional (commercially used) raw material sources and slightly better properties for confectionery production than the alternative fish gelatine. Not least, by introducing alternatives of the generated ABPs in the poultry slaughterhouses we also want to contribute to a sustainable animal husbandry.

5 MATERIALS, METHODS AND WORKFLOW

5.1 Raw material

The used CDRs for the experiments are ABPs from the production of mechanically deboned chicken meat, which were delivered from the Raciola poultry farm (RACIOLA Uherský Brod, sro. company, Czech Republic), whom field is poultry processing, production and sale of poultry specialties. The chicken production in the company is under strict hygiene conditions. [53] The composition of the CDR is the following:

- Dry matter: 38.15 %
- Ash content (based on the dry matter): 28.59 %
- Fat content (based on the dry matter): 25.97 %
- Nitrogen content (based on the dry matter): 6.45 %
- Total protein ((Nitrogen content)*6.25) (based on the dry matter): 40.31 %
- Collagen content from the total protein content (based on the dry matter): 68.3 %

The raw material arrived to the laboratory in frozen condition and were kept under -18°C.

5.2 Reagents and equipment

Equipment: Stevens LFRA Texture Analyser for measuring gelatine gel strength (Leonard Farnell and Co ltd., England), SPAR Mixer SP-100AD-B meat grinder (TH Industry RD, Taiwan), Rotina 35 centrifuge (Hettich, Germany), IKA T 25 digital Ultra-Turrax desintegrator (IKA-Werke, Germany), Memmert ULP 400 drying device (Memmert GmbH + Co. KG, Germany), LT 43 shaker (Nedform, Czech Republic), Kern 440 - 47 electronic scale, Kern 770 electronic analytical and precision balances (Kern, Germany), A 10 labortechnik analytical mill (IKA-Werke, Germany), ULP 400 drying oven (Memmert GmbH+Co. KG, Germany), Samsung fridge-freezer (Samsung, South Korea), Thermo Haake C 10 thermometer (Thermo Fisher Scientific, USA), Whatman No. 1 paper (Sigma Aldrich, UK), a metal filter sieve with the size of pores 1 and 2 mm (Labor-komplet, Czech Republic), Bosch rotating blade coffee grinder (Bosch GmbH, Germany), Forced convection chamber furnace NAT 15/65 as table-top model (Nabertherm GmbH, Germany), Hettich® EBA 20 centrifuge (Andreas Hettich GmbH & Co. KG, Germany), IKA HS 501 digital laboratory shaker (ProfiLab24 GmbH, Germany), Haake P5 Circulating Bath w/ Thermo

C10 Controller (Thermo Fisher Scientific, USA), Kavalier LT3 shaker (Sázava, Czech Republic), IKA LABORTECHNIK RCT BASIC magnetic mixer with heating plate (Staufen, Germany), WTW pH 526 pH meter (WTW, Oberbayern, Germany). .
Reagents: NaCl, NaOH, petroleum ether, ethanol and chloroform (Verkon, Czech Republic); all chemicals were of analytical grade, Protamex®, an endoprotease from Novozymes (Copenhagen, Denmark), was used for conditioning of defatted CDRs.

5.3 Workflow of processing CDRs into collagenous products

5.3.1 Preparation of purified collagen

During the sample preparation, the aim is to remove all of the non-collagenous substances, like fat and minerals, from the raw material.
Firstly, the frozen chicken bones were defrosted, chopped into smaller pieces and rinsed in fresh, cold water for 5 minutes for separating the albumins from the rest of the raw material. Then the 0,2 M NaCl treatment was applied in the ratio of 1:6 for approximately 1,5 hours for the globulin separations. It was followed by a 1:6 ratio 0.03 M NaOH treatment, which was applied for the elimination of glutelins from the raw material. During the treatment, the blend was occasionally stirred for 45 minutes and then filtered through a fine sieve. For sufficient clean material, the 0.03 M NaOH treatment was executed twice. When it is finished the material is dried at 35°C in a hot oven for 24-36 hours.
Afterwards, the defatting of the material was performed, by mixing the raw material with the 6-fold amount of mixture of petrolether/ethanol solvent in 1:1 ratio and shaking the blend for approximately 2 days at room temperature. During the shaking, the solvent mixture was changed twice on the material. At the end, the purified collagen was milled into smaller, approximately 3 mm diameter particles and kept in a closed vessel.

5.3.2 Preparation of demineralised collagen

The prepared purified collagen was blended with 3% HCl in a ratio of 1:7 in a bucket. Then it was placed on a shaker at room temperature for 4 days, but the 3% HCl was changed in every 24 hours. After 4 days, the demineralised collagen is filtered and rinsed in a cold water for 5 minutes. At the end, the product is dried at 35°C in an oven with air circulation for 24-36 hours.

Figure 14. The prepared purified and demineralized collagen.

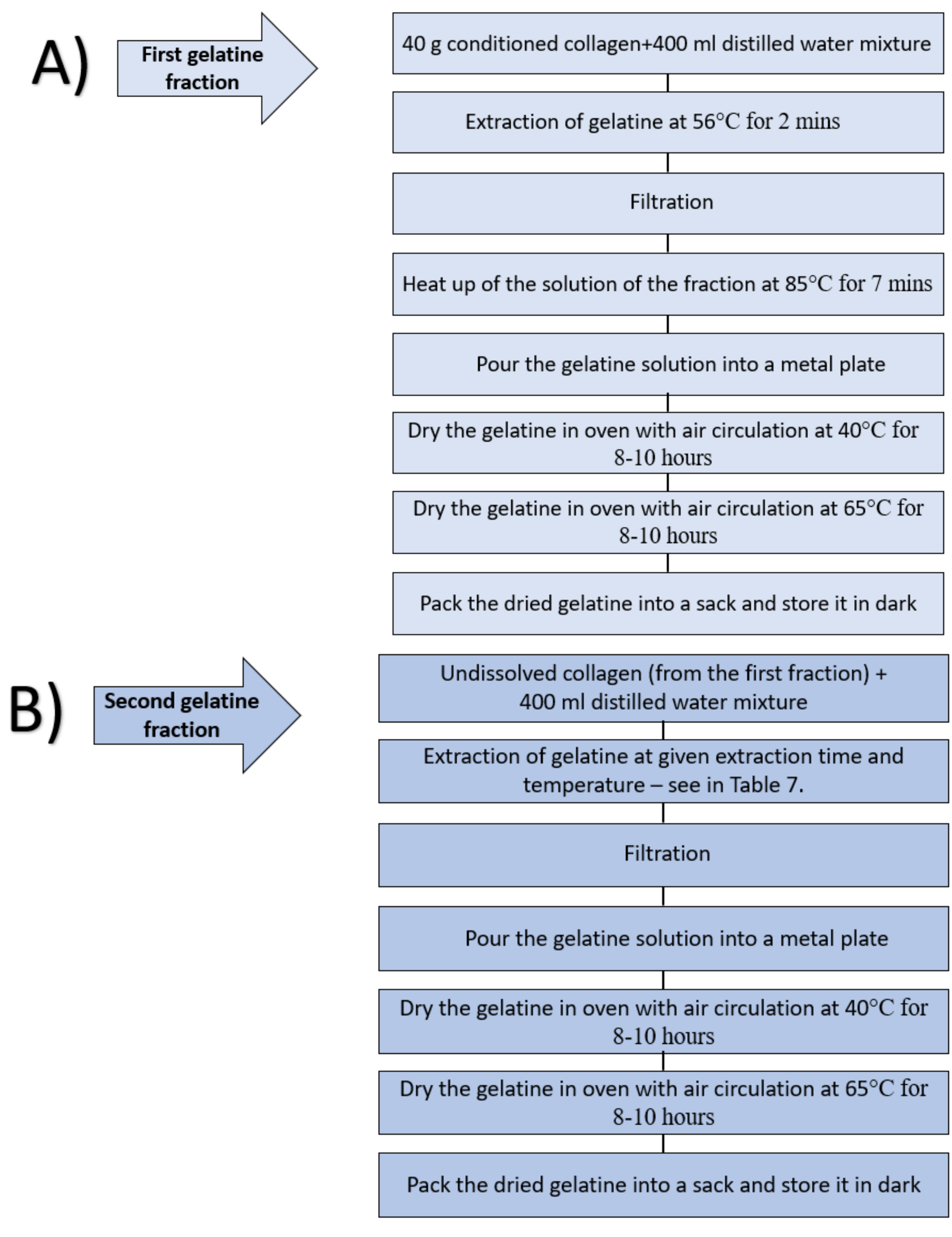
5.3.3 Preparation of gelatine

5.3.3.1 Enzyme conditioning of the purified collagen

40 g purified collagen was blended with 400 ml distilled water. Then it started to be shaken at pH 6.5-7, which is adjusted by 20% NaOH solution, for 24 hours. After 30 minutes of shaking, the Protamex® enzyme was added to the system in 0.4%, which was in our case 0.14192 g (it is based on the 0.4% of the dried matter amount of 40 g of purified collagen – dried matter amount is 35.48g) At the beginning in every 30 minutes the pH had to be controlled and when it was needed more 20% NaOH solution was added for reaching the desired pH value. When the 24 hours of shaking was over, the enzyme conditioned collagen matter was filtered by a 3-layers fine sieve and cleaned by fresh water to remove as much enzyme remnants as possible. The hydrolysate was poured into a metal plate, dried for 24 hours at 70°C and at the end weighted.

5.3.3.2 Gelatine extraction

During one experiment three extractions were performed. In term of each experiment, the first and the third extraction steps were the same, and only during the second extraction step there was a difference.



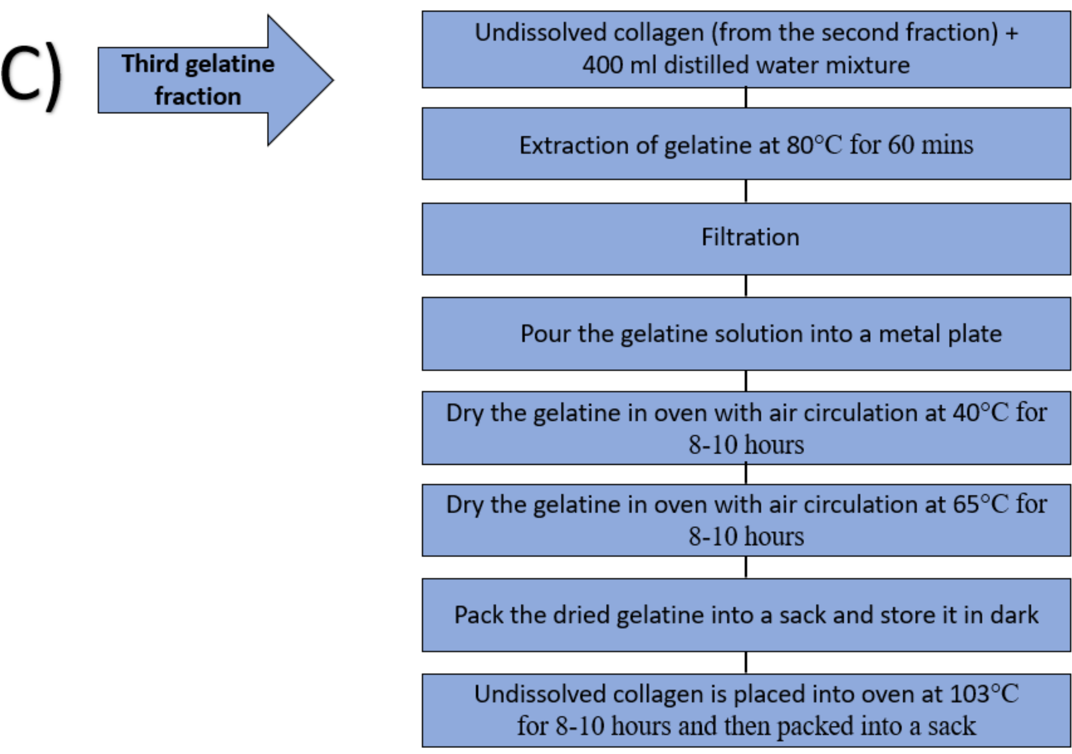


Figure 15. Schematic flowchart of the extraction of the first gelatine fraction (**A**), of the second gelatine fraction (**B**) and of the third gelatine fraction (**C**).

The extraction time and the extraction temperature were changed according to the values listed in Table 8. The three different extraction time were 20, 40 and 60 minutes, and the three different extraction temperatures were 60°C, 64°C and 68°C. All together ten experiments were accomplished which parameters are shown in Table 8. In the first nine experiments, all the possible combination of the three extraction temperatures and three extraction time were executed, whilst the tenth experiment was a comparison experiment to the middle temperature and time value without any added enzyme. The workflow of the extraction is shown in Figure 15.

Experiments	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**	**9.**	**10.**
Temperature (°C)	**60**	**60**	**60**	**64**	**64**	**64**	**68**	**68**	**68**	**64**
Time (mins)	**20**	**40**	**60**	**20**	**40**	**60**	**20**	**40**	**60**	**40**

Table 8. The given extraction temperature and time at each experiment in the second gelatine fraction.

5.4 Method of the work

Our gelatine extraction was planned according to the Design of Experiment (DoE) principles. DoE is used to understand the effects of several independent inputs and their interactions on the output responses. It consists of two steps:

1) Screening design for identifying the significant factors in the extraction,

2) Optimization design for optimizing the relevant factors in the extraction for the best efficiency.

During the optimization of the process, the main driving force is to obtain the maximum production or best quality with minimum cost. In our study, the aim was to gain the best gelatine properties, by optimizing the extraction time and temperature, which were found as significant factors during my supervisor's earlier experiments. DoE is the basic of the nowadays more and more used Six Sigma system also, which is applied by big companies as Nolato Hungary Ltd., General Electric or Eastman Kodak to improve their products' quality and decrease their waste products.[54]

The steps of a successful DoE are the followings[55]:

- Define the problem
- Plan the experiment
- Run the experiment
- Analyse the data by using statistical methods
- Report the results

Define the problem: In this study the raised problem was to get gelatine out of CDRs with good properties for confectionery products as jellies.

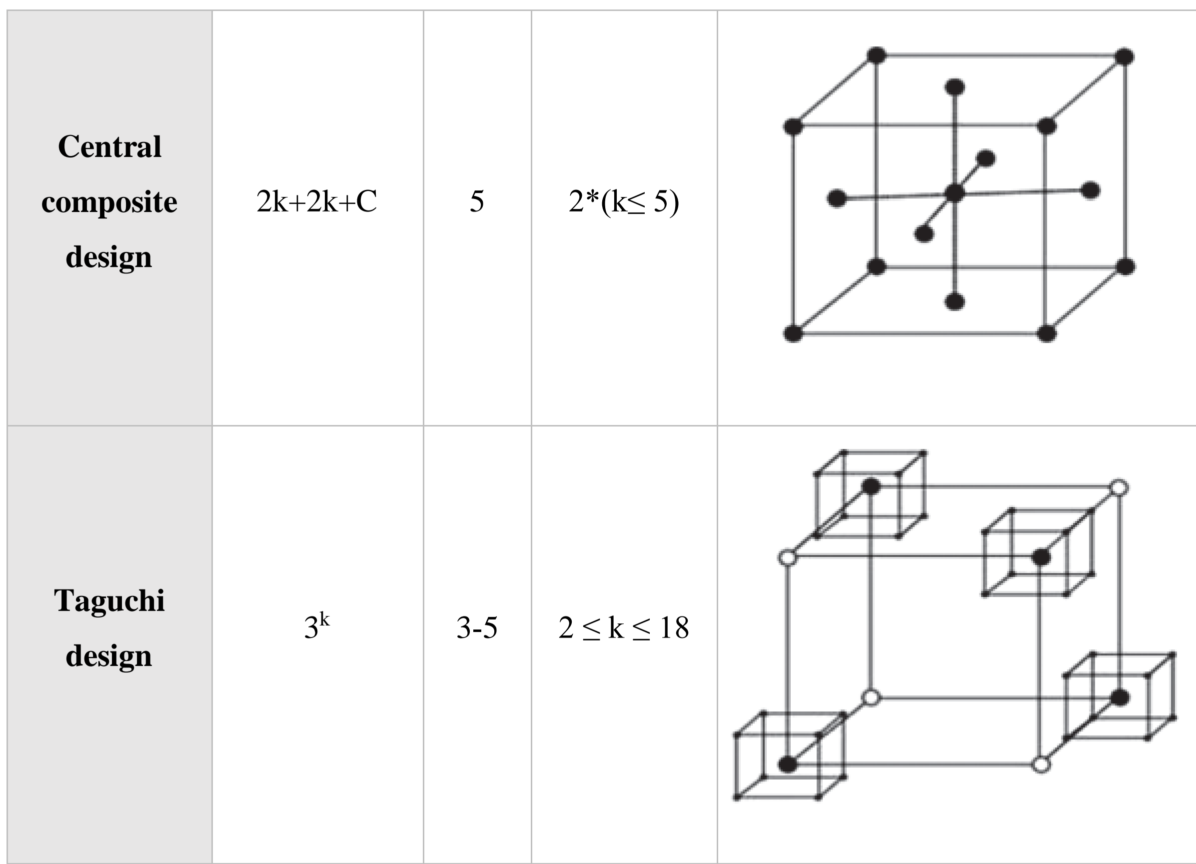
Plan the experiment: For process optimization the most often used DoEs are the three-level full factorial, Box-Behnken, central composite design and the Taguchi design methods (shown in Table 9.).[54,56,57]

- Three-level full factorial design (TLFFD): this design used for two or three different inputs. At increased number of input factors, the number of experiments, which should be done, are escalated exponentially. The number of experiments is calculated according to the following equation: 3^k, where k is the number of input factors.
- Box-Behnken design (BBD): this design is used at higher input numbers, due to its cost-effectiveness (less experiments must be done in this type of design than in the TLFFD).

The number of experiments is calculated according to the following equation: 2*k*(k–1)+C, where k is the number of input factors and C is the replicate number of centre points.

- Central composite design (CCD): this design is one of the most used optimization designs because here 5 level of each input factor is used, and it is still more cost-effective than the TLFFD. The number of experiments is calculated according to the following equation: 2k+2k+C, where k is the number of input factors and C is the replicate number of centre points.
- Taguchi design (TG): this design is for examining multiple factors (usually more than 2) with various factor levels based on the usage of orthogonal arrays. In this design method the results can be rapidly, accurately and precisely provided with a smaller number of experiments. The number of experiments is 3^k, where k is the number of input factors or at higher input factors it is maximum 0.3% of the original number of experiments in case of TLFFD. The limited number of experiments help to keep this design cost-efficient, but still reliable.

Experiment	Equation	Level	Factors	Illustration matrix at 3 input factors
Three-level full factorial design	3^k	3	$2 \leq k \leq 3$	
Box-Behnken design	2*k*(k-1)+C	3	$3 \leq k \leq 5$	



Central composite design	2k+2k+C	5	2*(k≤ 5)	
Taguchi design	3^k	3-5	2 ≤ k ≤ 18	

Table 9. Summary of optimization designs characteristics of TLFFD, BBD and CCD. The number of experiments, levels, factors, and their matrix illustration at three input factors are compared.[54,58]

Run the experiment: In this thesis, the TG was used with two independent input factors, which were the extraction temperature and the extraction time, and their influence on the gelatine properties was investigated. According to the TG's equation, all together $3^2 = 9$ experiments were implemented. In addition, one extra experiment was accomplished, which was a reference experiment with the mean input factors without added enzyme. This extra experiment provided results about the enzyme effectivity in the process.

Analyse the data by using statistical methods: After the experiments were done, the results were analysed in Minitab 17. During the analysing, the effect of the input factors can be visually represented and reliably predict the composition of the input factors where the gelatine properties meet the specifications or desired values or maximum. The conclusions of a study should be always relied on statistical analysis and confidence levels.

5.5 Evaluation of the efficiency of the process and the quality of the prepared products

5.5.1 Yield of gelatine

The yield of each fraction of the gelatine was calculated based on dry weigh basis according to the following equations:

$$m_{EG} = m_{FM} - m_{IM} \quad (1)$$

$$Y = \frac{m_{EG}}{m_{RMC}} * 100 \quad (2)$$

Where

m_{EG} – dry weight of extracted gelatine (g)
m_{IM} – initial mass (metal plate) (g)
m_{FM} – final mass (dry gelatine + metal plate) (g)
m_{RMC} – dry weight of collagen in the raw material (g)
Y – yield of gelatine fraction (%)

The summarized yield values are listed in Table 11.[39]

5.5.2 Determination of gelatine gel strength

For determining the gelatine GS, firstly a 6.67% gelatine solution was prepared. When the solution was done it had to be kept in a refrigerator for 8-10 hours to receive a gelatine gel.

Figure 16. Measuring of Bloom value.

Figure 17. The prepared gelatine gels for gel strength measurement.

Then with the help of a 0.5” diameter cylinder probe the surface of the gel was depressed by 4 mm.The measured value was the Bloom value. In our experiments, the yield of the gelatines was low, therefore only smaller amount of gelatine gel could be prepared for

testing. Due to this, in all the experiments the measured Bloom value had to be divided with a specified factor (calculated with the help of a known commercial gelatine in the standard glass and in the smaller glasses). According to the yield, four different method was used, shown in Table 10.[39]

Methods	Amount of gelatine (g)	Amount of added water (ml)	Used glass	Dividing factor
A	7.5	104.5	150 ml	1
B	3	42	75 ml	1.2627
C	1.5	21	37.5 ml	1.6372
D	0.94	13.16	30 ml	2.53

Table 10. According to the gelatine yield, there are four different types of methods for calculating the gel strength of each gelatine sample.

The results of the tested gelatines are listed in Table 13.

5.5.3 Gelatine dynamic viscosity

The same 6.67% gelatine solution as in the GS measurement, was used for measuring the DV of the gelatine samples. Firstly, the gelatine solutions were warmed up to 60°C and then the flow time of 100 ml solution was measured through a standard pipette.

Then the kinematic viscosity of the gelatine solution was calculated according to equation (3):[39]

$$\nu = A^{*}\tau - B/\tau \qquad (3)$$

Where

- ν – gelatine kinematic viscosity (mm^2/s)
- A – viscometer constant determined by a validated calibration fluid (0.5)
- τ – efflux time (s)
- B – kinetic energy correction constant determined from the dimensions of the viscometer (2.8)

Figure 18.The viscosimeter for measuring the dynamic viscosity of the gelatines.

And the DV of the gelatine solution was calculated according to equation (4):

$$\eta = \nu * d \quad (4)$$

Where

η – gelatine shear viscosity (mPa·s)
ν – gelatine kinematic viscosity (mm^2/s)
d – solution density (6.67% gelatine solution at 60°C, d=1.005 g/cm^3)

The results of the tested gelatines are listed in Table 13.

5.5.4 Determination of melting point of the gelatine

From each prepared 6.67% gelatine sample two cold (cooled down in refrigerator) samples were taken for determining the melting temperature. These samples were taken by a glass capillary which had a diameter of 2 - 4 mm, and the height of the gelatine column inside was between 5 and 10 mm. After the right amount of the sample was filled into the glass capillary, it was placed into cold water on a magnetic stirrer with a thermometer. Then the heating of the system was started. The temperature, when the gelatine column moved upwards (the gelatine dissolved and the water pressure pushed the sample out of the capillary), was the

melting point of the gelatine sample. At the end, out of the measured values an average was computed, which are shown in Table 13.[59,60]

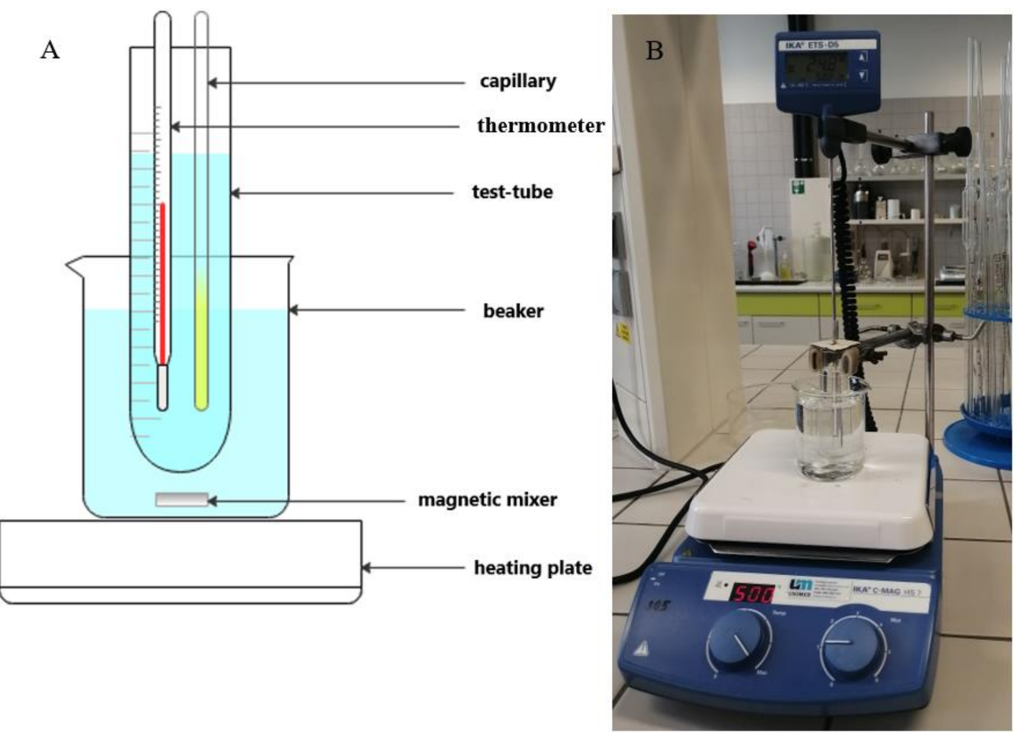


Figure 19. The equipment for the measuring of the gelatine melting point. In picture **A** the equipment is drawn schematically, while in picture **B** shows how it looks in our laboratory.

5.5.5 Determination of the gelling point of the gelatine

The GP was determined as the temperature at which the solidified gelatine solution retained a ball of defined weight on its surface without sinking to the bottom. For determining this temperature, the liquid gelatine solution was poured into a test-tube, which then attached to a holder which was connected to a heating plate. Then a thermometer was put into the gelatine solution and the whole test-tube was placed into a beaker with chilled water (in prior stayed in a refrigerator for at least two hours). After starting the measurement, continuously small balls were thrown into the gelatine gel until the ball retained on its surface. Each experiment was performed twice and then an average was calculated, which are shown in Table 13.[61]

Figure 20. The equipment for measuring the gelling point of the gelatine samples. The temperature, when the ball stays on the gelatine's surface, that is the gelling point.

5.5.6 Determination of ash content of the gelatine

It was important to determine the AC of the sample, in term of specifying the proper application of the gelatine. (In photographic applications low AC is needed, while in confectionary or pharmaceutical applications it can be a much higher value.) During the AC determination, the gelatine samples were firstly burnt at open fire till they did not smoke, then were placed into a 650°C hot oven for 8-10 hours. The rest of the sample, which stayed after the burning was the ash part of the gelatine. For the exact calculation of the AC, the equation (5) was used:[39]

$$AC = \frac{m_{end}}{m_{start}} * 100 \qquad (5)$$

Where

AC – ash content (%)
m_{end} – is the amount of the leftover of gelatine after burning (g)
m_{start} – is the beginning amount of the gelatine sample (0.5 g)

The results of each sample are shown in Table 13.

5.5.7 Water holding capacity and stability

1 g of gelatine sample was measured into a plastic test-tube and then dissolved in 25 g distilled water. Then it had to be shaken for 5 minutes at room temperature and placed into

a centrifuge for 30 minutes at 5000 rpm. After centrifugation, the supernatant (= liquid phase) was removed from the tube by a pipette and weighed. The WHC was calculated according to equation (6) in % and equation (7) in mL/g:[62]

$$WHC = \frac{m_{start} - m_{notabsorbed}}{m_{start}} * 100 \qquad (6)$$

Where

WHC – water holding capacity (%)
$m_{notabsorbed}$ – is the weight of the not absorbed water (g)
m_{start} – is the beginning weight of the water (25 g)

$$WHC = \frac{V_{absorbed}}{m_{gelstart}} \qquad (7)$$

Where

WHC – water holding capacity (mL/g)
$V_{absorbed}$ – is the volume of the absorbed water (mL)
$m_{gelstart}$ – is the beginning weight of the gelatine sample (1 g)

The results of each sample are shown in Table 13.

5.5.8 Fat binding capacity

0.1 g of gelatine sample was measured into a plastic test-tube and then dissolved in 10 g soybean oil. Then it had to be shaken for 5 minutes and rested for 30 minutes at room temperature, and then placed into a centrifuge for 30 minutes at 2500 rpm. After centrifugation, the supernatant (= liquid phase) was removed from the tube by a pipette and weighed. The FBC was calculated according to equation (8):[63]

$$FBC = \frac{m_{start} - m_{notabsorbed}}{m_{start}} * 10 \qquad (8)$$

Where

FBC – fat binding capacity of 1 g gelatine
$m_{notabsorbed}$ – is the weight of the not absorbed soybean oil (g)
m_{start} – is the beginning weight of the soybean oil (10 g)

The results of each sample are shown in Table 13.

5.5.9 Emulsification capacity and stability

0.05 g of gelatine sample was measured into a plastic test-tube and then dissolved in 5 ml distilled water. Then it had to be shaken for 5 minutes at room temperature, then 5 ml soybean oil was added and the system was well mixed. Afterwards the test-tube was placed into a centrifuge for 5 minutes at 1000 rpm. After the centrifugation, the height of the emulsion (= oil phase) and the total content of the tube were measured by a ruler, then the emulsification capacity (EC) was computed according to equation (9):[64]

$$EC = \frac{h_{emulsion}}{h_{total}} * 100 \quad (9)$$

Where

EC – emulsification capacity (%)
$h_{emulsion}$ – is the height of the emulsion (mm)
h_{total} – is the height of the total content (mm)

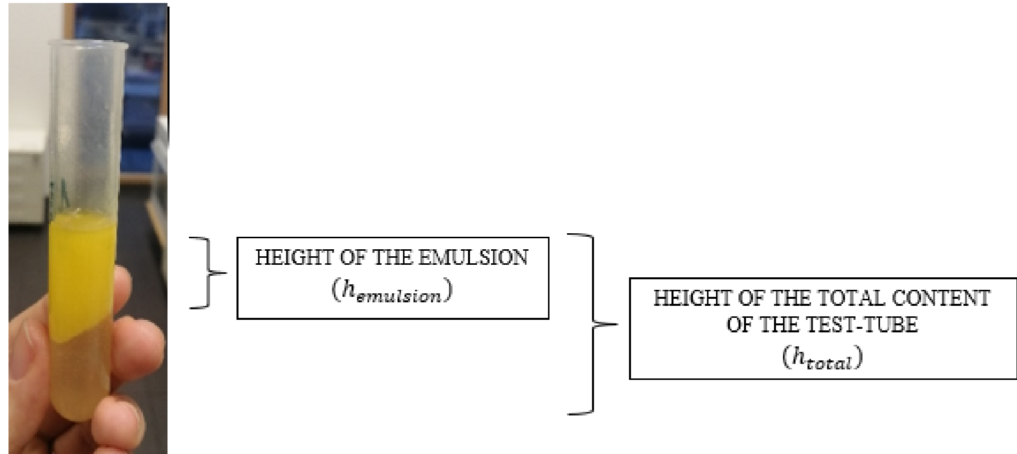


Figure 21. The water phase and the oil phase (emulsion) separation after the first centrifuge for 5 minutes at 1000 rpm.

For measuring the emulsification stability (ES) each test-tube was put into a warm water bath for 5 minutes at 55 °C. Then the test-tubes were replaced into the centrifuge for 5 more minutes at 2000 rpm. After the centrifugation, the height of the emulsion (= oil phase) and the total content of the tube were again measured by a ruler. The ES was computed according to equation (10):[64]

$$ES = \frac{h_{after}}{h_{before}} * 100 \quad (10)$$

Where

ES – emulsification stability (%)
h_{after} – is the height of the emulsion after the second centrifuge (mm)
h_{before} – is the height of the emulsion before the second centrifuge (mm)

The results of each sample are shown in Table 13.

5.5.10 Foaming capacity and stability

1 g of gelatine sample was measured into a 100 ml beaker and dissolved in 50 ml distilled water. Then the gelatine was melted on water bath at 45°C for 20 mins with constant stirring. When the gelatine solution was homogenous, the homogenizer was placed into it and the foaming of the solution was started at 10.000 rpm for 5 mins. After the foaming, the volume was measured for determining the FC of the gelatine by equation (11):

$$FC = \frac{A-B}{B} * 100 \qquad (11)$$

Where

FC – foaming capacity (%)
A – is the height of the foamed gelatine solution (ml)
B – is the height of the gelatine solution at the beginning (50 ml)

Then the foamed gelatine solution was placed on a table at room temperature for 30 mins for measuring the FS based on equation (12): [89]

$$FS = \frac{C-B}{B} * 100 \qquad (12)$$

Where

FS – foaming stability (%)
C – is the height of the foamed gelatine solution after 30 mins (ml)
B – is the height of the gelatine solution at the beginning (50 ml)

The results of each sample are shown in Table 13.

6 RESULTS AND DISCUSSION

The determination of the extraction of our gelatine was based on literature and earlier thesis results. As reported in these sources the mainly effecting factors on the physical and chemical properties of the gelatines are the extraction time (factor A, min) and extraction temperature (factor B, °C). However, the used raw material, animal age, type of collagen and method of manufacture, tissue type, species have also a significant influence on the properties. For determining the effect of these factors, TG of experiment was utilized, and all together $3^2 = 9$ experiments were executed. In case of receiving a higher yield of the extracted gelatines, during each experiment three gelatine fractions were performed. In addition, for improving the yield (according to literature) of gelatine, 0.4% (according to dry matter) enzyme (Protamex®) is added to the system. As a control group for controlling the added enzyme efficiency, an experiment is done at average extraction temperature and time without enzyme – this was the 10th experiments.

In each case, hydrolysates and three gelatine fractions are obtained under different conditions (more described in chapter 7.). The analysing of the results happened in Minitab 17, in which also a statistical evaluation of the results were done. The statistical significance of the A and B factors was measured by p-values for a 95% confidence level. If the p-value is lower than $\alpha = 0.05$ that means the factor has a significant effect on the evaluated variables with 95% probability. If the p-value is higher than $\alpha = 0.05$ value, in those cases the influence of the factor on the evaluated variables is not statistically detectable. The lower the p-value, the greater the influence of the given factor.[65] Each property (yield of gelatine, GS, WHC, FBC, EC, ES, FC, FS, AC, DV, MP, GP and digestibility) was evaluated and the most important ones (GS, DV and yield) were also graphically evaluated by layered graphs.

6.1 Yield of the gelatine fractions

Each experiment with enzyme started in the same way, therefore the yield of hydrolysate was similar, the average was 5.79% ± 0.6% (DS). In case of the 10th experiment, which was without enzyme the yield was 5.37%, which shows similar behaviour as the experiments with enzyme.

In case of the yield of the first fractions the same can be said, therefore the average was 1.97% ± 0.37%. In case of the 10th experiment, the yield was again really similar to the other experiments with its 1.97%. The obtained first gelatine fraction had poor properties and its amount was very low, therefore in this study no further evaluation was occurred on them.

The measured yields in each experiment are shown in Table 11.

Exp. No.		1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Extraction parameter of the 2nd gelatine fraction	Factor A, Extraction temperature (°C)	60	60	60	64	64	64	68	68	68	64
	Factor B, Extraction time (min)	20	40	60	20	40	60	20	40	60	40
Yield of hydrolysis (g / %)	-	2.1 / 5.92	2.2 / 6.2	2.1 / 5.91	2.5 / 7.05	2 / 5.64	2 / 5.64	1.8 / 5.07	2 / 5.64	1.8 / 5.07	1.9 / 5.37
Yield of the 1st gelatine fraction (g / %)	-	0.6 / 1.69	0.4 / 1.13	0.8 / 2.25	0.8 / 2.25	0.8 / 2.25	0.7 / 1.97	0.7 / 1.97	0.8 / 2.25	0.7 / 1.97	0.7 / 1.97
Yield of the 2nd gelatine fraction (g / %)	-	0.9 / 2.54	1.3 / 3.67	3.2 / 9.02	1.1 / 3.1	1.9 / 5.36	2.5 / 7.05	2 / 5.64	1.9 / 5.36	4.1 / 11.56	1.8 / 5.07
Yield of the 3rd gelatine fraction (g / %)	-	1.6 / 4.51	1.9 / 5.36	2.3 / 6.5	1.7 / 4.8	2.7 / 7.61	2.6 / 7.33	2.8 / 7.89	2.4 / 6.76	2.5 / 7.05	2.9 / 8.17
Undissolved collagen (g / %)	-	27.3 / 76.94	28.6 / 80.6	26.9 / 75.82	29 / 81.73	27.6 / 77.8	27.2 / 76.66	27.5 / 77.51	28.3 / 79.76	26.3 / 74.13	28.1/ 79.2
Total extraction yield (%)	-	8.74	10.16	17.77	10.15	15.22	16.35	15.5	14.37	20.58	15.21
Balance error (%)	-	8.4	3.04	0.5	1.07	1.35	1.35	1.92	0.23	0.22	0.22

Enzyme is used	-	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No

Table 11. The measured data at each experiment: yield of hydrolysate, yield of fractions, undissolved collagen in grams and in percentage, the total extraction yield and the balance error in percentage.

Total extraction yield (ΣY) and balance error (BE) were calculated, according to the (13) and (14) formulas:

$$\Sigma Y = Y_{G1} + Y_{G2} + Y_{G3} \tag{13}$$

$$BE = \left| 100 - (Y_H + Y_{G1} + Y_{G2} + Y_{G3} + UC) \right| \tag{14}$$

Where

Y_H – yield of the hydrolysate (%)

Y_{G1} – yield of the first gelatine fraction (%)

Y_{G2} – yield of the second gelatine fraction (%)

Y_{G3} – yield of the third gelatine fraction (%)

UC – undissolved collagen (%)

In Figure 22/1) the effect of factors A and B on the yield was shown. Visible that by increasing the extraction temperature and the extraction time, the yield is increasing as well. The highest values are measured at the highest extraction time (at 20 °C 9.02%; at 40 °C 7.05% and at 60 °C 11.56%). Obtaining the statistical influence of the factors an ANOVA-test is performed. The smallest level of the statistical influence of the factors is measured by

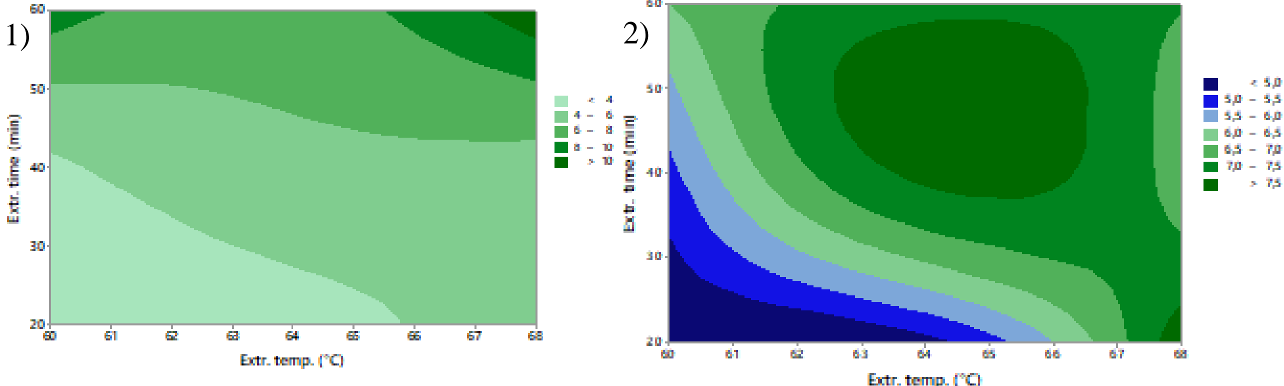


Figure 22. 1) Layered graph of the effect of A and B factors on the yield of the 2nd gelatine fraction. 2) Layered graph of the effect of A and B factors on the yield of the 3rd gelatine fraction.

of the different condition parameters, a bigger yield difference was noticeable between the experiments The yield of this fraction ranged from 2.54% to 11.56%, which was in line with the results in the literatures and earlier results of our research group. The yield in the comparative experiment (5.07%) was only slightly lower than in the enzymatic pre-treated experiment (5.36%), which suggests that the added enzyme did not play a remarkable role. Contrasting the 2nd fraction with the 3rd fraction, at the 3rd fraction the lowest gained yield was at the 1st experiment also (4.51%), but the highest yields were at the 5th experiment (7.61%) and at the 7th experiment (7.89%), which means at medium extraction time. The statistical influence of factors A and B was shown again by the p-value. According to the p-value, neither the extraction temperature nor the extraction time had a statistical influence (p-value of factor A = 0.393; p-value of factor B = 0.086) on the yield of the 3rd fraction. Unexpectedly, the yield of the 3rd fraction was the highest in the comparative experiment (8.17%), however just a bit.

Summarizing the overall extraction efficiency, it was not high, just 14% ± 3.45% in average. In case of the 10th experiment, it was 15.51%, which was slightly higher than the average of the enzymatic extractions, but almost the same as the 5th experiment, which outcome was 15.22%. In contrast of our expectations, the enzyme did not increase the gelatine extraction from the collagen. For this detection one explanation could be that during the enzymatic treatment of the collagen the set pH was not ideal, thus the enzyme could not be activated well. The optimal interval of the pH of the Protamex® endoprotease enzyme is really narrow and the processing lasted for 24 hours with regular, but not constant control. The processing equipment, which was used, could provide another,explanation. The set of the temperature was not punctual, ± 2°C differences from the set temperature could happen, which could cause relevant influence on the gelatine yield, taking that in account that the extraction temperature differences were undoubtedly small. In addition, the time interval for heating up the gelatine fraction till the desired temperature could be also crucial - significantly change the yield if the heating part to the final temperature took longer or shorter period. As well as, the raw material size was not equal in our storage bottle, which means that the used raw material size differed in each experiment. On the top of the storage bottle the pieces were bigger, while on the bottom, they were smaller. From smaller pieces the extraction is better, therefore it can have a notable effect on the yield.

Previous study of our research group presented an optimized process for gelatine extraction with similar technical processing conditions like in this thesis, just they used an enzymatic

defatting step with a lipolytic enzyme Lipolase 100 T® and their used enzyme amount was higher, they used 1% of enzyme based on the dry matter of the raw material and during each gelatine extraction the conditioned raw material was mixed with water in a ratio of 1:8 (w/v), and their gained yield was remarkably higher. Their minimum yield of gelatine (21.1%) was at the minimal extraction time and temperature (60 mins and 64°C), while the maximum yield (32.3%) was reached at maximum extraction time and temperature (180 mins and 80°C).[80] Rafieian et al. also found that the extracted gelatine from CDR was not considerable, their yield prediction was 10.2%, but on the other hand they observed that the gained gelatine showed high quality.[26] Erge et al. also reported in their study that the extraction time and temperature affected the most the gelatine yield, and in their optimized process (24 hours with 3% HCl at 10°C, followed by alkaline conditioning with 4% NaOH for 48 hours at room temperature and finally extracted by water extraction for 250 minutes at 80 °C) their maximum yield was 15.34%.[77] Rammaya et al. observed a very similar yield of gelatine, 16.03% (but it was based on wet weight basis, which indicates that the dry based yield is lower, but without exact data about the water content no more can be said). They defatted the mechanically deboned chicken meat just with water at 35°C, which was followed by a 25°C running water. The demineralization of the material went under the same conditions like in this study, but the conditioning occurred with an alkaline method (4.0% NaOH, 72 hours at room temperature) and the extraction was with water at pH 4 at 80°C for 2 hours.[81]

	Degree of Freedom	**Sum of Squares**	**Mean Squares**	**F-Value**	**p-Value**
Response: Yield of the 2nd fraction (%) = -2.59 + 0.0519 factor B (°C) + 0.02387 factor A (min)					
Regression	3	59.21	19.737	11.43	0.011
Factor A (Extraction Time)	1	8.955	8.955	5.19	0.072
Factor B (Extraction Temperature)	2	50.255	25.127	14.56	0.008*
Error	5	8.632	1.726		
Total	8	67.841			
Response: Yield of the 3rd fraction (%) = -0.56 + 0.0347 B (°C) + 0.00478 A (min)					
Regression	3	7.098	2.366	2.27	0.199
Factor A (Extraction Time)	1	4.735	4.735	4.53	0.086
Factor B (Extraction Temperature)	2	2.363	1.181	1.13	0.393
Error	5	5.222	1.044		
Total	8	12.32			

Table 12. Analysis of variance of the experimental design for gelatine yields.

6.2 Gelatine gel strength

The gained gelatine fractions physicochemical characterisation was examined to determine the quality and the possible field of usage of them. All the properties were measured at least three times and then an average was calculated.

The results are shown in Table 13.

Number of experiments	Number of extractions	GS (Bloom)	DV (mPa·s)	WHC (%)/ WHC (mL/g)	FBC (mL/g)	EC (%)	ES (%)	AC (%)	FC (%)	FS (%)	GP (°C)	MP (°C)	Temperature interval of viscous state (°C)
1.	2nd	208	3.2	-	6.9	43.3	96.2	-	-	-	15.6	35.2	19.6
	3rd	231	5	-	7.2	46.6	94.5	-	-	-	22.1	35.5	13.4
2.	2nd	241	3.6	-	7.7	48.1	98.1	-	54	12	18.9	35	16.1
	3rd	297	6.9	33.2/ 8.3	5.0	44.1	96.2	-	60	4	22.2	34.2	12
3.	2nd	334	4.5	38.4/ 9.6	4.6	44.1	100	0.01	36	2	19.9	37.8	17.9
	3rd	281	5.6	36.8/ 9.2	4.2	45.8	92.6	-	52	2	22.8	37.2	14.4
4.	2nd	217	3.9	-	5.4	45.6	100	-	-	-	21.8	36.4	14.6
	3rd	295	7.6	32.4/ 8.1	5.3	50	94.7	-	42	0	23.8	34.6	10.8
5.	2nd	256	4.1	37.2/ 9.3	5.6	44.8	100	-	32	0	19.9	37	17.1
	3rd	200	4.4	41.6/ 10.4	7.6	45.8	100	-	36	0	19.9	35.4	15.5
6.	2nd	278	4.9	37.6/ 9.4	7.8	45.8	100	-	50	4	21.8	35.3	13.5
	3rd	267	7.2	38.9/ 9.7	8.3	43.9	100	-	30	0	23.7	35.5	11.8
7.	2nd	271	4.4	37.7/ 9.3	8.8	47.5	96.4	-	42	0	19.3	37.5	18.2
	3rd	217	4.4	30.9/ 7.7	7.6	46.6	92.6	-	40	0	19.1	35.7	16.6
8.	2nd	341	4.5	39.3/ 9.8	9.5	45.6	96.2	-	44	4	20.9	32.3	11.4
	3rd	274	5	24.2/ 6.1	8.7	46.6	98.1	-	44	2	20.2	35.5	15.3
9.	2nd	289	3.4	34.3/ 8.6	8.1	44.8	96.2	0.004	42	4	18	34.4	16.4
	3rd	268	4	21.7/ 5.4	7.7	44.8	100	-	44	4	22.4	32.8	10.4

10.	2nd	304	4.2	36.0/ 9.0	6.2	46.6	96.3	-	46	2	20.6	35.5	14.9
	3rd	308	5.5	30.3/ 7.6	7.9	44.8	96.2	-	46	0	18.8	32.4	13.6

Table 13. Properties of the 2nd and 3rd gelatine fractions in each experiment. In all cases the following parameters were measured: gel strength, dynamic viscosity, water holding capacity, fat binding capacity, emulsification capacity and stability, ash content, foaming capacity and stability, gelling point, melting point, clarity and digestibility. Some parameters could not be measured due to the lack of the gelatine sample.

The range of the GS of the 2nd fraction was from 208 to 341 Bloom and the 3rd fraction was between 200 and 297 Bloom, which means the gained Bloom values were mostly in the high Bloom value category (medium Bloom values are between 150-220 Bloom, high Bloom values are above 220 Bloom).[78] In general, the quality of the 3rd gelatine fraction was weaker than the 2nd gelatine fraction. The 341 Bloom, the highest GS, was measured at 68°C and at 40 mins. But at 60°C and at 60 mins the gained GS had a very similar value, 334 Bloom. It was a surprising result, because we expected the highest GS at the highest A and B factor value. However, the possible human errors which were discussed at the yield could occur here also, which could cause inaccuracy in the circumstances and affected the gelatine quality. In the comparative experiment the GS was 304 Bloom, which was also in the high Bloom value category. The effects of both factors in both cases are shown in Figure 23. and in Table 13. None of the factors had statistical influence on the results (2nd fraction: p-value of factor A = 0.176; p-value of factor B = 0.346; 3rd fraction: p-value of factor A = 0.72; p-value of factor B = 0.518).

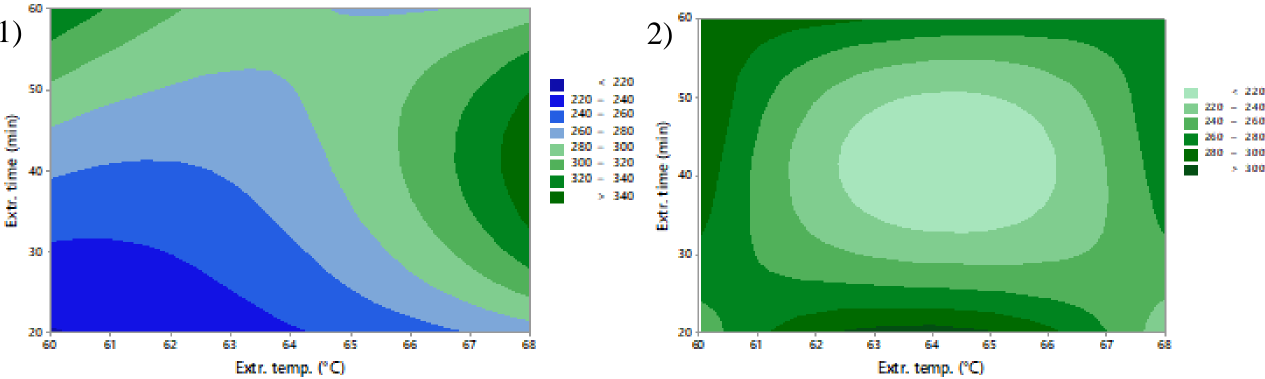


Figure 23. 1) Layered graph of the effect of A and B factors on the gel strength of the 2nd gelatine fraction. 2) Layered graph of the effect of A and B factors on the gel strength of the 3rd gelatine fraction.

Compared with the results in the literatures about CDRs gelatine, Rafieian et al. measured even higher Bloom values, their overall range was between 320 and 570 Bloom.[26] This interval was from 281 to 1176 Bloom in case of Erge et Zorba study, whom found that NaOH concentration during the alkaline pre-treatment and the extraction time had the most important influence on the GS.[77] Similar extraction conditions were used by Rammaya et al. with a slightly modification, that they used during conditioning of the raw material a higher concentration of NaOH solution for a longer time than Erge and Zorba, and their results became outstandingly lower, around 62 Bloom, which value was in the low Bloom value category.[78,80,81] In previous studies by our research group, the obtained Bloom values were between 8 and 158 Bloom.[80] In an earlier thesis in our research group, where the effect of the extraction temperature (from 60°C till 80°C) was determined at constant extraction time (60 mins), the observed GS were also low, between 50 and 146 Bloom.[82]

	Degree of Freedom	**Sum of Squares**	**Mean Squares**	**F-Value**	**p-Value**
Response: Gel strength of the 2nd fraction (Bloom) = 4.528 + 0.01383 factor B (°C) + 0.00545 factor A (min)					
Regression	3	8127	2709	2.03	0.228
Factor A (Extraction Time)	1	1442	1442	1.08	0.346
Factor B (Extraction Temperature)	2	6686	3343	2.51	0.176
Error	5	6662	1332		
Total	8	14790			
Response: Gel strength of the 3rd fraction (Bloom) = 6.177 + 0.0105 B (°C) + 0.00194 A (min)					
Regression	3	1838.8	612.9	0.4	0.762
Factor A (Extraction Time)	1	748.2	748.2	0.48	0.518
Factor B (Extraction Temperature)	2	1090.7	545.3	0.35	0.720
Error	5	7751.2	1550.2		
Total	8	9590			

Table 14. Analysis of variance of the experimental design for gelatine gel strength.

6.3 Gelatine viscosity

The viscosity is the second most important commercial physical property of a gelatine.[72] For determining the effects of the factors on the viscosity, the p-value was checked, which were the followings in term of the 2nd fraction: 0.695 in case of factor A and 0.595 in factor B; and in term of the 3rd fraction: 0.981 in case of factor A and 0.32 in factor B. It means none of these factors had a statistically relevant influence on the DV outcome. The overall range of the DV of the 2nd fraction was between 3.2 and 4.9 mPa·s. The low-viscosity gelatines usually yields less and a more brittle texture gel, while gelatines with high viscosity obtain a tough and extensible gel. Therefore, the high-viscosity gelatines have a greater commercial value and preferred at many applications. The comparative experiment result

was 4.2 mPa·s, which showed a little bit higher viscosity profile then the enzymatic pre-treated experiment (4.1 mPa·s). It could be explained by the fact, that the enzyme could break the collagen chains and caused lower MW peptide chains. According to Figure 24. the trend was that the viscosity was higher by the increased extraction temperature and time, but at the highest extraction temperature and time the DV suddenly dropped back. In case of the 3rd gelatine fraction the range was from 4 to 7.6 mPa·s. This fraction showed a much better viscosity property than the 2nd fraction. At the 3rd fraction, by the increasing extraction temperature a reduction could be observed in the DV. From previous studies, well-known that on the DV of the gelatine, the degree of polydispersity, the MW of the collagen chains

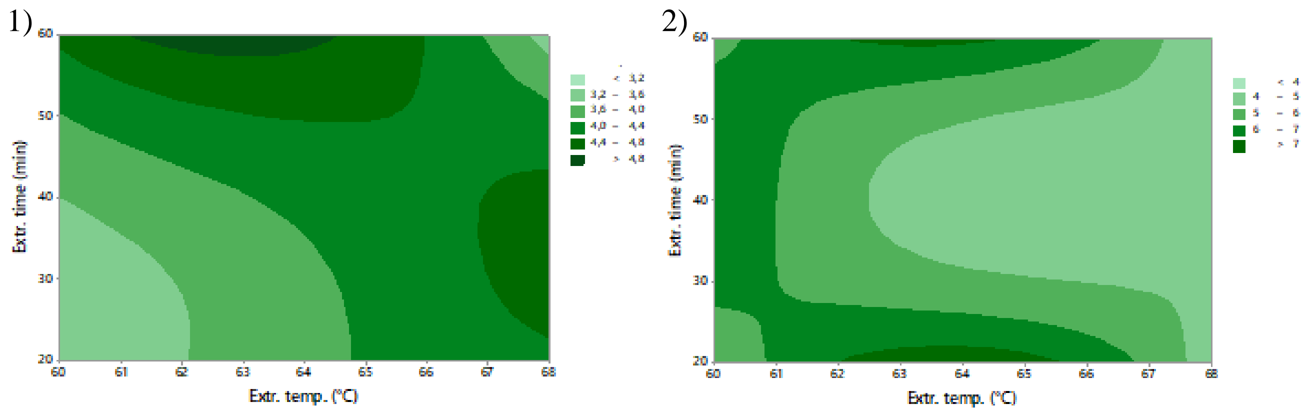


Figure 24. 1) Layered graph of the effect of A and B factors on the dynamic viscosity of the 2nd gelatine fraction. 2) Layered graph of the effect of A and B factors on the dynamic viscosity of the 3rd gelatine fraction.

and the MWD have a great impact. Lower values were performed at higher extraction time and temperature, which could mean that in those gelatine fractions the MWD was greater, which can be explained by partially broken collagen chains, thus resulted shorter peptide chains. Rafieian et al. produced gelatine solution with 5.55 ± 0.19 mPa·s, which was a little bit better than the gained gelatine in this thesis, but the difference is insignificant.[72] In previous thesis, done at our research team, the gained viscosity was worse, only around 1.96 and 2.48 mPa·s.[82] In an earlier published study by our research group, the measured DV of CDRs gelatine was also much lower, in the best case it was only 2.75 mPa·s.[80] Contrasting with this report, the acquired viscosity in this study showed a great improvement.

	Degree of Freedom	Sum of Squares	Mean Squares	F-Value	p-Value
Response: Dynamic viscosity of the 2nd fraction (mPa·s) = 0.69 + 0.0093 factor B (°C) + 0.0029 factor A (min)					
Regression	3	0.4639	0.1546	0.37	0.78
Factor A (Extraction Time)	1	0.135	0.135	0.32	0.595
Factor B (Extraction Temperature)	2	0.3289	0.1644	0.39	0.695
Error	5	2.0983	0.4197		
Total	8	2.5622			
Response: Dynamic viscosity of the 3rd fraction (mPa·s) = 3.69 + 0.0308 B (°C) + 0.0003 A (min)					
Regression	3	2.8883	0.96278	0.42	0.748
Factor A (Extraction Time)	1	2.8017	2.80167	1.22	0.32
Factor B (Extraction Temperature)	2	0.0867	0.04333	0.02	0.981
Error	5	11.5117	2.30233		
Total	8	14.4			

Table 15. Analysis of variance of the experimental design for gelatine dynamic viscosity.

6.4 Ash content

Due to the lack of the amount of samples, the AC measurement was done by only two samples, which had the highest yield. The preparation of the raw material was similar in all cases; therefore, it could be assumed that there was no high vary in the AC among the samples (AC mainly depends from the raw material and the preparation process). As we expected due to the profound purification of the CDRs before the gelatine extraction, the AC in our gelatines were really small 0.01 and 0.004%. Comparing with our research team earlier study (between 0.63 and 0.67%), it was a much cleaner gelatine. In contrary, Mokrejš et al. obtained a significantly higher AC value, their overall range was from 2.1 to 6.7%.[80] Rafieian et al. acquired high AC gelatine (4.41%), as a result of poor purification pre-treatment on the raw material (just filtration was done by a Whatman No. 4 filter paper).[72] Erge et Zorba, also gained similar low AC to our study, their result was 0.14%.[77]

6.5 Gelling and melting points

The overall range of melting point was very similar at both fractions, they were between 32 and 38°C. At both fractions, the highest melting point was measured at the 3rd experiment, where the extraction temperature was the lowest (60°C), but the extraction time was the highest (60 mins). In term of the 2nd fraction, the lowest value was performed in the 8th experiment, while at the 3rd fraction the lowest value was at the 9th and the 10th experiment (without enzymatic pre-treatment). As known, the melting point highly depends from the MW, MWD, the used NaOH concentration during the pre-treatment of the raw material and the interactions, which are determined by the amino acid composition and the ratio of α/β

chains present in gelatine.[77,83] The GS also shows a significant dependence on these properties, which can cause that the highest Bloom values connected with the highest melting points. On this physicochemical property there was only one presented result in literature by Erge et Zorba, whom maximum obtained melting point was 33.71°C (at 3.0 g/100 ml NaOH, 70°C, 150 mins).[77] Comparing with this result, the gained melting points were higher in this study. At our laboratory, the earlier gained melting point on the same raw material was between 29.8 and 33.2°C, which are close to the results found by Erge et Zorba.[82]

Generally, the GP was at least 10.4°C and maximum 19.6°C less than the melting point at the fractions, however the 2nd fraction (range: 11.4-19.6°C) showed a wider range and higher difference between the melting and the GP than the 3rd fraction (range: 10.4-16.6°C). Results obtained by an earlier master student showed similar values, in that study the range was from 15.55 till 18°C.[82] In previous literatures, there was just one research team, Erge et Zorba, who measured the GP of the gained gelatine out of CDR, and their maximum result was 25.15°C (at 3.0 g/100 ml NaOH, 58°C, 150 mins), which was higher than our results (the maximum in our research was 23.8°C). Unfortunately, their minimum value was not reported, therefore the two methods can not be completely compared. In their measurement, they first heated the gelatine solution from 10ºC to 45ºC and then cooled back to 10ºC by a scan rate of 2ºC/min. They also found that the effecting factor on the GP is the concentration of the NaOH used at the raw material chemical pre-treatment.[77] In our experiments we heated the gelatine solution up to only 35ºC and the cooling rate was not defined. Primarily, the GP divergence between the gelatines could be explained by the intrinsic difference in the protein structure of the raw material, the used distinct pre-treatments and the range of the MWD. If the range of the MWD is bigger, the GP is lower, cause the lower MW fractions prevents the higher MW fractions from the formation of the triple helixes.[77,88]

6.6 Water holding capacity and fat binding capacity

Although for food applications the WHC is an important parameter because it affects on the flavour and texture of food, just some studies are devoted to its determination. Our gelatine fractions showed an overall range between 5.4 and 10.4 mL/g. Earlier master thesis result at our research team had similar result, its interval was between 5.8 and 6.7 mL/g.[82] Rafieian et al. received very similar results, their gelatine fraction's WHC was 8.59 ± 0.6 mL/g. The gelatine's ability for binding water is strongly depending on the size, shape, amino acids

composition, protein conformation, surface hydrophobicity/polarity and the presence of lipids, carbohydrates and amino acid residues on the surface. [72]

Similarly, the FBC is a rarely measured property, although it has a significant importance at the food product texture and other food quality (as the interaction between oil and other components). The FBC value is mainly affected by the raw material, processing conditions, composition of additives, particle size and temperature. In this study the gained FBC values were between 4.2 and 9.5 mL/g., and the lowest values were measured in the 3rd and 4th experiments. Comparing with the earlier result of our research team, where they gained values between 2.9 and 3.45 mL/g, it was high.[82] Rafieian et al. observed an even lower fat-binding ability for CDRs, only 0.67 mL/g. The difference can be due to variation in the presence of nonpolar side chains, which bind the hydrocarbon side chain of oil.[72]

6.7 Surface properties of the gelatines

The FC and FS are important properties of the gelatine due to its application as a foaming agent in commonly food products as foamy jellies, marshmallows and premixed coffee beverages. Foaming properties of the gelatine could be influenced by raw material, intrinsic properties of protein, its compositions and conformation in solution and at the air/ water interface. Comparing the FC and FS results of our gelatines with the available data from CDR gelatines, our samples had a lower FC values, ranged between 30 and 60%. Comparing the results with Rafieian et al. results (only study where the FC and FS properties were measured), our results had to be recalculated according to their calculation formula – the FC was calculated by the followings: the volume after whipping was expressed as a percentage of the initial (before whipping) volume. According to this equation, our samples had an FC value between 130 and 160%, while Rafieian et al. gained 323%, which is double of our result. The FS property was also measured differently by Rafieian et al. (they determined the FS as the volume of foam remaining after 30 min, expressed as a percentage of the initial foam volume). In this term, our FS were between 0 and 22%, while in Rafieian et al.'s report it was a higher value, around 44%.[72]

The emulsifying properties of the prepared gelatines did not show significant differences, neither the EC and the ES. They were characterized by very good EC values, in a range of 43.3 and 50%, and excellent ES values, from 92.6 to 100.0%. Unfortunately, no previous literature was published about the emulsifying properties of the gelatines out of CDRs, thus no comparison can be done according to the same raw material source.

7 EVALUATION OF THE RESULTS AND BENEFITS OF THE MASTER THESIS

7.1 Evaluation of the results

In the focus of this thesis is the CDRs, which are so far largely ignored in the gelatine production despite of their high collagen content. The very few literatures, which dealt with this raw material for gelatine production, determined only limited physicochemical properties as GS, yield, AC and viscosity. However, in this study a wider functionality prospect is obtained including WHC, FBC, FC, FS, EC, ES, MP and GP, which are important characteristics in the further processing of gelatines, especially in the food industry. Table 16. shows a comparison of the individual analyses performed in this study with other works dealing with alternative raw materials under different processing conditions. The selected values, obtained in this study, into Table 16. were according to the highest yield, highest GS and closest GS to 260 Bloom, the ideal Bloom value for jelly products. Due to this selection the 9th experiment has both the highest yield and closest GS to the ideal 260 Bloom, while the 8th experiment 2nd fraction had the highest Bloom value. The appendix XI. introduces the extraction conditions in each case.

Reference literature	Yield (%)	GS (Bloom)	DV (mPa·s)	WHC (mL/g)	FBC (mL/g)	EC (%)	ES (%)	AC (%)	FC (%)	FS (%)	GP (°C)	MP (°C)
Diploma thesis sample (DTS) 9th experiment 2nd and 3rd fractions	20.6	289/ 268	3.4/ 4	8.6/ 5.4	8.1/ 7.7	44.8/ 44.8	96.2/ 100	0.004/ -	42/ 44	4/ 4	18/ 22.4	34.4/ 32.8
DTS 8th experiment 2nd fraction	14.4	341	4.5	9.8	9.5	45.6	96.2	-	44	4	20.9	32.3
[66] average	15.5[b]	119	6.9	-	-	-	-	0.25	-	-	-	-

[67] at 60°C	13.8	79	6.52	-	-	-	-	-	-	-	-	-
[68] at 70°C	-	-	3.0	5.0	1.06	36.8	85.7	-	20.0	4.4	-	-
[69] 14th experiment	19.8	310	6.9	-	-	-	-	1.45	-	-	-	-
[70] Test 4	7.83[b]	295	-	-	-	-	-	1.91	-	-	-	-
[72]	-	520	5.55	8.59	0.67	-	-	4.41	323	44	-	-
[74] 3rd experiment	33.9	248	5.82	-	-	-	-	-	-	-	-	39.3
[76] Chicken skin gelatine (CSG)	2.2[b]	352	7.5	-	-	-	-	0.4	190	47	23	30
[77]	15.3	1176	-	-	-	-	-	0.14	-	-	25.2	33.7
[79] Chicken head gelatine (CH)	52.3[c]	248/ 200	-	-	-	-	-	0.05/ 0.03	20/ 25	5/ 4	26.2	33.7
[80] 72 h: enzyme conditioning, 80°C: extraction temperature, 180 h: extraction time	38.6	158	2.17	-	-	-	-	4.24	-	-	-	-
[82] at 70°C	-	76	1.96	5.8	3.45	-	-	0.63	-	-	15.2	33.2
[68, 71] Commercial porcine gelatine (CPG)	-	260	2.4	4.43	0.42	30.7	94.4	-	62.3	14.4	~31.8	~32.6

[68, 71] Commercial bovine gelatine (CBG)	-	260	3.5	6.42	0.71	57.7	88.9	-	55.1	13.2	~31.6	~32.2
[71] Common carp skin gelatine (CC)	-	181	5.91	1.76	3.28	-	-	1.1	2.45	1.83	17.9	28.2
[76] Tuna skin gelatine (TSG)	11.3[b]	336	5.0	-	-	-	-	1.2	46	68	22	30
[73] optimum	21.3	206	-	-	-	-	-	-	-	-	-	-
[75] on water bath	11.7	250	-	-	-	-	-	-	-	-	-	33.9
[76] Frog skin gelatine (FSG)	11.3[b]	363	14.1	-	-	-	-	1.2	143	59	28	43
[79] Turkey head gelatine (TH)	62.8[c]	368/ 333	-	-	-	-	-	0.06/ 0.03	33/ 36	7/ 6	28.2	34.2

Table 16. Comparison of the individual analyses performed in this study with other works dealing with alternative raw materials under different processing conditions. In the table grey colour indicates camel gelatine, yellow the fish gelatines, blue the chicken gelatines, green the commercial beef gelatine, red the commercial porcine gelatine, purple the duck gelatine, orange the frog gelatine and the aqua the turkey gelatine. [b] means that the yield was calculated based on wet weight basis, [c] means that the yield was calculated based on the dry weight of the collagen content.

7.1.1 Yield

In this study the total yield of the gelatine varied between 8.74–20.58%. The gained gelatine yield showed no significant difference with most of the gelatines out of alternative sources: duck (11.7% by Kim et al.)[75], frog (11.3% by Aksun Tümerkan et al.)[76], tuna (11.3% by Aksun Tümerkan et al.)[76], and camel (21.33% by Al-Kahtani et al)[73]. Although, the comparison was not always clear due to the fact, that in the studies performed by Aksun

Tümerkan et al., Taufik et al. (the gelatine was extracted from chicken feet skin and the yield was 15.5%)[66], Almeida et al. (the gelatine was extracted from skins and tendons of chicken feet and the yield was 7.83%)[70] and Rammaya et al. (the gelatine was obtained from mechanically deboned chicken meat and the yield was 16%)[81], the yield was computed by the percentage of the weight of the dry gelatine to the wet weight of the fresh skin. Mokrejš et al. with the enzymatic pre-treatment by 0.4% Polarzyme enzyme for 48 hours obtained a significantly higher yield, 37.15%.[74] Very high yield was reported by Du et al. from TH (62.8%) and CH (52.3%), who applied both alkaline (0.1M NaOH) and acidic (0.05M CH_3COOH) pre-treatment on the raw material. However, in this study the yield was calculated in the percentage of the dry weight of the collagen in the raw material, which explains the higher value in the result. The yield of 15.3% was achieved out of chicken (mechanically deboned chicken meat) after alkaline pre-treatment by 4% NaOH in the study executed by Erge et Zorba.[77] In contrast, after the acidic pre-treatment the gained gelatine yield was significantly less, varied from 2.2% till 13.75%. The lowest yield (2.2%) was performed from chicken skin by Aksun Tümerkan et al. by using 5% HCl for 24 h at room temperature.[76] Almeida et al. got a higher yield (7.83%) with 4% CH_3COOH acidic pre-treatment for 16 h from skins and tendons of chicken feet, and the highest yield (13.75%) was from the chicken legskin treated with 3% CH_3COOH for 24 h from Sompie et al.[67,70] Using combination of alkaline and acidic pre-treatment on chicken legskin (regardless of the chicken age) and on mechanically deboned chicken meat, the observed yield (15.3-16.5%) was similar as the yield after alkaline treatment.[66,81] The highest yield was undoubtable reached by enzymatic pre-treatment, which was also used in this study. Mokrejš et al. also used Protamex® enzymatic pre-treatment on chicken feet and CDR, and the yields were 19.8% and 23.2% respectively, which is remarkably similar to our results.[69,80] Compared to the yield after utilizing Polarzyme enzyme, the yield was almost double higher (37.15%) than after Protamex® enzyme, which indicated that by Polarzyme enzymatic treatment the yield could be outstandingly enhanced.[74]

In conclusion, our three-stage gelatine extraction out of CDRs led to the preparation of three different gelatine fractions with an overall yield up to 20.6%, which corresponded to the most observed yield value in literature, where gelatine was gained from chicken or other alternative ABPs. The scientific hypothesis of gelatine yield has thus been confirmed.

7.1.2 Gel strength

Well-known from the available literatures that the quality of the alternative gelatines is influenced by several factors, including species, age of the animal, their living conditions, the type of tissue being processed (skin, bone, paw, feet), the method of conditioning (acids, alkalis, enzymes), extraction conditions (temperature, time, pH), the method of drying the obtained product and mostly by the representation of chains with MW approximately 100 kDa. (The higher proportion of lower MW than 100 kDa chains causes a weaker forming helix-like structure during the cooling, while the lower proportion cause stronger helix-like structure.)[90] The commercially used gelatines (porcine and bovine) in the food industry for jelly production have usually 260 Bloom value, which is classified as a high Bloom value. This gelatine is likely to use, due to its versatile properties; it is good also for soft jellies and hard jellies depending on the added amount of it. The 9th experiment 3rd fraction had a very similar GS to this ideal value, and on the other hand, the highest yield was also obtained at this experiment, which made this fraction a great alternative instead of the mammalian gelatines. Similarly, the gelatine extracted from duck skin, CH from both research teams (Du et al. and Gál et al.) showed a very identical Bloom value to this ideal 260 Bloom; 250 Bloom, 248 Bloom (extracted in the first stage at 50°C) and 248 Bloom respectively.[74,75,79]

The overall range of our samples GS was between 200 and 341 Bloom. Contrasting our results to other alternative raw materials, the gelatines gained from fish tissues mostly had a much lower Bloom value (low or medium Bloom value).[90] As shown in Table 16., the CC skin gelatine obtained by Ninan et al. had 181 Bloom (medium Bloom value), while the TSG gained by Aksun Tümerkan et al. had a significantly higher Bloom value, 336 Bloom (high Bloom value).[71,76] The highest Bloom values, apart the chicken gelatines, were prepared from frog skin (363 Bloom) and TH (368 Bloom).[76,79] Due to the increased interest in effective utilization of underutilized resources and industrial waste in order to reduce production cost and environmental hazards, several new alternative sources appeared in literatures as camel-bone. It occurred to be proven as a possible replacement for commercial gelatine with its high GS (206 Bloom), which is higher than most fish gelatine Bloom value, but lower than our results.[73,90] In term of chicken gelatines, the highest GS was performed by Erge et Zorba from mechanically deboned chicken meat (1176 Bloom) by alkaline pre-treatment method, which was almost four times higher than our and other literature results.[77] The acidic treatment on the chicken raw materials prior to the extraction step resulted a wide range of Bloom values, from 63 to 520 Blooms.[67,70,72,76] The lowest values

belong to the experiments from chicken feet skin and mechanically deboned chicken meat pre-treated by both alkali and acid; 119 Bloom and 63 Bloom respectively.[66,81] Earlier studies, where the enzymatic pre-treatment was used, showed very similar results to our outcomes; range between 158 Bloom and 310 Bloom.[68,69,74,80]

7.1.3 Dynamic viscosity

The gelatines according to their DV are categorized into three group: 1) low-viscosity gelatines (<3.5 mPa·s); 2) medium-viscosity gelatines (3.5–5.5 mPa·s); and 3) high-viscosity gelatines (5.5< mPa·s).[90] The prepared gelatines viscosity in this study varied from 3.2 to 7.6 mPa·s, which could be classified as medium and high viscosity gelatines, while the CPG and CBG have much lower (2.4 to 3.5 mPa·s) values. Very high viscosity value was reported by Aksun Tümerkan et al. for frog gelatine (14.1 mPa·s).[76] In term of fish gelatines, the viscosity was also high, proved by Aksun Tümerkan et al. with tuna (5 mPa·s) and Ninan et al. with CC (5.91 mPa·s).[71,76] The chicken gelatines viscosity from different chicken parts with different pre-treatment conditions did not show a significant difference (range is from 5.5 to 7.5 mPa·s), except in two cases: 1) gelatine gained from chicken skin by Mokrejš et al. used Polarzyme enzymatic pre-treatment (3 mPa·s); and 2) gelatine from CDR by Protamex® enzymatic pre-treatment from Mokrejš et al. (2.17 mPa·s).[68,80]

7.1.4 Ash content

During the utilization of gelatines in the food industry, the AC of the gelatine (based on the dry matter) can not exceed the 2%, while the pharmaceutical industry allows up to 3%.[69,91,92] Generally, the gelatines gained from alternative sources (where the AC was measured) were characterized by a low AC, varied from 0.004% till 1.91%, which means all of them can be a good replacement of the commercial gelatines for food applications. Exceptions were the studies on the preparation of gelatines from CDRs, where Mokrejš et al. and Rafieian et al. reported 4.24% and 4.41% AC respectively, due to the high mineral content in the bones.[72,80] In this study, prior to the enzymatic pre-treatment on the raw material,, a thorough purification was done (all non-collagenous substances, fat and minerals were removed) to avoid the high AC in the obtained gelatines in order to fulfil our aim and be able to use these gelatines for jelly production.

7.1.5 Water holding capacity

However, the WHC of the gelatine was an important parameter in term of food applications, just a very few studies dealed with its determination. The gelatine fractions in this study showed WHC values in the range of 5.4 to 10.4 mL/g. In contrast to the CBG (6.42 mL/g) and CPG (4.43 mL/g), our gelatines had a very similar values.[72,90] Gelatines prepared from CC skin showed a very low WHC value; 1.76 mL/g.[71] Mrázek et al. obtained a significantly lower WHC (from 3.85 to 5.58 mL/g) by using Polarzyme enzymatic pre-treatment. Although, in this study the extraction temperature had a significant positive influence on the WHC value.[68] As previously mentioned, Rafieian et al. executed similar results (8.59 ± 0.6 mL/g) from CDRs to our values.[72]

7.1.6 Fat binding capacity

In terms of FBC, our samples ranged between 4.2 and 9.5 mL/g. These values were high compared to the commercially used gelatines, which had a value of 1.23 ± 0.08 mL/g.[72] Among previous literatures, only limited determinations could be found. Gelatines presented by Ninan et al. out of CC skin showed also a higher FBC value; 3.28 mL/g.[71] Mrázek et al. obtained approximately identical results to the commercial gelatines (from 0.87 to 1.26 mL/g) by using Polarzyme enzymatic pre-treatment.[68] As above mentioned, Rafieian et al. reported a low FBC value (0.67 ± 0.08 g/g) from CDRs.[72]

7.1.7 Melting point

Compared to CPG and CBG, where the range of the melting point was usually between 21 and 34°C, the melting point of our prepared gelatines was slightly higher, from 32.3 to 37.8°C.[72] Gelatine samples prepared out of fish had a lower melting point than our result; CC skin gelatine had 28.2°C, while tuna skin had 30°C.[71,76] Beside the TSG, Aksun Tümerkan et al. also determined gelatine out of frog, which had a significantly higher melting point comparing with any raw material, its value was 43°C.[76] The gelatines made of poultry sources (duck and turkey) had a similar results to each other; 33.9°C and 34.2°C respectively.[75,79] Gelatines from CH had an overall range between 33.7°C and 39.3°C; while gelatine out of chicken skin and CDR had 30°C and 33.7°C respectively.[74,76,77,79] Generally, it can be said that frog and poultry gelatines had significantly higher melting point than commercial gelatines, nevertheless fish gelatines had similar values to the commercial ones.

7.1.8 Gelling point

In case of standard bovine and porcine gelatines, the GP was usually 5°C lower than the melting point.[90] In term of our gelatines, this difference was significantly higher, between 10.4°C and 19.6°C, which means all our prepared gelatines from any fraction had a notable lower GP value (15.6-23.8°C). In contrast to the commercial gelatines, all the gelatines from other raw material had a much higher melting and GP difference, similarly to our results, except in term of Du et al., where the gelatine was extracted from TH (difference was only 6°C).[79] Compared to other alternative sources, frog gelatine had 15°C, chicken gelatines had 7-7.5°C, while fish gelatines had 8°C (tuna skin) and 10.3°C (CC skin) difference.[71,76,77,79]

7.1.9 Foaming capacity and stability

Our prepared gelatines FC values were ranging from 30 to 60%. These values mean that our samples had medium and good foaming capability. Regarding the stability of the whipped foams (FS), the gelatine fractions showed a very low stability (from 0 to 4%) except the 2nd fraction in the 2nd experiment, which had 12%. Comparing the FC and FS results of our gelatines with the available data about alternative and traditional types of gelatines, our prepared samples foaming properties are comparable. CPG had 62.3% of FC and 14.4% of FS, while the CBG had 55.1% of FC and 13.2% of FS.[68] Depending on the preparation conditions and chicken sources, the FC and FS values showed a great variety. CSGs had FC 17.8–61.1% and FS 4.4–38.9%, CH gelatines had FC 20.1-24.7% and FS 4.4-4.8%, CDR gelatine had FC 323% and FS 44% (in this study the FC and FS were calculated by a different equation as above-written in the 6.7 chapter.)[68,72,79,90] In term of fish skin gelatines, FC was between 2.45 and 46% and FS was from 1.83 to 68%. According to data, the worst foaming abilities belong to CC.[71,76,90] TH gelatines had FC 33-36% and FS 6-7%, while FSG had a much higher FC value, 143% and FS 59%, but here again a different calculation was applied; the foam formation ability was calculated as the volume ratio of foam liquid, and the foam stability was calculated as the ratio of the initial volume of foam to the volume of foam after 30 min.[76,79]

7.1.10 Emulsification capacity and stability

Information on EC and ES of alternative gelatines are rare in the literature. In this study, the emulsifying properties of the highest yield gelatines were very good: the EC was 44.8% at both fractions, while the ES were 96.2% and 100%, which were excellent values. In term of

the gelatine with the highest Bloom value, the EC was 45.6% and the ES was 96.2%. The obtained results in this study were slightly higher than the results reported by Mrázek et al. from chicken skin (EC: 36.8%; ES: 85.7%).[68] According to Gál et al. study most of the fish gelatines had higher EC (between 51-57%), but lower ES (59-92%) values than our gelatines.[90] Contrasting our results with the commercial gelatines, the values were higher than in case of CPG (EC: 30.7%; ES: 94.4%), but lower than in term of CBG (EC: 57.7%; ES: 88.9%).[68]

7.2 Benefits and importance of the master thesis

The importance of our work lies in the fact that it deals with the processing of chicken waste as CDRs into gelatines; this represents a new alternative gelatine raw material, which is processed by a non-traditional biotechnological method using a commonly available food proteolytic enzyme. Another new element was in this study, that the gelatine was obtained by three-stage extraction, which significantly increased the overall yield of the prepared gelatines. Each fraction had different surface and gel-forming properties, however all of them had high GS and relatively similar gelling and melting points to mammalian gelatines, which nominates them as a good replacement for mammalian gelatines in the food industry.[83,90]

7.2.1 The applications of the CDR gelatine

Generally, the usage of the gelatines depends on the GS. Chicken gelatines with a high GS and a viscosity of about 4.0–5.5 mPa·s are suitable, for example, for the production of gelatine desserts, confectionery products as gummy bears, extruded marshmallows, candy jellies due to its gel formation property, also great for the utilization in the meat industry as aspics, binder for meat emulsions, ham, jellies, canned meat, sausages, broths due to its emulsion stabilizer and binding agent properties, in the manufacture of dairy products as low-fat butter spreads, panna cotta, jelly items, whipped cream, yoghurts, cheese as a syneresis stabilizer, in the production of frozen food products as ice creams, frozen desserts for its reducing water loss agent property, and in the baking industry in the production of foams in cakes, pies and breads as a stabilizer. In the biomedical industries as encapsulating material for drugs or chemicals. For the preparation of hard gelatine capsules, all the prepared chicken gelatines with a viscosity of 4.5–6.5 mPa·s are suitable; for producing soft gelatine capsules, gelatines with a viscosity of 2.5–4.5 mPa·s are sufficient. Due to the chicken gelatines high GS, there are also various applications in the biomedical field. For

example, they can be used as hydrogel carriers for delivering bioactive macromolecules, producing membranes, microspheres, and nanoparticles, encapsulating carriers for the controlled release of biologically active substances, or delivering cell transplants for tissue repairs. In addition, it can be used in the photographic industry for preparing emulsions.[39,69,83,84]

On other hand, the AC of the gelatine solution is also important; almost 0% of AC allowed at the photography industry, up to 2% allowed in the food industry and up to 3% is permitted in the pharmaceutical industry.

7.2.2 Sensory properties of the produced jellies

Including in this thesis, from the obtained CDR gelatines, jelly candies were prepared based on a recipe from The Candy Plus Sweet Factory, s.r.o. (Rohatec, Czech Republic). Eight ingredients were used during the jelly production (in same ratio at each case, just the type of the gelatine or the mould shape changed): water, gelatine powder, sugar, glucose syrup, flavour, colorant, juice concentrate and citric acid. In one dish, the gelatine powder was dissolved in water in a warm water bath at 50°C until a homogeneous viscous liquid was formed. At the same time, in another dish the glucose syrup was mixed with sugar and water. When its Brix value was around 80°, the two pots were mixed. Then the colorant, flavour, citric acid and juice were added to the mixture and stirred for approximately 1 min. The prepared solution was poured into a starch mould and placed on a table for 12 hours at room temperature. After 12 hours, the jellies were purified from starch and polished with carnauba wax. The prepared jellies went under sensory testing to contrast their crucial properties to the commercially available jellies. In our case, these jellies were made from 260 Bloom porcine skin gelatine with the same preparation method. As defined by the Institute of Food Technologists (IFT), "sensory evaluation is a scientific method used to measure, analyses and interpret responses as perceived through the senses of sight, hearing, touch, smell and taste". During the sensory testing the following properties were examined by 13 people aged between 26 and 65 with similar cultural background (all of them were from Central Europe): 1) appearance; 2) chewiness; 3) colour; 4) smell; 5) taste and finally 6) the overall acceptability. [85,86,87]

The average results at each sample is visible in Table 17., where the **A** sample was the jelly from 260 Bloom porcine skin gelatine in a bottom-like shape; **B** sample was also from 260 Bloom porcine skin gelatine in a sea creatures form; **C** sample was made out of 289 Bloom

CDR gelatine (9th experiment 2nd fraction) in a bottom-like shape; and **D** sample was out of 268 Bloom CDR gelatine (9th experiment 3rd fraction) in a bottom-like shape.

Jelly	Appearance	Chewiness	Colour	Smell	Taste	Overall acceptability
A	2.3	2.8	1.8	2.9	2.5	2.4
B	1.7	2.9	1.8	2.2	2.5	2.3
C	4.8	2.7	4.5	3.8	3.1	3.8
D	4.8	4.6	4.3	3.9	3.5	4.8

Table 17. The average results at each sample at each criteria.

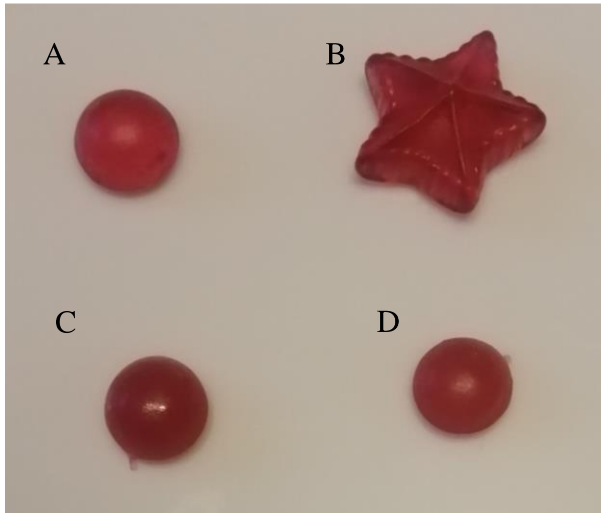


Figure 25. Samples: **A** sample was the jelly from 260 Bloom porcine skin gelatine in a bottom-like shape; **B** sample was also from 260 Bloom porcine skin gelatine in a sea creatures form; **C** sample was made out of 289 Bloom CDR gelatine in a bottom-like shape; and **D** sample was out of 268 Bloom CDR gelatine in a bottom-like shape.

During sensory analysing a seven-point hedonic scale was used. (The survey, which the testers had to fulfil is attached in the appendix X.):

CONCLUSION

This master thesis was inspired by the annually generated huge amount of food waste (one third of the food which are produced for human consumption is waste), especially the can be avoided type of food waste, which are usually the ABPs as the CDRs. The theoretical part first chapter deals with the food waste, their categories, life cycle and the conditions of their utilization. The second chapter is devoted to the type of ABPs, their potential utilization as a secondary raw material and the collagen processing types and conditions out of ABPs. In addition, this chapter introduces the CDRs, which is the main raw material in this diploma thesis for alternative gelatine production. Lastly, the third chapter is dedicated to gelatine, its preparation, analysing, properties and applications.

The aim of this work was to show the utilization of CDRs by-product, which has a high collagen proportion and is an affordable sustainable source for quality gelatine production. During processing, a new biotechnological (enzymatic pre-treatment by Protamex® enzyme) pre-treatment was executed in order to minimize the usage of unnecessary chemicals. Furthermore, the CDR raw material meets the principle of the 21st century waste-free (“green”) management due to the gained gelatines high (~99%) biodegradability and the available utilization of the generated by-products during the gelatine production (for example, the calcium phosphate dihydrate at the bone demineralization can be used in feed mixtures, while the remaining undissolved residues after gelatine extraction can be used as an N-type fertilizer in agriculture).

On the processing parameters of the gelatine extraction an optimization was done by TG, in order to determine greater gelatine properties (yield, GS, WHC, FBC, EC, ES, FC, FS, DV, AC, GP and MP).

Relied on the obtained results, two optimum conditions were selected: the highest yield and at the same time the best usable Bloom value (260 Bloom) at the confectionary industry and the highest Bloom value gelatine fraction. The highest yield was processed in the 9th experiment (68°C extraction temperature and 60 mins extraction time), where the gained Bloom value in the 2nd fraction was 289 Bloom and in the 3rd fraction was 268 Bloom. The highest bloom was received in the 8th experiment 2nd fraction (68°C extraction temperature and 40 mins extraction time), which value was 341 Bloom.

At the end, the results were compared with studies on chicken and other alternative gelatines, and they were comparable, which nominates them as a possible substation for other gelatines

on the market in the pharmaceutical (as nanomicrosphere containers, hydrogels), medical (as encapsulating material for drugs or chemicals) and food industry (as jellies, gelatine desserts, meat emulsions), which is proven by the pioneer sensory testing made on jellies out of CDR gelatines. However, during the candy jelly production for obtaining similar texture to the commercial jellies, a higher bloom value CDR gelatine had to be applied.

Further considerations are suggested on the storage time and particle size of the demineralized raw material and their effect on the extracted gelatine properties. Another future step should be to determine the chemical composition, amino acid content and MWD of the CDR gelatine. In addition, the jelly production could be optimized for gaining better properties for the jelly products and wider the CDR gelatine applicability opportunities in the food and other industries.

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LIST OF ABBREVIATIONS

ABPs	Animal by-products
AC	Ash content
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BBD	Box-Behnken design
BE	Balance error
CBG	Commercial bovine gelatine
CC	Common carp
CCD	Central composite design
CH	Chicken head
CDR	Chicken deboner residue
CSG	Chicken skin gelatine
CPG	Commercial porcine gelatine
CQC	Chain Quality Control
DNA	Deoxyribonucleic acid
DoE	Design of Experiment
DT	denaturation temperature
DTS	Diploma thesis
DV	Dynamic viscosity
EC	Emulsification capacity
EDTA	Ethylene amino tetraacetate
ES	Emulsification stability
EU	European Union
FAO	Food and Agriculture Organization of the United Nations United Nations
FBC	Fat binding capacity

FBPs	Fish by-products
FC	Foaming capacity
FS	Foaming stability
FSG	Frog skin gelatine
GMP	Good Manufacturing Practise
GP	Gelling point
GS	Gel strength
HACCP	Hazard Analysis Critical Control Point
HCl	Hydrogen chloride
IFT	Institute of Food Technologists
MMPs	Matrix metalloproteinases
MP	Melting point
MW	Molecular weight
MWD	Molecular weight distribution
NADH	Nicotinamide adenine dinucleotide
RNA	Ribonucleic acid
SDB	Sodium dodecyl benzene
SDS	Sodium dodecyl sulfate
TC	Thermoplastic collagen
TG	Taguchi design
TH	Turkey head
TLFFD	Three-level full factorial design
TSG	Tuna skin gelatine
UV	Ultraviolet
WHC	Water holding capacity

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APPENDIX I: FOOD WASTE IN AFRICA

Country name	Reference	Study area	Food waste estimate (kg/ capita)
Ethiopia	(Assefa, 2017)	Laga Tafo Laga Dadi town, Oromia	92
Ghana	(Miezah et al., 2015)	Nationwide	84
Kenya	(JICA, 2010)	Nairobi	100
	(Takeuchi, 2019)	Nairobi	99
Nigeria	(Orhorhoro et al., 2017)	Sapele	189
Rwanda	(Mucyo, 2013)	Kigali	164
South Africa	(Chakona & Shackleton, 2017)	Richards Bay, Dundee and Harrismith	18
	(Nahman et al., 2012)	Johannesburg and Ekurhuleni	8-12
	(Oelofse et al., 2018)	Johannesburg	12
	(Ramukhwatho, 2016)	Nationwide	134
United Republic of Tanzania	(Oberlin, 2013)	Kinondoni municipality, Dar es Salaam	119
Zambia	(Edema et al., 2012)	Ndola	78

Annually food waste per kg per capita in Africa.

APPENDIX II: FOOD WASTE IN LATIN AMERICA AND THE CARIBBEAN

Country name	Reference	Study area	Food waste estimate (kg/capita)
Belize	(Inter-American Development Bank, 2011)	Belize City	34
		Caye Caulker	45
		San Ignacio / Santa Elena	95
		San Pedro	36
Brazil	(Araujo et al., 2018)	Nationwide	60
Colombia	(JICA, 2013)	Bogota	70
Mexico	(Kemper et al., 2019)	Nationwide	94

Annually food waste per kg per capita in Latin America and the Caribbean.

APPENDIX III: FOOD WASTE IN ASIA AND THE PACIFIC

Country name	Reference	Study area	Food waste estimate (kg/capita)
Australia	(Arcadis, 2019)	Nationwide	102
Bangladesh	(Salam et al., 2012)	Chittagong	74
	(Sujauddin et al., 2008)	Chittagong	57
China	(Gao et al., 2013)	Beijing	26
	(Gu et al., 2015)	Suzhou	67
	(Li et al., 2021)	Shandong	21
	(Lo & Woon, 2016)	Hong Kong	101
	(Qu et al., 2009)	Beijing	59
	(Song et al., 2015)	Nationwide	23
	(Zhang et al., 2020)	Urban China Total	150
India	(Grover & Singh, 2014)	Dehradun	73
	(Ramakrishna, 2016)	Rajam, Andhra Pradesh	58
	(Suthar & Singh, 2015)	Dehradun	20
Indonesia	(Dhokhikah et al., 2015)	Surabaya	77
Japan	(Food Industry Policy Office, 2017)	Nationwide	64
Malaysia	(Jereme et al., 2013)	Nationwide	112
	(Watanabe, 2012)	Bandar Baru Bangi	71
New Zealand	(Sunshine Yates Consulting, 2018)	Nationwide	61
Pakistan	(JICA, 2015)	Gujranwala (urban)	88
		Gujranwala (rural)	60
Sri Lanka	(JICA, 2016)	Jaffna	118
		Nuwara Eliya	95
		Kataragama	95
		Thamankaduwa	79
		Katunayake	78
		Moratuwa	75
		Kesbewa	75
		Dehiwala Mt Lavinia	75
		Kurunegala	47
		Trincomalee	21
Viet Nam	(Thanh et al., 2010)	Mekong Delta	85
	(Vetter-Gindele et al., 2019)	Da Nang	67

Annually food waste per kg per capita in Asia and the Pacific.

APPENDIX IV: FOOD WASTE IN WEST ASIA

Country name	Reference	Study area	Food waste estimate (kg/capita)
Bahrain	(Alayam, 2018)	Nationwide	132
Georgia	(Denafas et al., 2014)	Kutaisi	101
Iraq	(Al-Maliky & ElKhayat, 2012)	Baghdad	75
	(Al-Rawi & Al-Tayyar, 2013)	Mosul	85
	(Al-Mas'udi & Al-Haydari, 2015)	Karbala	142
	(Sulaymon et al., 2010)	Al-Kut City	138
	(Yasir & Abudi, 2009)	Nassiriya	163
Israel	(Elimelech et al., 2018)	Haifa	94
	(Leket Israel, 2019)	Nationwide	105
Lebanon	(Chalak et al., 2019)	Beirut	105
Saudi Arabia	(SAGO, 2019)	Nationwide	105

Annually food waste per kg per capita in West Asia.

APPENDIX V: FOOD WASTE IN NORTH AMERICA

Country name	Reference	Sector	Food waste estimate (kg/capita)
Canada	(Environment and Climate Change Canada, 2019)	Household	79
United States of America	(U.S. Environmental Protection Agency, 2020a)	Household	59
		Food service	64
		Retail	16

Annually food waste per kg per capita in North America.

APPENDIX VI: FOOD WASTE IN EUROPE

Country Name	Reference	kg / capita food waste estimate
Austria	(Environment Agency Austria, 2017)	39
Belgium	(Flemish Food Supply Chain Platform for Food Loss, 2017)	50
Denmark	(Danish Environmental Protection Agency, 2018)	79
	(Edjabou et al., 2016)	83
Estonia	(Moora, Evelin, et al., 2015)	78
Finland	(Katajajuuri et al., 2014)	67
	(Stenmarck et al., 2016)	64
France	(ADEME, 2016)	85
Germany	(Schmidt et al., 2019)	75
Greece	(Abeliotis et al., 2015)	142
Hungary	(Kasza et al., 2020)	94
Ireland	(Stenmarck et al., 2016)	55
Italy	(Giordano et al., 2019)	67
Luxembourg	(Luxembourg Environment Ministry, 2020)	89
	(Caldeira et al., 2019)	91
Malta	(Caldeira et al., 2019)	129
Netherlands	(The Netherlands Nutrition Centre Foundation, 2019)	50
Norway	(Hanssen et al., 2016)	79
Poland	(Steinhoff-Wrześniewska, 2015)	56
Russian Federation	(Tiarcenter, 2019)	33
Slovenia	(Republic of Slovenia Statistical Office, 2020)	36
	(Republic of Slovenia Statistical Office, 2019)	33
Spain	(Caldeira et al., 2019)	77
		78
Sweden	(Swedish Environmental Protection Agency, 2014)	81
United Kingdom of Great Britain and Northern Ireland	(WRAP, 2020b)	77

Data points relating to households from studies in Europe per year per kg per capita.

Country Name	Reference	kg / capita food waste estimate	Confidence level
Austria	(Caldeira et al., 2019)	31	High
	(Environment Agency Austria, 2017)	26	High
Belgium	(Flemish Food Supply Chain Platform for Food Loss, 2017)	20	Medium
Denmark	(Danish Environmental Protection Agency, 2014)	21	High
Estonia	(Moora, Piirsalu, et al., 2015)	17	High
Finland	(Katajajuuri et al., 2014)	23	Medium
	(Stenmarck et al., 2016)	24	Medium
France	(BIO Intelligence Service, 2010)	17	Medium
	(ADEME, 2016)	32	Medium
Germany	(Schmidt et al., 2019)	21	High
Ireland	(Stenmarck et al., 2016)	56	Medium
Luxembourg	(Luxembourg Environment Ministry, 2020)	21	Medium
Norway	(Stensgård et al., 2019)	5	Medium
Serbia	(Bogdanović, et al., 2019)	6	Medium
Slovenia	(Republic of Slovenia Statistical Office, 2020)	20	Medium
	(Republic of Slovenia Statistical Office, 2019)	20	Medium
Sweden	(Swedish Environmental Protection Agency, 2014)	20	High
		21	High
Switzerland	(Beretta et al., 2013)	40	Medium
United Kingdom of Great Britain and Northern Ireland	(WRAP, 2020b)	17	High

Data points relating to food service from studies in Europe per year per kg per capita.

Country Name	Reference	kg / capita food waste estimate	Confidence level
Austria	(Environment Agency Austria, 2017)	9	High
Belgium	(Flemish Food Supply Chain Platform for Food Loss, 2017)	10	Medium
Denmark	(Danish Environmental Protection Agency, 2014)	30	High
Estonia	(Moora, Piirsalu, et al., 2015)	5	Medium
	(Caldeira et al., 2019)	5	Medium
France	(ADEME, 2016)	26	Medium
Germany	(Schmidt et al., 2019)	6	High
Greece	(Stenmarck et al., 2016)	7	Medium
Italy	(Cicatiello et al., 2019)	4	High
Luxembourg	(Luxembourg Environment Ministry, 2020)	9	Medium
	(Stenmarck et al., 2016)	4	Medium
	(Caldeira et al., 2019)	9	Medium
Netherlands	(Stenmarck et al., 2016)	11	Medium
Norway	(Stensgård et al., 2019)	14	Medium
	(Stensgård & Hanssen, 2016)	14	Medium
	(Caldeira et al., 2019)	13	Medium
		14	Medium
Russian Federation	(Tiarcenter, 2019)	14	Medium
Slovenia	(Republic of Slovenia Statistical Office, 2020)	7	Medium
Sweden	(Swedish Environmental Protection Agency, 2020)	10	High
United Kingdom of Great Britain and Northern Ireland	(WRAP, 2020b)	4	High

Data points relating to retail from studies in Europe per year per kg per capita.

APPENDIX VII: NUTRIENT COMPOSITION AND MINERAL CONTENT OF THE FISH-BY-PRODUCTS

	By-Product Nutrient Composition																								
	(g/100 g) *	Head				Gills				Intestines				Trimmings				Bones				Skin			
		Large		Small		Large		Small		Large		Small		Large		Small		Large		Small		Large		Small	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Meagre	Moisture	64.0	0.5	68.9	0.7	68.3	0.2	74.3	0.4	73.0	0.2	59.2	0.4	63.1	0.7	57.0	0.1	63.2	0.7	40.6	0.2	58.4	0.3	65.3	0.2
	Ash	20.95	0.37	21.27	1.04	15.59	0.98	19.18	0.32	4.77	0.08	2.25	0.05	49.12	1.91	48.51	2.55	21.00	0.58	23.30	1.23	20.24	0.53	15.23	1.06
	Protein	40.41	0.16	47.50	1.25	45.62	0.16	48.46	0.32	59.62	0.08	29.79	0.64	45.87	0.91	45.98	1.92	32.07	0.25	36.41	0.13	75.16	1.87	75.15	0.03
	Fat	28.88	1.60	23.34	0.74	19.71	1.33	21.31	0.45	17.09	0.19	54.05	4.94	3.00	1.50	4.35	0.69	34.96	0.10	31.07	2.10	6.12	0.42	9.61	1.60
	Carbohydrates	9.76	1.65	7.89	1.78	19.08	1.66	11.05	0.63	18.52	0.22	13.91	4.98	2.01	2.59	1.16	3.27	11.97	0.64	9.22	2.44	1.01	0.01	0.99	0.01
	(g/100 g) *	Head				Gills				Intestines				Trimmings				Bones				Head			
		Large		Small		Large		Small		Large		Small		Large		Small		Large		Small		Large		Small	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Gilthead Sea Bream	Moisture	57.3	0.7	62.4	0.2	66.6	0.3	62.9	0.8	67.1	1.0	57.15	0.5	48.6	0.1	53.1	0.2	53.3	0.7	74.5	0.8	53.0	0.5	61.2	0.1
	Ash	18.11	1.24	21.39	1.33	16.60	0.40	17.49	0.30	3.57	0.06	2.62	0.07	45.76	2.29	47.26	0.73	26.62	0.10	27.70	0.58	6.02	0.17	4.36	0.17
	Protein	32.40	0.45	37.19	0.67	31.49	0.42	38.50	1.47	37.23	0.75	26.87	0.35	41.85	1.00	45.10	2.30	34.02	0.98	40.74	1.57	43.16	0.89	49.67	0.11
	Fat	37.08	4.19	28.76	0.47	37.46	1.16	26.69	0.23	43.19	0.35	55.12	0.98	5.45	0.09	4.09	0.33	30.56	0.11	21.47	0.54	46.39	3.45	45.94	0.54
	Carbohydrates	12.41	4.39	12.66	1.56	14.45	1.30	17.32	1.52	16.01	0.83	15.39	1.04	6.94	2.50	3.55	2.44	8.80	0.99	10.09	1.75	4.43	3.56	0.03	0.02

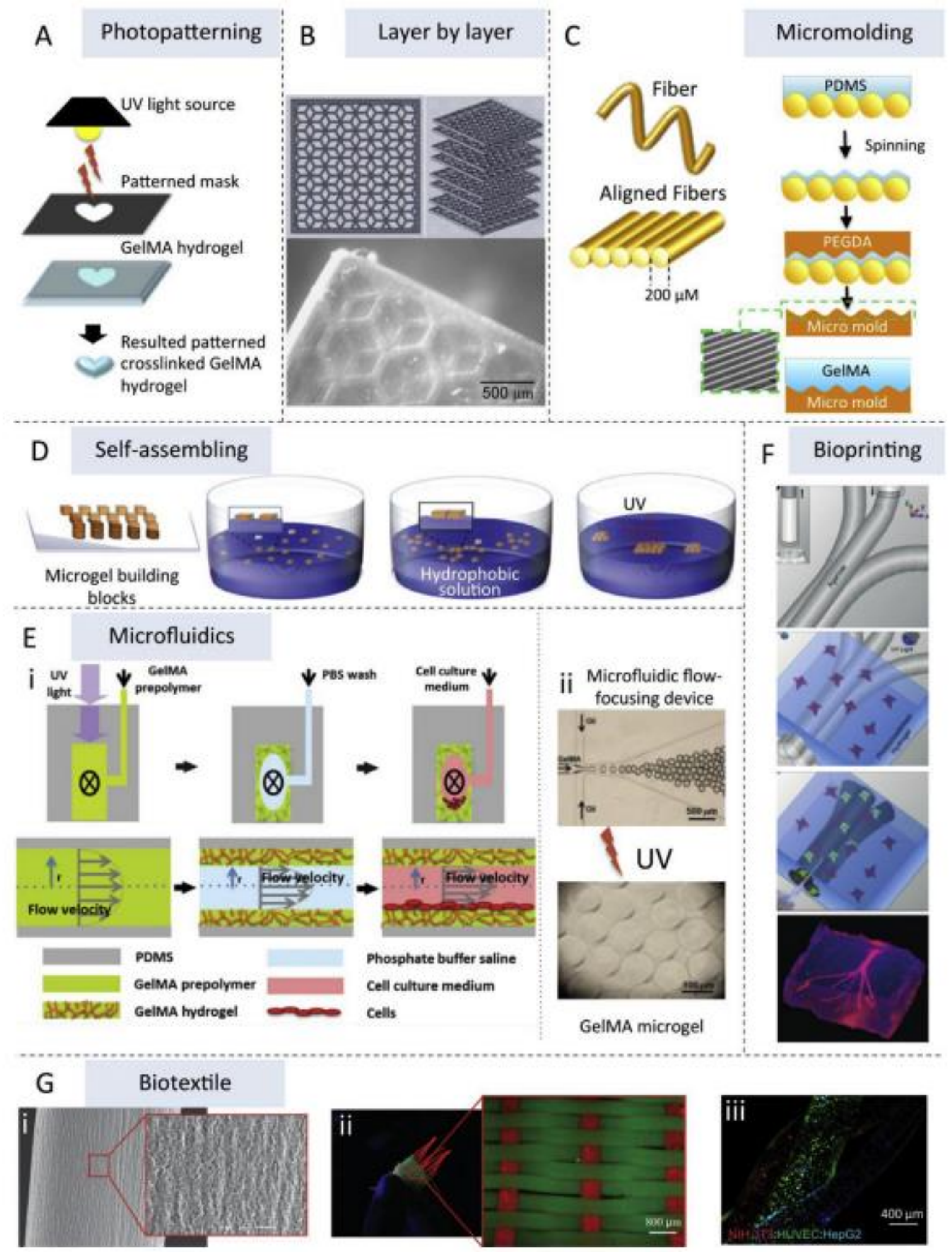
* ash, protein, fat, and carbohydrate contents are expressed on a dry weight basis; carbohydrates were calculated by difference; no statistically significant differences were observed between the different by-products, either between fish species or size classes.

	By-Product Nutrient Composition																								
	(mg/g)	Head				Gills				Intestines				Trimmings				Bones				Skin			
		Large		Small		Large		Small		Large		Small		Large		Small		Large		Small		Large		Small	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Meagre	Calcium (Ca)	5.02	0.72	8.59	1.13	5.88	0.23	6.80	0.12	0.61	0.04	0.58	0.10	12.82	0.34	46.58	1.34	6.39	0.93	9.58	0.57	5.59	0.71	3.90	0.08
	Sodium (Na)	4.07	0.37	4.92	0.56	5.52	0.13	5.92	0.27	2.53	0.12	2.12	0.57	5.87	0.99	3.72	0.43	3.46	0.11	2.76	0.73	4.42	0.28	3.15	0.17
	Potassium (K)	6.31	1.05	8.30	0.87	8.55	1.06	9.34	0.77	8.92	1.03	4.86	1.13	6.92	1.02	6.65	0.66	7.62	0.24	8.92	0.23	8.46	1.03	9.33	1.12
	Magnesium (Mg)	0.24	0.19	1.34	0.44	1.37	0.17	3.52	0.82	2.51	0.55	1.49	0.67	0.71	0.04	2.46	0.25	0.67	0.13	0.45	0.12	0.43	0.03	0.76	0.32
	% RDA/AI (Ca) *	50		86		59		68		6		6		128		466		64		96		56		39	
	% RDA/AI (Na) *	27		33		37		39		17		14		39		25		23		18		29		21	
	% RDA/AI (K) *	13		18		18		20		19		10		15		14		16		19		18		20	
	% RDA/AI (Mg) *	8		42		43		110		78		47		22		77		21		14		13		24	
Gilthead Sea Bream	Calcium (Ca)	8.59	0.43	5.62	1.02	4.52	0.72	7.59	0.33	0.24	0.09	0.67	0.25	42.38	0.11	11.49	0.45	9.23	0.34	7.92	1.10	2.10	0.92	0.50	0.02
	Sodium (Na)	3.68	0.21	3.28	0.21	4.26	0.82	4.54	0.72	3.01	0.79	2.39	1.01	6.81	0.35	6.08	0.26	3.43	0.14	3.79	0.99	3.52	0.13	1.34	0.22
	Potassium (K)	6.47	0.92	6.40	1.02	7.04	0.11	8.16	0.13	4.65	0.15	7.83	1.73	7.09	0.13	8.09	1.00	6.92	0.88	8.35	0.37	7.44	0.32	8.66	0.64
	Magnesium (Mg)	0.86	0.43	0.28	0.29	0.64	0.31	2.49	0.46	1.61	0.17	2.84	0.12	3.34	1.04	0.77	0.25	0.33	0.06	0.30	0.09	0.58	0.36	2.10	0.03
	% RDA/AI (Ca) *	86		56		45		76		2		7		424		115		92		79		21		5	
	% RDA/AI (Na) *	25		22		28		30		20		16		45		41		23		25		23		9	
	% RDA/AI (K) *	14		14		15		17		10		17		15		17		15		18		16		18	
	% RDA/AI (Mg) *	27		9		20		78		50		89		104		24		10		9		18		66	

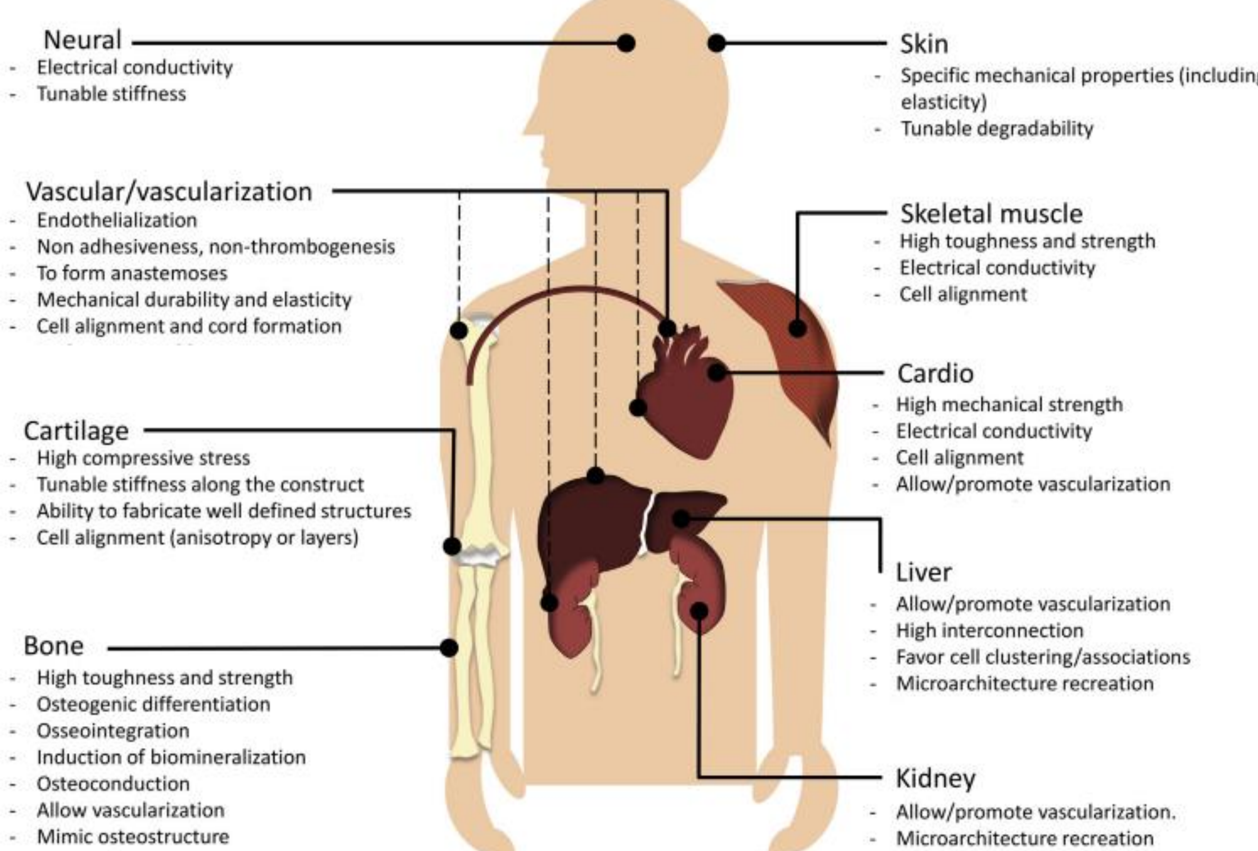
* Mineral concentration was calculated as mg/g of dry matter; RDA/AI percentages for the content of 100 g of dried sample were calculated based on the following values: 1000 mg for Ca, 4700 mg for K, 329 mg for Mg, and 1500 mg for Na

Data for the nutrient composition and mineral composition of fish-by-product samples (head, gills, intestines, trimmings, bones, skin) of meagre and gilthead sea bream fishes.[24]

APPENDIX VIII: OTHER BIOMEDICAL APPLICATIONS OF GELATINE



Microfabrication techniques used to produce gelatine methacryloyl (GelMA) hydrogels constructs.[93]



The specific characteristics and functions of each tissue impose particular tissue engineering requirement.[93]

APPENDIX XI: CONTRASTING THE DIFFERENT GELATINE EXTRACTION METHODS

Reference	Authors	Year	Raw material	Extraction condition
[82]	Tereza Novotná	2022	Chicken bone	Purification of the raw material happened by 1:6 0.2M NaOH at 23°C for 1.5 h and 0.03M NaOH by 1:6 for 3 shaking circles, each for 45 mins, then it was defatted by 1:1 petrolether/ethanol for appr. 2 days. The demineralisation was done by 1:7 3% HCl for 4 days (the HCl was changed in every 24 h). The enzymatic pre-treatment of the purified raw-material was with 0.5% Protamex® in distilled water at pH 6.5-7. The extraction happened in water bath at 4 different temperatures, at 60°C for 3 mins, and at 70, 80 and 90°C for 60 mins.
[77]	Erge, A., Zorba, Ö.	2018	Mechanically deboned chicken meat gelatine	The raw material demineralization occurred by 3% HCl for 24 h at 10°C, which was followed by the residues elimination by different concentration (from 1.8 to 4.2%) of NaOH for 48 h at 25°C. The extraction was carried out in water bath at different temperatures (from 58 to 82°C) and at different time (from 30 mins to 250 mins).
[80]	Mokrejš, P., Gál, R., Pavlačková, J.,Janáčová, D.	2021	Chicken deboner residue	The collagen is purified by 0.2M NaCl at 25°C for 1.5 h and by 0.003M NaOH at 25°C for 15 h. Two defatting steps, one enzymatic one with Lipolase 100 T® enzyme for 48 h at 25°C and the second one is with 1:1 petroleum ether and ethanol in 1:9 ratio at 25°C for 20 h. The conditioning of the purified material was by 1% of Protamex® enzyme at 23°C from 24 to 72 h. Extraction temperature was from 64 to 80°C and time was from 60 to 180 mins.

Reference	Authors	Year	Raw material	Extraction condition
[81]	Rammaya, K.; Ying, V.Q.; Babji, A.S.	2012	Mechanically deboned chicken meat gelatine	Defatting of the raw material by water at 35°C under constant shaking, demineralization by 3% HCl for 24 h at 10°C, then alkaline pre-treatment by 4% NaOH at 25°C for 72 h and the end the extraction was in distilled water under constant shaking at pH 4 for 120 mins at different temperatures (from 60 to 80°C).
[66]	Taufik, M., Triatmojo, S.†, Erwanto, Y.†, Santoso† U.	2010	Chicken feet skin	The purification of the raw material was by 0.1% NaOH for 40 mins, 0.1% H_2SO_4 for 40 mins, 0.4% $(CH_2CO_2H)_2$ for 40 mins in 1:5 ratio in each case. Extraction was in water bath for 24 h at different temperatures (from 45°C to 55°C).
[67]	Sompie, M., & Triasih, A.	2018	Chicken legskin	For curing the raw material 3% CH_3COOH was used for 24 h, then the samples were neutralized and then extracted in five steps from 50 to 70°C, each step lasted for 5 h.
[68]	Mrázek, P., Mokrejš, P., Gál, R., Orsavová, J.	2019	Chicken skin	Separation of non-collagen parts was performed by 1M NaCl and 0.5% NaOH, defatting was obtained by 1:1 petroleum ether and ethanol. Enzymatic pre-treatment of the purified raw-material was with 0.5% Polarzyme 6.0T in distilled water at pH 7.5. The extraction happened in water bath at 5 different temperatures (40, 50, 60, 70 and 80°C) for 60 mins.
[69]	Mokrejš, P., Mrázek, P., Gál, R., & Pavlačková, J.	2019	Chicken feet	Purification of the raw material happened by 1:8 (w/v) 0.1% NaOH at 23°C for 4 shaking cycles, each for 45 mins, then it was defatted by 1:1 petroleum ether and ethanol in 1:6 ratio at 23°C for 32 h. Enzyme conditioning happened by different concentration (0.2–0.8 %, w/w) of Protamex® at 23°C from 24 to 120 h. Extraction was in water bath at 80°C from 1 to 4 h.

Reference	Authors	Year	Raw material	Extraction condition
[70]	Almeida, P. F., Lannes, S. C. da S.	2013	Skins and tendons of chicken feet	Pre-treatment of the raw material was with 4% CH_3COOH for 16 h. Then the extraction was in water bath at 55°C for 6 h.
[71]	Ninan, G., Joseph, J., Aliyamveettil, Z. A.	2012	Common carp skin	The raw material was pre-treated by 0.2% NaOH for 45 mins, 0.2% H_2SO_4 also for 45 mins and then 1% CH_3COOH. Extraction was carried out in water bath at 45°C for 10 h.
[72]	Rafieian, F., Keramat, J., Shahedi, M.	2015	Chicken deboner residue	Salt and alkaline-solutions of the raw material were extracted by 1% (w/v) NaCl solution, then firstly it was soaked in 6.73% (w/v) HCl solution for 24 h at 25°C and at the end it was soaked in distilled water with a residue/water ratio of 1:3 (w/v). The final extraction was carried out in a water bath at 68.8°C and for 1.95 h.
[73]	AL-Kahtani, H. A., Jaswir, I., Ismail, E. A., Ahmed, M. A., Monsur Hammed, A., Olorunnisola, S., Octavianti, F.	2016	Camel-bone	Demineralization occurred in different concentration HCl solution (0, 1.5, 3 and 6%) for 1 to 5 days at 25. It was followed by a 6% HCl pre-treatment for 3 days at room temperature. Extraction conducted under different conditions (temperature was between 40 and 80°C, pH was from 1 to 7, duration was from 0.5 to 3.5 h).
[74]	Gál, R., Mokrejš, P., Mrázek, P., Pavlačková, J., Janáčová, D., Orsavová, J.	2020	Chicken head	Non-collagenous parts were separated by with 1:8 ratio of 0.1% NaOH for 45 mins at room temperature, repeated for 4 times. defatting was obtained by 1:1 petroleum ether and ethanol for 58 h. Enzymatic pre-treatment of the purified raw-material was with 1:10 ratio of different concentration (0.4% or 1.6%) of Polarzyme 6.0T in distilled water at pH 7.5 at room temperature for 24 or 72 h. Two extraction steps were executed: firstly at 80°C for 1 or 4 h, then it was heated at 100°C for 5 mins to inactivate the remaining enzyme, and it was followed by the second extraction was performed at 95°C for 15 or 60 mins.

Reference	Authors	Year	Raw material	Extraction condition
[75]	Kim, T.-K., Ham, Y.-K., Shin, D.-M., Kim, H.-W., Jang, H. W., Kim, Y.-B., Choi, Y.-S.	2019	Duck skin	The raw material was purified by 0.1 N HCl, and then 0.1 N NaOH at 18°C for 24 h at different pH. For neutralization it was washed in tap water at 18°C for 48 h. The gelatine extraction was performed in 4 different methods: 1) in water bath at 60°C for 10 mins; 2) sonication extraction method at 60°C with 40 kHz for 10 mins; 3) superheated steam extraction method at oven temperature of 150°C and steam temperature of 150°C for 10 min; 4) microwave extraction method at 2450 MHz and 200 W power for 10 mins.
[76]	Aksun Tümerkan, E. T., Cansu, Ü., Boran, G., Regenstein, J. M., & Özoğul, F.	2019	Tuna skin	Prior to the extraction step, skins were soaked in 0.1 M NaOH at 27°C with 1:10 skin/solution (w/v) for 1 h and in 0.2 M CH_3COOH 1:10 skin/solution (w/v) ratio at 4°C for 12 h. The extraction was performed in water bath at 45°C for 12 h at 1:10 (w/v) skin/water ratio.
[76]	Aksun Tümerkan, E. T., Cansu, Ü., Boran, G., Regenstein, J. M., & Özoğul, F.	2019	Frog skin	Prior to the extraction step, skins were soaked in 0.2 M NaOH with 1:10 skin/solution (w/v) at 4°C for 30 min three times and in 0.05 M CH_3COOH 1:10 skin/solution (w/v) ratio at 25°C for 3 h. The extraction was performed in water bath at 45°C for 12 h at 1:10 (w/v) skin/water ratio.
[76]	Aksun Tümerkan, E. T., Cansu, Ü., Boran, G., Regenstein, J. M., & Özoğul, F.	2019	Chicken skin	The raw material was defatted by 30% isopropanol at a solid/solvent ratio of 1:10 (w/v) at room temperature for 2 h. Then the material was demineralized with 1% (w/v) NaCl with 1:4 skin/solution (w/v) by gentle stirring for 30 min at room temperature. Then it was soaked in 5% HCl at a ratio of 1:2 skin/solution (w/v) for 24 h at room temperature. The extraction was performed in water bath at a ratio of 1:3 skin/water (w/v) from 45 to 65°C for 15 h.

Reference	Authors	Year	Raw material	Extraction condition
[79]	Du, L., Khiari, Z., Pietrasik, Z., Betti, M.	2013	Turkey and chicken head	Defatting of the raw material occurred by 15 mM $NaHCO_3$ solution at a ratio of 1:4 (w/v) 1 h at 4°C and then centrifuged at 10,000 × g for 10 min at 4°C. Then the material was purified by 0.1 M NaOH at a ratio of 1:10 (w/v) for 6 h at 4°C and 0.05 M CH_3COOH solution at a ratio of 1:10 (w/v) for 18 h at 4°C. Final extraction was performed in 2 stages in water bath: 1) at 50°C for 18 h; 2) at 60°C for 6 h.

APPENDIX X: SENSORY TESTING SURVEY

7-point hedonic scale survey on jellies

Appearance: the physical shape of the product.

Chewiness: indicates the amount of energy needed to chew the product before it can be swallowed.

Colour: the colouring of the product (the colour is a primary characteristic of its palatability because it dominates the way we perceive products and it also has a substantial impact on customer appetite).

Smell: the pleasant of the product's aroma

Taste: the pleasant of the product's flavour

Overall acceptability: is the parameter that informs which product meets the respondents' taste and preference the most.

Jelly	Appearance	Chewiness	Colour	Smell	Taste	Overall acceptability
A						
B						
C						
D						

1 – I like extremely

2 – I like very much

3 – I like

4 – Neither like nor dislike

5 – I dislike

6 – I dislike very much

7 – I dislike extremely