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Laying Hens Heads as an Untraditional Source of Collagen

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Master's Thesis
2021

Tomas Bata University in Zlín
Faculty of Technology

Tomas Bata University in Zlín
Faculty of Technology
Department of Polymer Engineering

Academic year: 2020/2021

ASSIGNMENT OF DIPLOMA THESIS

(project, art work, art performance)

Name and surname:	**Stephen Boahene**
Personal number:	**T19877**
Study programme:	**N0722A130002 Polymer Engineering**
Field of study:	**Polymer Engineering**
Type of Study:	**Full-time**
Work topic:	**Laying Hens Heads as an untraditional Source of Collagen.**

Theses guidelines

1. In the theoretical part of the thesis work out a literature study about technological conditions of gelatines extraction from the different raw collagenous materials, about properties of gelatines with respect to their processing techniques and applications.
2. In the practical part, study the influence of selected parameters of processing of laying hens collagenous tissue on process efficiency.
3. Focus on detailed study of properties of prepared gelatines, which are of high importance for their processing and applications, especially in pharmacy and food industry.
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.

Form processing of diploma thesis: **printed/electronic**
Language of elaboration: **English**

Recommended resources:

Schrieber R.; Gareis H.: *Gelatine Handbook. Theory and Industrial Practice*. Wiley-VCH, Weinheim 2007.
Ockerman H. W.; Hansen C. I.: *Animal By-Product. Processing & Utilization*. Woodhead Publishing, London 2000.
Sarbon, Mhd.N.; Badii, F.; Howell, K.N. Preparation and characterisation of chicken skin gelatin as analternative to mammalian gelatin. *Food Hydrocoll.* 2013, *30,* 143-151.
Widyasari, R.; Rawdkuen, S. Extraction and characterization of gelatin from chicken feet by acid and ultrasound assisted extraction. *Food Applied Biosci. J.* 2014, *2*, 83-95.

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Date of assignment of diploma thesis: **February 1, 2021**
Submission deadline of diploma thesis: **May 14, 2021**

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In Zlín April 1, 2021

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ABSTRAKT

Při produkci drůbežího masa vzniká velké množství vedlejších produktů, jako jsou kůže, hlavy, kosti, krev, vnitřnosti, běháky, peří; mnohé obsahují vysoký podíl bílkovin, zejména kolagenu. Práce se zabývá zpracováním hlav ze slepic nosnic na želatiny. Ze suroviny je nejprve nutné odstranit nekolagenní bílkoviny a tuky. Přečištěná suroviny byla kondicionována proteolytickým enzymem Protamex; želatinové frakce byly extrahovány destilovanou vodou při teplotách 60, 70 a 80 °C za různých dob extrakce (60, 90 a 120 minut). Byl hodnocen vliv podmínek zpracování na výtěžek želatin a na funkční vlastnosti želatin. Pevnost želatinových gelů byla 50–130 Bloom, viskozita 2,0–4,0 mPa.s, obsah popela 1,2–2,3%, vodu zadržující kapacita 11,1–25,0 g/1 g, tuk vázací kapacita 11,0–15,20 g/1 g, pěnivost 1–10%, stabilita pěny 0–2%, emulgační kapacita 40–47% a stabilita emulse 90–100%. Podmínky přípravy a vlastnosti připravených želatina byly srovnány s drůbežími želatinami připravených z jiných případových studií. Želatiny získané z hlav slepic nosnic mohou představovat alternativu k tradičním vepřovým a hovězím želatinám a lze je využít např. v potravinářství či farmacii.

Klíčová slova: extrakce, funkční vlastnosti, kolagen, kuřecí hlavy, procesní podmínky, proteolytický enzym, výtěžek, želatiny

ABSTRACT

The substantial manufacture of poultry meat produces large amounts of by-products such as feathers or skins, heads, bones, blood, viscera, feet viscera, bones and legs) containing significant volumes of proteins, particularly collagen. The preparation of gelatin through the partial hydrolysate of collagen can be a merit since it is derived from the utilization of poultry by-product. The processing of the head of hens were purified and separated from non-collagenous proteins, and fats. Collagen was treated with Protamex enzymes and further extracted by the use of distilled water at temperatures 60, 70 and 80˚C during varying extraction time 60, 90 and 120mins to obtain gelatin fractions. The influence of the processing conditions on the gelatin yield and functional properties of gelatin were evaluated. The gel strength prepared from the hens head was in the range from 50-130 Bloom, the viscosity from 2.0-4.0mPa.s, ash content from 1.2-2.3%, water holding capacity from 11.1-25.0g/1g, Fat binding capacity from 11.0 to 15.20g/1g, foaming capacity from 1-10%, foaming stability from

0-2%, emulsification capacity from 40-47% and emulsification stability from 90-100%. Gelatin were to other gelatins produced from other case studies. Hence, gelatin from hens head has the possibility as an alternative to traditional gelatins (pork and bovine)

Keywords: chicken heads, collagen, extraction, gelatin, functional properties, processing conditions, proteolytic enzyme, yield

ACKNOWLEDGEMENTS

My sincerest and profound gratitude goes to the Almighty God for giving me the strength during this hard times to complete this task. To my supervisor Prof. Ing. Pavel Mokrejs, Ph.D., I want to express my heartfelt and truest appreciation for your guidance, advice and faith you had in me to finish this task successfully. I owe all that you and with that, I am forever be grateful. A big thanks to the laboratory technicians especially Petra Elsikova for the support you gave to me during the course of work.

Not forgetting my study coordinator Miroslava Milickova, I will always be indebted to you. Your quick response to my email, text messages and also your counselling are something that I am never going to forget. Thank you so much Miroslava.

Finally to my family back home in Accra, Ghana, you raised a dreamer and I dedicate this to you. Thank you for imparting so much into me. This is definitely not the end and will still continue to make you all proud.

I hereby declare that the print version of my Master's thesis and the electronic version of my thesis deposited in the IS/STAG system are identical.

Zlin 14th May, 2021 ……

STEPHEN BOAHENE

CONTENTS

INTRODUCTION

The most abundant protein found in mammals is Collagen and these protein can be located in various products of animal origin, such as bird feet (Nomura et al., 1996; Lin & Liu, 2006a; Liu et al, 2001; Alves & Prudencio-Ferreira, 2002); fish scales, mammalian skin and tendons (Nimni & Huang, 1993). In regards to the skin, the most abundant collagen is the Type I collagen, with ligaments, bones and tendons, representing 90% of the dry matter (Shimokomaki & Olivo, 2002). Some studies have shown the commonness in extract of this type of collagen in chicken feet. (Liu et al, 2001; Hashim et al, 2014; Lin & Liu, 2006a).

The substantial manufacture of poultry meat produces large amounts of by-products such as feathers or skins, heads, bones, blood, viscera, feet (Zhu et al., 2010). The production of livestock feeds are mostly produced by the usage of these byproducts. It is quiet unfortunate that the most common practice in some developing countries is by incineration or landfill of the byproducts. Also on the other hand, poultry byproducts such as heads, paws and stomachs are mostly fried or cooked and consumed as traditional meals (Toldra et al., 2016). Poultry by-products are rich in enzymes, proteins and lipids (Hansen and Ockerman, 2000; Raju et al., 1997) and contains economic and nutritional potential (Rintala and Salminen, 2002).

Collagen used for gelatin production as traditional sources includes skins, bones, and connective tissues from beef or pork origin (Morrison et al., 1999). The pig skin accounts 46% of the production of gelatin gained, from bones 23%, 2% accounts gelatine made from other sources, and from beef skin 29% (Gelatine Manufacturers of Europe's, 2008).

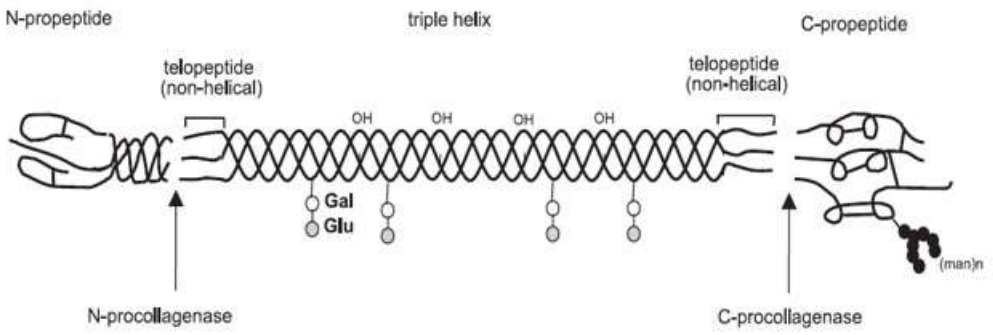


Fig. 1. Molecular structure of fibrillary collagens with the various subdomains as well as the cleavage sites

This situation turn to generate the usage of alternative sources of collagen such as skins, fish, bones and scales, which are becoming more important. Chicken, duck skin or turkey are another alternative source of poultry by-products. About 15% of live weight of the animal are represented by chicken skins (Sheu and Chen, 2002). Thus, chicken skins should be considered as by-products with significant economic potential. Another options of chicken skins application and byproducts from converting chicken meat to chicken breast, is the use as a raw material for the removal of gelatine. Gelatine can be defined as a partial collagen hydrolysate with a wide range of possible functions based on its distinct structure (Norziah et al., 2009). Elasticity, viscosity and stability in foods are supplied by this distinctive biopolymer (Zhou et al., 2006). Gelatine gel has the capability of "melting in the mouth" with glutinous agents of plant origin, such as pectin, alginate, agar, carrageenan and starch, lack (Bazawine and He, 2003). If the concentration of solution made from gelatin is appropriate for network forming, transition from sol to gel takes place (Kaur et al., 2002). Gelatine is, due to its unique properties, used as a food ingredient in various types of products (e.g. marshmallows, ice creams, desserts, aspics, lunch meats, yogurts, coating, puddings, sauces), also in the biomedical field (three-dimensional tissue regeneration products and wound dressing) or in many applications of non-food products (e.g. paper manufacture, photography, coating, sizing, matches) (Chatterjee and Bohidar, 2005; GMIA, 2012). The Use of Gelatine is also applied in the pharmaceutical industry for soft and hard capsules production (Karim and Bhat, 2008). In 2011, the global gelatin consumption was 348,000 tons and was expected to be as much as 450,000 tons in 2018 (Sheela, 2014). The quality of gelatine is determined mainly by the gel strength of the gelatin which is expressed in the Bloom value (Binsi et al., 2009). Also other important functional characteristics of gelatine mainly in the food industry includes water holding and fat binding capacity, emulsifying, clarity, foaming properties and viscosity.

I. THEORY

1 ANIMAL BY-PRODUCTS PROCESSING AND UTILISATION

Animals are reared and killed to provide nutrition from meat for humans, and few of what we consider 'meat' animals would be allowed to exist except as examples of species in zoos without this utilization. There is often a shift in its diet and nutrition to include a greater percentage of tasty, well-balanced protein from animal sources as the economic stature of a country or race increases. The people's size (particularly height) usually tends to increase with this consumption of a well-balanced protein from meat.

There still remains a great quantity, often in excess of 50%, of animal by-products of rather unusual physical and chemical characteristics which are not part of the normally consumed steaks and roasts regardless of the natural advantages of animal food products. By the quantity of fat and bone that traditionally remains with the carcass at the slaughter stage, this large quantity of material can then be increased; therefore, it is obvious about the involvement of enormous tonnage of this material.

There is the demand by the economics of the world's meat industry that in order for the livestock industry stay economically competitive with vegetable protein sources, there is the need for animal by-products to be utilized. A valuable source of potential revenue is lost if animal by-products are not effectively utilized, and the increasing and added cost of disposal of these products is sustained by the industry. The cost of the live animal often surpass the selling price of its carcass currently; therefore, to generate the profit for the meat slaughtering operation, the value of the by-products must pay the expense of slaughter.

The meat industry, in addition to the economics involved has the commitment to get rid of waste by retrieving as much of the animal as possible, since this is an important natural resource and people who are responsible are expected to be productive agents of the resources placed at their allocation. It takes energy to organize these chemical by-product structures, since much of the world's vegetation can only be harvested by animals. In the organized form, it would befit humans to utilize these by-products, where possible, and not allow the conversion of these by-products into to a lower energy state. Animal by-products which are non-utilized would, of course, create a major inventive and disastrous public-health related problem. The major influences in upgrading public health in the last century have probably been the effective utilization of animal by-products and water- and sewage-treatment plants.

An effective utilization of by-products has been a major source of the modern livestock industry and often been stated that ‘‘all of the pig is used except the squeal and the curl in the tail’’. But a great deal more can be done, in spite of this, since more than 2% of the remains is often unexplained for and is lost mostly to the server.

Animal by-products are easy to be classified by some products such as hides, and products such as steaks can easily be eliminated from such a classification. However, there is the difficulty to classify other products such as lard or liver.

1.1 Poultry By-products

Poultry processing industry by-products are significantly edible tissue and bone from the remains, inedible remains that are provided, feathers and eggshells (see Table 1). From the production phase of the industry, poultry droppings are considered as a by-product.

Separation of meat from bone by mechanical means is discussed. Large amounts of mechanically deboned poultry residues are obtained in this process. This residue may differ depending on the tissue deboned and the process of deboning, but an average composition is 13% fat and 17% protein (Jackson et al., 1982). The protein is mainly of collagen and protein extracted may be attained or achieved with solvents such as sodium chloride (NaCl) or treatments by alkali followed by precipitation of acid. The tumbling of deboning residue at a pH of 10.5 at 23°C (73°F) for 30-60 minutes are involved in one extraction procedure. The liquid extract can be separated from the solid residue by centrifugal force and then the pH can be adjusted to 5.5 by adding 1N HC1 to precipitate the soluble protein after the previous treatment. Centrifuging or screening is done to separate the coagulated protein from the liquid as a protein curd. By-product meal from poultry is produced by wet or dry rendering of ground clean parts of the remains such as racks, condemned chickens from deboning operations, feet, head, underdeveloped eggs and viscera, but not including feathers, except in low amounts as might occur in processes of normal procedures. The material is highly rough due to grit from gizzards. Back priming with fat is sometimes used to facilitate the material, improve heat transfer and to reduce wear. Continuous processing equipment (Anon, 1979) containing vertically mounted double-walled disc with a tubular shaft fitted and a temperature of 113-116 °C (235-240 °F) given by a steam jacket is used by some of the larger processors. Two cookers are run in series in some operations. Firstly, the product is usually broken down (often with cage mill grinders), then it is screened (often pellet-mill whirly

cleaners) through a rough screen to take off ferrous material and then ground to an equilibrium between those of flour and cornmeal and then finally passed through a fine screen. The rough material from the screening is returned to the grinder. The moisture level is reduced in a separate dryer to approximately 8% in rendering and then to remove excess fat so usually a fat level of approximately 10% remains, the product is pressed.

Fig 2. Flow chart showing Broiler offal by-product processing. 1 lb = 0.454kg

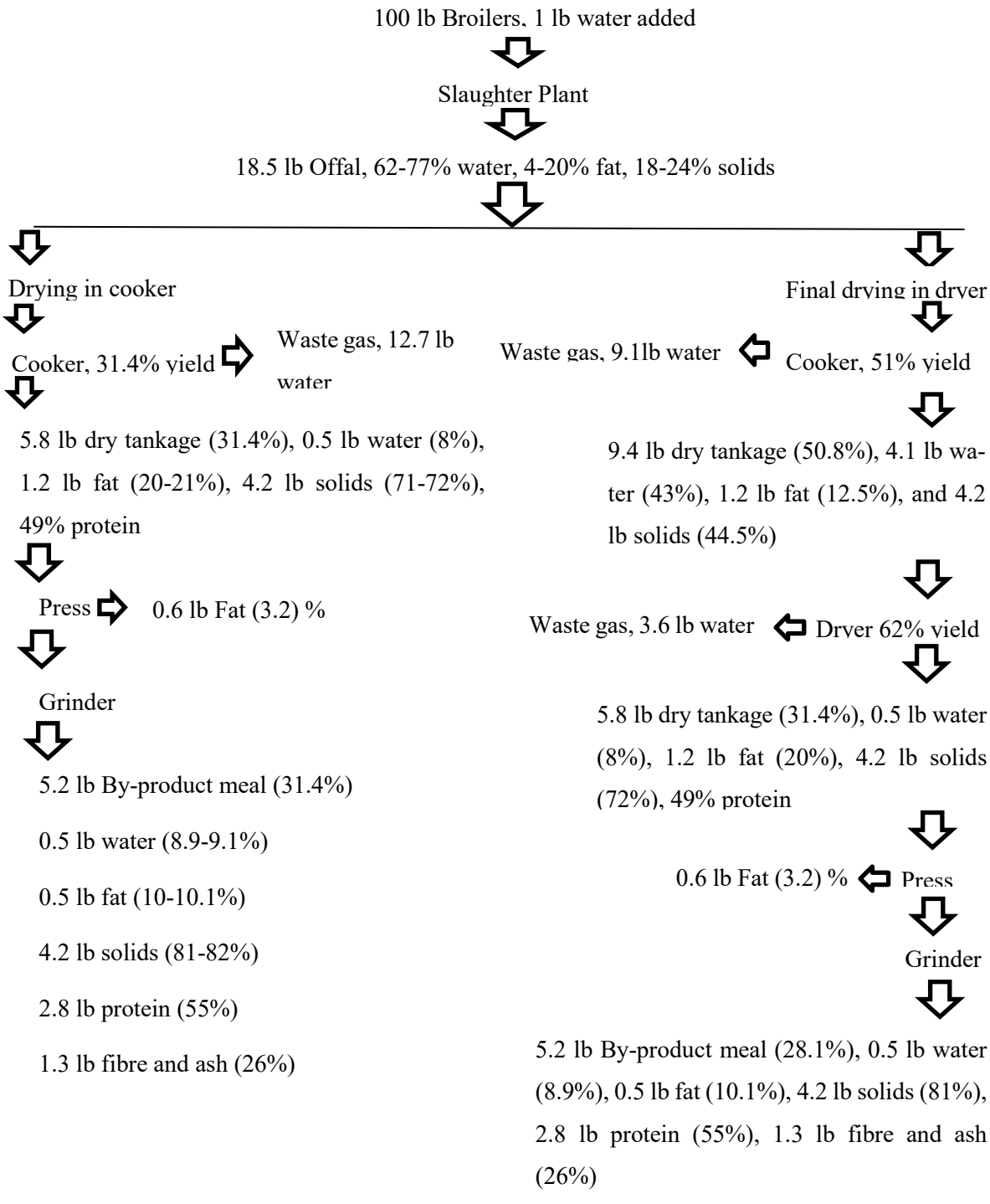


Table 1. Yield of by-products from broilers, fowl and turkeys

MATERIAL	**PERCENTAGE OF LIVE WEIGHT**		
	BROILERS	FOWL	TURKEY
Waste yield from Pantry			
Offal	17.5 (15-20)	17.0 (17-18)	12.5
Blood	3.5 (3.2-4.2)	3.0	3.5
Feathers	7.0 (4.8-7.5)	7.0	7.0
Feathers, wet	22.0	20.0	14.0
Mixed (dry feathers)	28.0	A	23.0
Water pick up			
Offal	1.0	1.0	-
Blood	-	-	-
Feathers	15.0	13.0	7.0
Mixed	16.0	A	7.0
Total waste yield			
Offal	18.5	18.0	12.5
Blood	3.5	3.0	3.5
Feathers	22.0	20.0	14.0
Mixed	44.0	A	30.0
Water evaporated			
Offal	12.7	10.6	7.5
Blood	2.7	2.3	2.7
Feathers	16.5	14.5	8.1
Mixed	31.9	A	18.3
Dry product(8% Moisture)			
Offal	5.8	7.4	5.0
Blood meal	0.8	0.7	0.8
Feather meal	5.5	5.5	5.9
Mixed	12.8	A	11.7
Pressed product (1% fat)			
By-product meal	5.2	4.3	4.2
Grease	0.6	3.2	0.8

α No advantage to mixing prior to cooking, since fat level will require pressing prior to grinding. Source: Lortschet et al. (1957)

After slaughter and before scalding, the more important feathers of the wing and tail are removed, but currently, in the U.S, this is not done in operations of commercial base. The remains then proceed to the scalder. The scalding normally requires 90-120 seconds with a slightly higher temperature (60°C (140°F)) and longer time being used for turkeys and the scalding water temperature for chickens is normally between 53° and 58°C (127-136°F).

In the range 60-63°C (140-145°F) waterfowl carcasses are scalded for 2 minutes, and they are dipped in wax at 91°C (195°F) if the feathers are not shaved, and then into water which is cold. In the ease of feather removal, the age of ducks is also important. They may be held for as long as 12 hours if soaked in a solution containing 6.8 kg (15 lb) of salt (NaCl), 473 ml (1 pint) hydrochloric acid (HC1) and 113 L (30 gallons) of water after feathers have been shaved. Using a mild soap, the feathers are usually washed to remove dirt and blood. If suitable, potassium permanganate ($KMnO_4$), hydrogen peroxide (H_2O_2) or chlorine (Cl_2) which are examples of bleaching agent can also be utilized. To remove objectionable odours, Stoddard's solvent (a gasoline of high flash-point) is sometimes used. Finally, to encourage fluffing, the feathers are thoroughly rinsed in clean water, blow-dried and then into groups size (length and weight), they are sorted by air currents. Some feathers are lightly sprayed with mineral oil to replace some of the natural oils that have been removed in processing depending upon their ultimate use.

Because of the development of synthetic fibres and plastic foam, the use of feathers in bedding has declined, but body feathers, generally from waterfowl are used for good-quality bedding. When in use, basic requirements for good bedding feathers are maximum volume and for storage, minimum volume is mostly used. Ability to return to their original volume, fluff ability, low absorption, softness, drap ability, warmth, cleanliness, fire-resistance, and durability are other desirable characteristics. Also, when feathers are used for decorative purposes, shape, size, plumage patterns and colour play an important role. Cock pheasants are in demand because of their brightly coloured feathers for this reason. In most cases, the feathers are trimmed, dyed and bent to desired patterns.

The feathers are carefully hand-selected for sporting equipment. For example in application for fletching arrows, sturdy feathers from mature turkeys are used. To assure proper rotation of the arrow, feathers on an individual arrow must all come from either the right or left wing. In badminton, stiff feathers are also used for shuttlecocks and for the manufacture of artificial lures for fishing, other selected feathers are used.

Feathers can also be applied in fertilizer and mulch production. They gradually and slowly release their nitrogen as a result of their decomposition. They should be ploughed under to prevent unwanted distribution by the wind.

Feathers are made up of protein (keratin) which is complex, which must be dilapidated by hydrolysis to make them digestible. The feathers are washed with water after collection from the processing plant. In some operations, by mechanical pressure rather than heat, they are dewatered. They are steamed, wet-cooked for hydrolysis under pressure with constant agitation after the removal of some of the water, and under 2-3 atmospheres pressure for 1-2 hours, they are processed in dry-renderers (cookers). After this, the feathers are then cooled and dried, in a tube-like drier that has been changed to an air drier, and then ground. The meal which is ground goes through a detector made of metal and then is screened to remove rough-like particles. The cooking time and pressure (amount of hydrolysis) affects the digestibility of feather meal, usually with more thorough processing proceeding in higher availability of biological values and amino acids.

Table 2 Composition of Poultry by-products

Protein	Minimum as specified
Moisture	Maximum 10%
Fibre	Maximum 4%
Ash	Maximum 15%
Acid	
Insoluble ash	Maximum 4%
Fat	Maximum or minimum as specified
Grind	95% through U.S. No. 10 screen 100% through U.S. No. 7 screen

1.2 Statistical data regarding by-products from animals

The contribution of animal meat and animal product to a crucial part of the human diet is very effective because they give essential nutrients from vegetables and their products which

cannot be easily derived. They however, provide a way for malnutrition reduction and household food increase and food security [1]. Over the last two decades, there has been an increased in many parts of the world (including Africa, Asia, Europe and United States of America), the demand for meat and meat products and this has led to quick flow in production of livestock for sustainable food security [2]. The process where livestock is converted to meat in slaughter-houses often produces a lot of by-products and further utilized by humans as food or secondary by-products which are reprocessed for both industrial and agricultural uses [3]. Although animal by-products account for about two-third of the animal after slaughter, the yield of these by-products has been reported to account for about 10% to 15% of the value of the live animal in developed countries, [4]. Dressed remains such as heart, liver, blood, spleen, kidney, rumen contents, meat trimmings and fats are primarily by-products from animals that include all parts of a live animal that are not part.

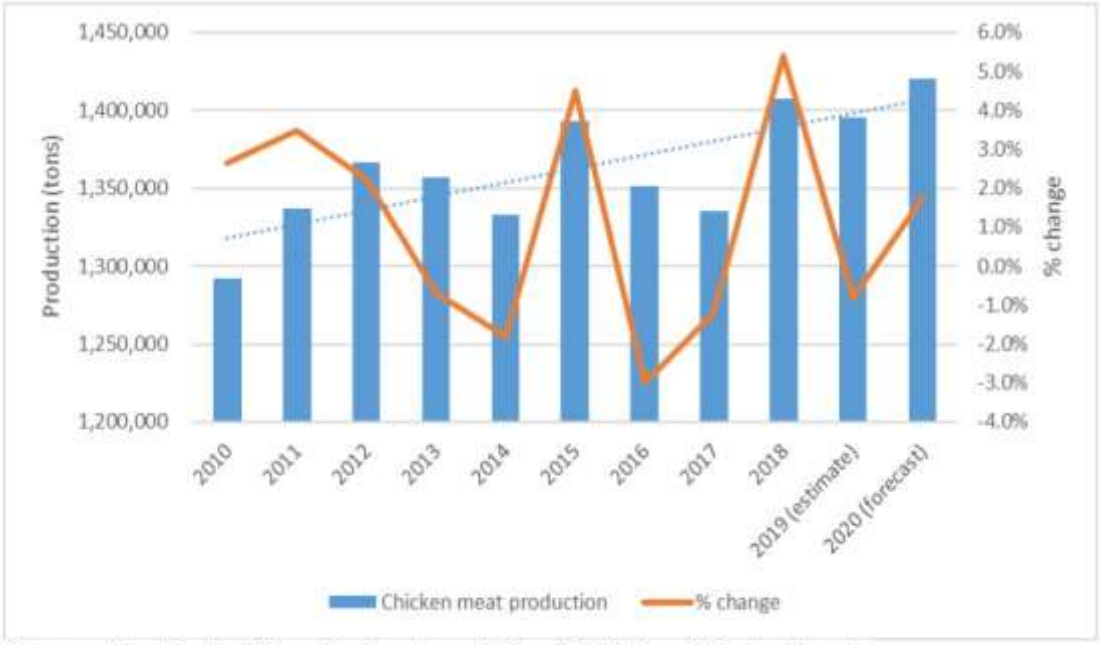
1.2.1 Africa

In Ghana, regarding slaughtering and processing, there is a very much limit of facilities for poultry. Mostly, killing of live birds takes place at the household level or in live bird markets. There are very few slaughter-houses in the country, but these services are mostly for goats, cows, sheep and pigs. Some of the large commercial farmers do have their own facilities for processing but lack modern equipment. Today, there are only 2 modern certified poultry processing facilities in Ghana [5]

Recently, due the pernicious drought, the outburst of highly pathogenic avian influenza (H5N8) (also see Southern Africa countries suspend imports of poultry from South Africa) and a Listeriosis food scare (see also SADC countries suspend processed imports of meats from South Africa), the South African broiler industry had been under considerable pressure. However, in 2018, the broiler industry regained its maximum growth, thereby increasing production by 6% to a record of 983 million broilers slaughtered. The broiler industry exhibiting a gross value of about R47 billion (US$3 billion) and contributing about 17% to the total gross value of agricultural products is the country's largest individual agricultural industry. Approximately, 90% of the chicken meat industry in South Africa, with the remaining 10% comprising of subsistence farming production and used flock is responsible for commercial broiler meat production. In 2018, the 983 million broilers slaughtered equaled 1.27 million tons of chicken meat (excluding offal). South Africa's total chicken meat production for 2018 is calculated at 1.41 million tons if depleted flock and subsistence farming

production are added which is actually a 5% increase from the previous year (see also Figure 3). In addition, an under recovery of higher input costs are being led due to an expected decrease in exports putting downward pressure on producer prices. As a result, broiler producers are expected to decrease production to 970 million broilers slaughtered in 2019. In 2020, chicken meat production is projected to increase by 2 % to reach 1.42 million tons, under the assumption of normal weather conditions [6]

Fig. 3. Chicken Meat Production and Percentage Change per annum in South Africa

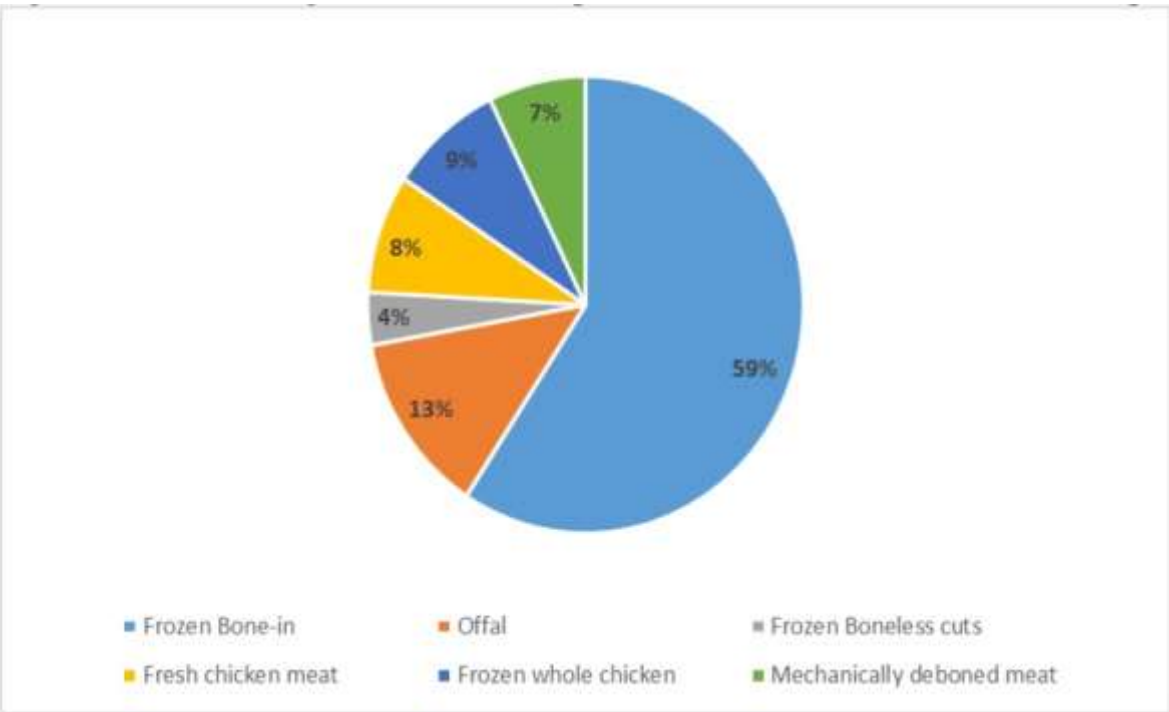


Source: The South Africa Poultry Association (SAPA) and Post estimates

Consumption of chicken meat per capita grew by more than 7 percent per annum in the same period. As South Africans' income grew, their buying behavior reflected their strong fondness for meat. The growth in meat consumption was impacted due to a slowdown in South Africa's economic growth since 2010. The per capita consumption growth rate of meat slowed down to about 1% per year. The economic growth of South Africa was expected to remain sluggish in 2019 due to structural and policy constraints. It was estimated that the economic growth of less than 1% in 2019, which will reduce an excessive increase in the demand for chicken meat. As already mentioned, economic growth is the main overall driver for the increased demand in meat. As a result, Post estimates only a 1% increase in the demand for chicken meat (excluding offal's) in 2019 to 1.90 million tons. According to the South African government economic growth should get back in 2020 on increased local and foreign direct investments. Hence, Post predicted that in 2020, there will be a 2% increase

in chicken meat consumption to 1.93 million tons. There was an introduction for the regulation to restrict the brined content to a maximum of 15% of the mass sold in 2016. Brining levels of up to 43% were recorded prior to 2016, and this resulted in higher water content per package of frozen chicken meat. Less than 10% of total consumption of chicken meat in South Africa is represented by fresh chicken meat [6].

Fig 4. The Percentage Contribution of Specific Chicken Meat Products to Consumption



1.2.2 Europe

A new and high record of 15.2 million tonnes of poultry meat was produced by the European Union (EU) in 2018. Since 2010, this represents a collective rise of about one quarter or 3.2 million tonnes.

In 2018, about 70 % of the EU production of poultry meat came from just six Member State with Poland, having the highest percentage (16.8 %), next was the United Kingdom with a percentage of 12.9 %, then France with 11.4 %, followed by Spain with a percentage of 10.7 %, Germany follows with 10.4 % and lastly, Italy with 8.5 %.

Fig. 5. Poultry meat production in Europe, 2018

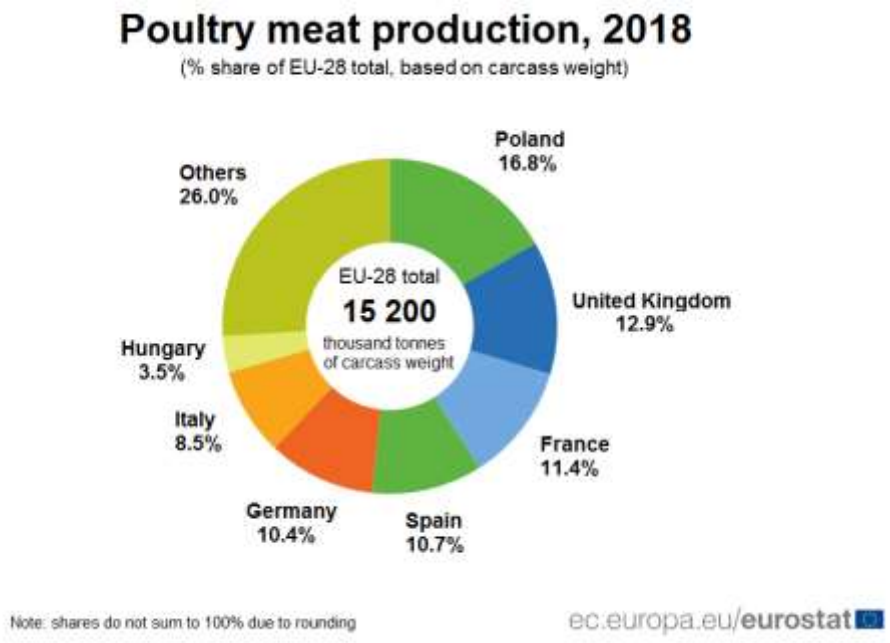


Table 3.showing the collective rise of poultry meat production in Europe in tonnes

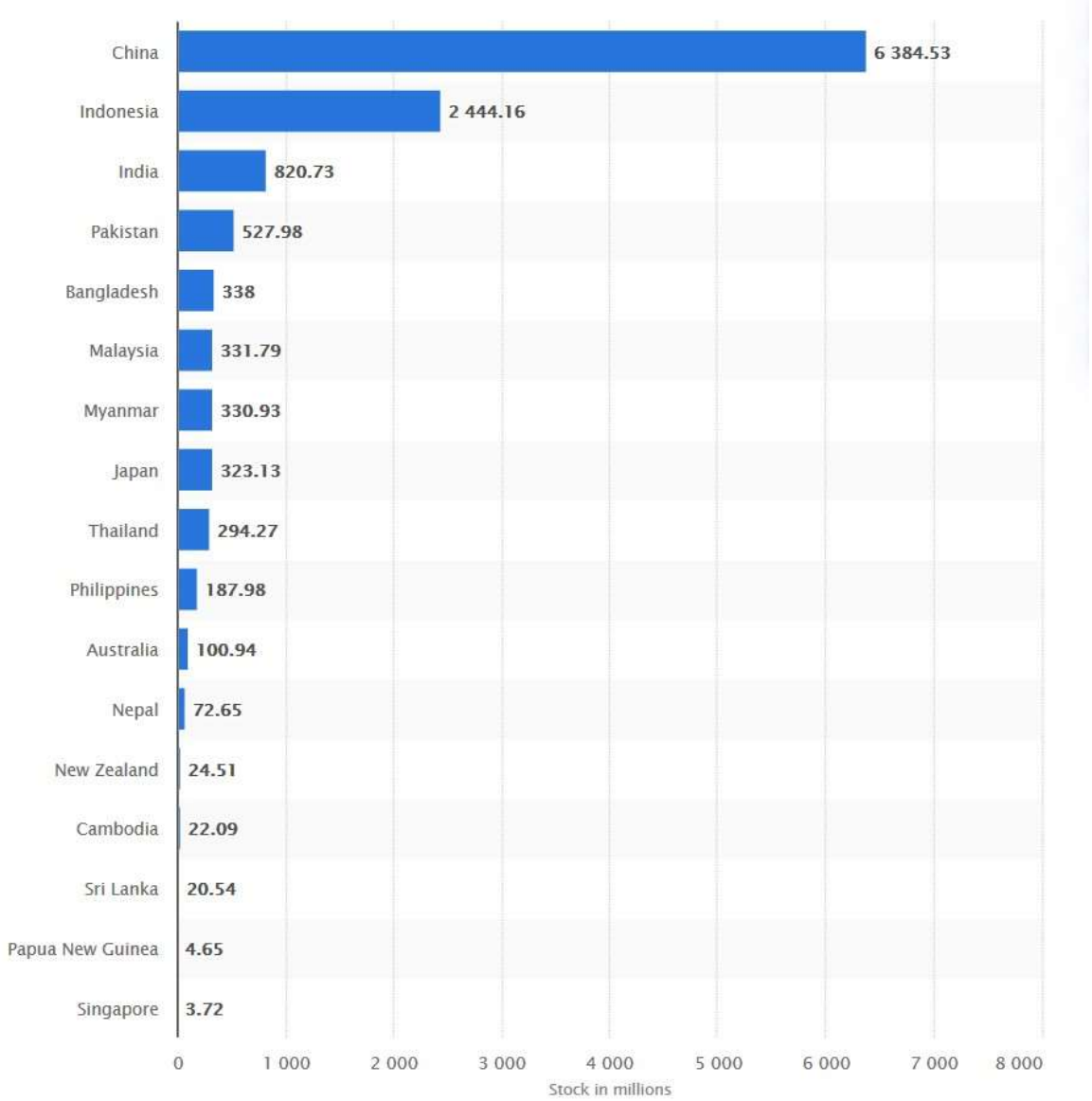
	2012	2013	2014	2015	2016	2017
Austria	125	121	122	122	123	129
Belgium / Luxembourg	411	389	433	453	461	465
Bulgaria	108	98	102	106	109	111
Croatia	74	68	68	83	81	83
Cyprus	25	24	24	23	24	25
Czech Republic	181	171	175	175	177	178
Denmark	180	168	173	147	156	159
Estonia	17	18	19	20	20	21
Finland	108	111	113	116	121	131
France	1.859	1.842	1.827	1.828	1.829	1.855
Germany	1.695	1.714	1.775	1.807	1.817	1.717
Greece	118	180	190	189	173	175
Hungary	488	515	543	577	620	599
Ireland	124	129	129	125	132	134
Italy	1.261	1.259	1.261	1.321	1.388	1.372
Latvia	24	26	29	29	30	31
Lithuania	85	93	100	111	112	115
Malta	4	4	4	4	4	5
Netherlands	838	848	941	1.057	1.097	1.097
Poland	1.712	1.798	2.022	2.173	2.871	3.110
Portugal	324	324	327	336	343	346
Romania	335	360	382	353	370	417
Slovakia	64	64	59	58	66	66
Slovenia	59	57	60	59	67	69
Spain	1.251	1.299	1.390	1.453	1.526	1.511
Sweden	116	128	137	147	155	163
United Kingdom	1.607	1.606	1.587	1.663	1.739	1.789
EU	**13.191**	**13.413**	**13.991**	**14.534**	**15.611**	**15.872**

Sources: MEG according to EU Commission, national data, FAO and Eurostat.

1.2.3 Asia

In the year 2018, China had the highest poultry bird stock throughout the Asia Pacific region. This edge was attained with a stock of over 6.38 billion. By comparison, Singapore also had a poultry bird stock of 3.72 million in 2018

Fig. 6. Poultry bird stock through Asia Pacific region



Source: asia-pacific-poultry-bird-production-by-country/statistic Container

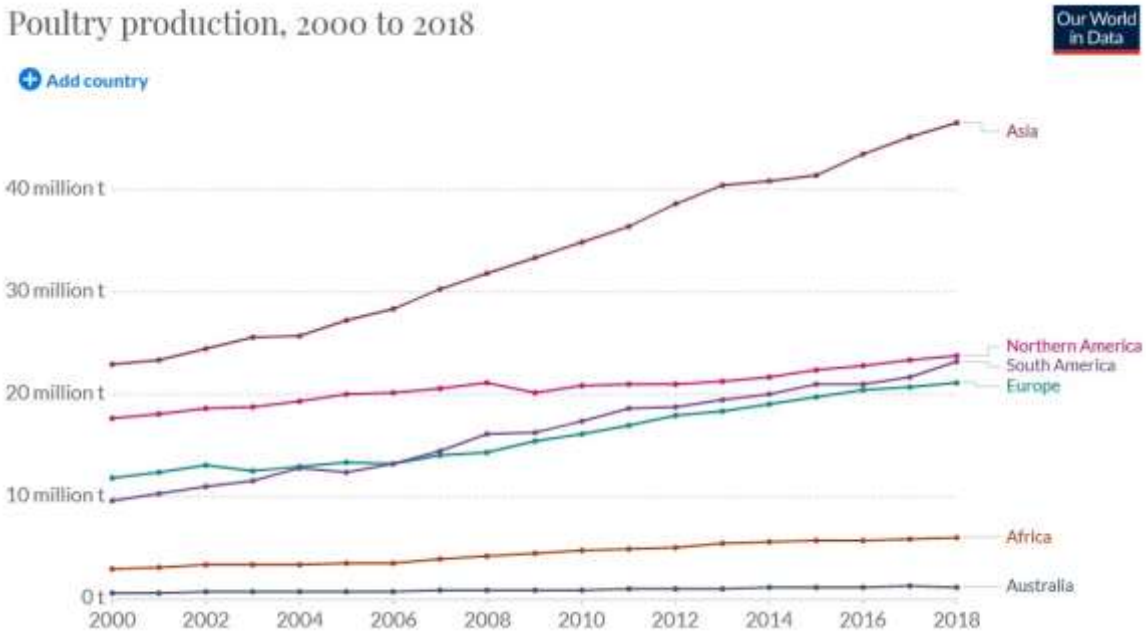
1.2.4 Worldwide

As the growth of poultry processing and egg production industries depends largely on waste management, waste products from these industries must be efficiently dealt with. The conversion of wastes to valuable products is participated by Animal and poultry waste management center, at North Carolina State University, North Carolina, USA and the work being assisted by various agencies, companies, organization etc. (Anon 1995).

The Generation of an enormous disposal problem for the animal industry has been as a result of the intensive and large scale production of food animals and animal. These wastes, including mortalities, animal excreta, feathers, hair, and processing wastes are convertible to

convenient resources. There was a report by Shih (1993) of an efficient thermophilic anaerobic digester system that converts animal manure to methane for an energy source. A digestible protein source produced from the properties of a feather degrading bacterium, Bacillus licheniformis, which can ferment and convert feathers to feather lysate, for feed use. Keratinase, which is an enzyme, secreted by this bacterium, was purified and characterized. The keratinase is a strong proteinase that hydrolyses collagen, elastin and feather keratin

Fig. 7 poultry production from 2000-2018, worldwide



Source: Food and Agriculture Organization of the United Nations (FAO) (2020)

An evaluation by Kondaiah et al. (1993) on Emulsion—based mutton nuggets, incorporating chicken by-products, i.e., skin, gizzard and heart (SGH) from spent hens were made. Better acceptability of mutton nuggets as compared to that containing mutton fat came as a result of incorporating SGH. The breakdown of proteins into smaller polypeptides or amino acids and amino acid breakdown of heated and irradiated poultry by-products of muscle tissue was studied by Urlings et al. (1993) and he concluded that enzymic activity has to be reduced or eliminated to ensure safe and high quality products during processing of poultry meat and poultry wastes. The main by-products from the poultry industry are shown in Table 4.

Table 4. Poultry industry by-products and their potential uses

TYPE OF BY-PRODUCT	% OF LIVE WEIGHT	USES
By-product from production phase		
Poultry litter and manure	-	Recycled feed, surface dressing of agricultural land
Hatchery by-products		
Egg shells, infertile eggs, un-hatched eggs and dead as well as culled chicks	-	Hatchery by-product meal up to 3-5% into feed, Egg shell meal as high calcium diet
By-products of poultry dress-ing plant		
Feathers	7-8	Bedding material, decora-tive purpose, sporting equipment, manure or ferti-lizers, feather meal
Heads	2.5–3.0	Poultry meal
Blood	3.2–3.7	Blood meal
Gizzard and proventriculus	3.5–4.2	Edible, source of chi-tinolytic enzyme
Feet	3.5–4.0	Soup, technical fat/poultry grease
Intestines and glands	8.5–9.0	Sportgats,, meat meal, poul-try grease and active princi-ples (hormones and en-zymes)

1.3 Legislative lay-out regarding tackling with by-products

Animal by-products comes as a result of the slaughtering of animals for human consumption, during the product production from animal origin such as dairy products, and in the course of dead animals' disposal and during control measures of diseases. They pose a potential risk to public and animal health and the environment regardless of their source. There is the need to adequately control this risk, either by means of directing such products towards safe disposal or as a result of using them for another purposes, provided that strict conditions are ensured to reduce the health risks involved.

Animal by-products disposal are not a realistic option, as this contributes to risks for the environment and unsustainable costs. Contrarily, provided the health risks are minimized, there is a clear interest for all citizens that, a wide range of animal by-products are safely used for various applications in a sustainable manner. A wide range of by-products from animal are indeed commonly used in important applications, such as the feed, leather and pharmaceutical industries.

The classification of animal by-products into three categories according to the degree of risk involved was introduced by the Regulation (EC) No 1774/2002. Animal by-products of different categories separate from each other are to be kept as required by operators if they wish to make use of by-products from animal which do not contribute a risk to animal or public health, in particular if such products are obtained from raw materials good for consumption by humans.

The principle that high-risk material should not be fed to farmed animals was also introduced by the regulation, and that material obtained from animals is not to be fed to other animals of the kind from which it is derived. In addition, Rules for processing standards which helps in the reduction of risks were laid down. [7]

2 PROCESSING CONDITIONS OF GELATIN EXTRACTION

The product obtained by the partial collagen hydrolysis derived from the bones, skin and other connective tissues of animals is known as Gelatin. These gelatin can be made from many distinct collagen sources. The main commercial sources of collagen include pig skins, cattle hides, bones, fish and poultry. With the above statement, it can be denoted that collagen comes from either agricultural or non-agricultural sources. There are currently no sources of gelatin from plants, and there is no relationship between gelatin and other materials referred to as vegetable gelatin, such as seaweed extracts chemically [GMIA 2012].

2.1 Pig skin Gelatin

Pigskin, is today's most important raw material for gelatine worldwide and was first used for the gelatine manufacture in the 1930s [8]. Some processable skin of about 3–4 kg is obtained from every slaughtered pig. First is separation of the pigskin from the layer of fat during processing of the meat, and then, if processing into sausages or luncheon meat is not required, it is transported (frozen or cooled) to the gelatine manufacturing factory. The process of cooling or freezing prevents oxidation of any fat remaining in the skin during transport and microbial degradation. The tissue made of fats from the pigskin also contains protein made up of collagens that can be used by the manufacturer for gelatin production after melting out the fat in the form of ''greaves''. However, a special process using special equipment is required because of the turbidity caused by emulsified fat. In countries such as China having a huge pigskin leather industry, the skin of the pig is split as outlined above for hide from cattle and then gelatin produced after further processing [8].

The animal connective tissue containing collagen dissolves even in boiling water at a very slow rate. The cross-linked nature of the collagen is the main reason for the above statement. Thus, gentle chemical treatment is necessary to break down these cross-links prior to extraction. The degree of cross-linking and type are dependent on the animal's age. The manufacturer for gelatin processing must therefore modify or regulate the parameters of the process for conditioning as well as the successive or following extraction conditions exactly to the raw material being made to be able to obtain the desired properties from the gelatin (see Table 5) [8]

In principle, by a process of slow ''cooking'', the cross-links could be split, a method used in the preparation of aspic for centuries. However, negative influence of all the parameters

which affect quality comes as a result by subjecting the material to longer periods of higher temperature. Thus, a much better quality of gelatin are produced by a gentle chemical cleavage. Only highly dilute acids and alkalis are used for this reason. In this way, the chains of the protein from collagen remain fundamentally intact, but there is a cleavage of the cross-links. In this case, only partial chemical breakdown of a compound due to reaction with water has taken place [8]

Table 5. Process parameters for conditioning as well as the subsequent extraction conditions precisely to the raw material being processed to be able to obtain gelatin with the desired properties

Raw material Type	Raw Material Conditioning	
	Acid	Alkali
Bones	X	X
Cattle hide splits	X	X
Pig skin splits	X	X
Pig skin	X	
Fish skin	X	
Poultry skin	X	
Poultry feet	X	

For animals older, more alkaline treatment is intensely preferred, while for the ones younger, a short conditioning period with very dilute acid is certain. Pigskin, because of its content of high fat, is mainly suited for acid digestion to prevent the process that involves conversion of fat, oil or lipid into soap and alcohol by the action of heat in the presence of aqueous alkali that would occur otherwise.

Any gelatine would however, be useless for most use for sensory and visual reasons, produced with materials of such. However, all other raw materials used in the gelatine production can also take a series of actions or steps in order to achieve a particular end using the acid method. This also applies to splits of the hide which, during the dehairing process and

swelling, are treated to treatment with alkaline; this is the equivalent of brief conditioning with alkaline.

Apart from enzymes, alkalis and acids, or an enzymes combination and chemicals, are also used for the cross-links cleavage. Special enzymes that break the peptide bonds in collagen are required for such a process, however, as native collagen are not able to digest by conventional proteinases. Thus, gelatine can be digested in the human body but not collagen.

This reveals just how the resulting gelatin types obtained and changing today's production processes, are. In accordance with the required properties of the gelatine to be produced, the manufacturer can thus select the production process [8]

2.1.1 Alkaline process

Both the split material chopped (see Fig. 6) and the prepared ossein from bone are treated in a tank or pits up to 125 m^3 with alkali, with or without agitation. The conditioning process takes from a few days depending on the temperatures and concentrations used. For example, sodium hydroxide solution with 1% concentration at 20 ˚C to four months with milk of lime that is supersaturated. Conditioning process is always speed up by agitation. The gelatin quality with respect to the viscosity and Bloom can be a result of the relationship between duration of conditioning and concentration of sodium hydroxide, temperature. Higher viscosity normally comes as a result of stronger conditioning [8]

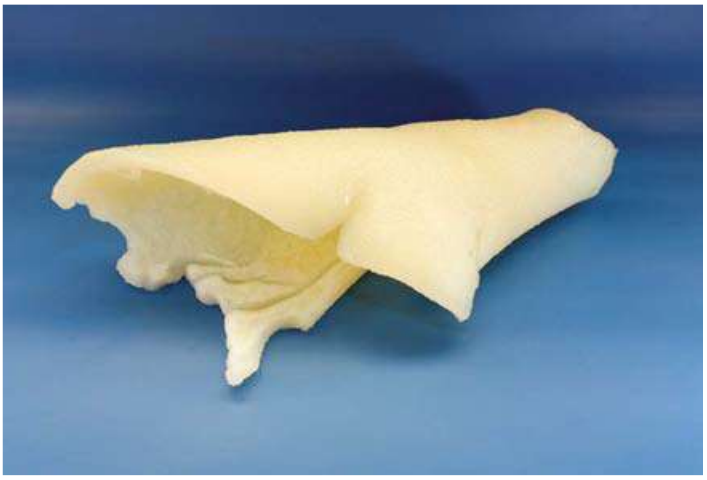


Fig. 8 Cut hide splits ready for extraction of type B gelatin

During treatment where the process lasts for several months, sulfur-containing compounds as well as non-collagenous proteins, especially albumin and globulin and the non-protein

substances such as mucopolysaccharides, are reliably dissolved out as they are always contained in the raw material,. This results in effective purification of the raw material and the relatively mild calcium hydroxide at the same time helps to balance out certain raw materials by their differences. This applies to the thickness of the hide pieces, the particle size of the bone chips, and the age of the animals. In this way, losses of undesired yield can be avoided.

The collagen becomes soluble in cold water if treatment with alkali is excessive. Therefore, collagen will dissolve in the aqueous phase as the raw materials are being washed and thus contribute to lower yields [8]

The solution is constantly enriched with dissolved non-collagenous protein and other substances during the alkaline treatment process, and it is therefore changed over the months repeatedly, daily at first and weekly basis later on. Afterwards, the treated material is washed free of alkali and neutralized by acid addition. During this process, most of the neutral salts produced are then removed essentially by washing numerous times.

2.1.2 Acid Pretreatment

The methods of breeding used today mean that approximately pigs are slaughtered when they are five to seven months old. The collagen of their skin as a consequence is relatively cross-linked weakly. The collagen warm-water solubility is to soak the hand-sized pieces (see Fig. 7) for up to 24 h, for example in 2–4% dilute sulfuric or hydrochloric acid at room temperature and thus this is all that is then required to ensure. At the same time, this process separates off most of the fat, which floats to the surface because of the mechanical agitation involved and hence it removes easily. The content of fat in the skin of pigs, separated carefully from the bacon fat, is about 30%; in comparison, and only about 20% for the protein content [8]

Fig. 9 Piece of fresh pigskin for type A gelatin

Organic acids and phosphoric are also appropriate for this processing step, but they tend to influence the odor, taste of the final product negatively and they are more expensive.

The manufacturer normally allows the acid to react over a period of 48–72 h if the hide splits of bovine are conditioned with acid. However, extraction must be carried out in an acid medium subsequent to acid conditioning.

After the acid treatment, the pH is rose to about 2–4 with the addition of an alkali by the gelatine manufacturer. By using frequent changes of water, most of the salts formed are then washed out over a period of 24 hr.

During subsequent extraction, the ratio of gel firmness to viscosity is regulated by temperatures used, the extraction time and the pH.

There is an influence exerted by both parameters, on the speed of extraction. Therefore, the optimal ratio between undesired chemical/thermal hydrolysis of the gelatine already melted out and the desired rapid extraction must be found by the manufacturer [8]

2.2 Cattle Hide Gelatin

The second major collagen source for gelatin manufacture is fresh cattle hide. The climate where the cattle are raised are depended by the thickness of the hide. This is to say, the warmer the climate, the thinner is the hide. The external side of this material is almost exclusively used for the manufacture of leather because it contains less collagen. The side of the meat is made up of tissues made of fats, which is removed by an expert.

The central layer, is thus an excellent raw material for the manufacture of gelatine and however, is practically pure collagen. (see Fig. 8).

A process that expands the hide and allows it to be dehaired after which the hide is cut using horizontal cutters into three separate layers. The process occurs after pretreatment with alkali. This process result to the ‘‘split’’ designation and used throughout the industry.

This ‘‘split’’ is cut firstly using a cutting machines into hand-sized pieces in the gelatine factory and conditioned with acid or alkali immediately. In principle, processing the hide pieces that have not been subjected to the splitting process is possible, but they will also need the use of alkali/sulfide solution during the dehairing stage before being processed by the gelatine manufacturer. Their low content in collagen reduces the yield greatly and lowers the operating efficiency and productivity consequently. [8]

The reduction size of the hides is essential for good conditioning. The acid and alkaline treatments are more uniform and the extraction is made possible.

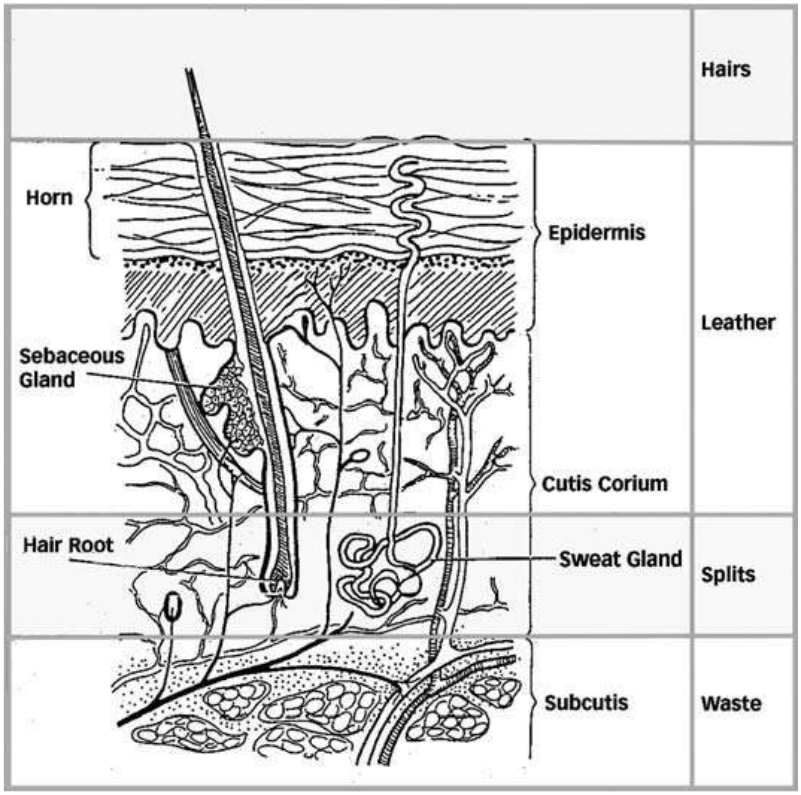


Fig. 10 The central layer of the cattle hide (‘‘split’’) is an excellent source of collagen for the manufacture of gelatine.

2.3 Gelatin from Bones

Fresh meat and also fresh bone material are obtained mostly in slaughterhouses and meat processing plants. A small portion of the bone material obtained are used as soup bones when they are sold to butchers. However, gelatin manufacturers are the main group where the major part of this valuable source of collagen goes to. The process is carried out under strict hygienic conditions and it is a rapid one. During the manufacturing process, about 0.5cm of a cube size is gently chipped from the bone and further washed for 30 mins by removal with hot water between a temperature from 85–90˚C under strong agitation by mechanical means. Any residual flesh and bone skin that may still be attached is completely removed under this process [8]

The bone that is chipped is dried with hot air in driers that are continuous, sieved and particle size sorted afterwards. The various chip grades are then separately processed. During this process, by-products such as meat, bone and fat are produced, where mostly the bones are

being used as fertilizers in North America and Europe. The chips that are cut from the bone and that have been blurted for processing further to gelatine are treated with dilute (4–6%) hydrochloric acid in a process in which there is a crossover of some property, usually heat or some chemical, between two flowing bodies flowing in opposite directions to each other in a battery of tanks for about a week at 10–20 ˚C. During this time, the calcium carbonate and calcium phosphate, which are bone material bound and provide it with its firmness, are converted into their soluble forms and are later precipitated by separation. This demineralization process is called maceration.

After maceration completion, the ones left is the proteinaceous structural framework of the bone which is the ‘‘ossein’’or the actual raw material that is used for the gelatin production (see Fig. 9). Here, initially, the freshly chipped and removed bone material is treated with an already strong-salt acid solution. In the final process step, the demineralized bone significantly is then treated with fresh acid. This scheme which is the counter-current scheme increases considerably the final effectiveness of the maceration process and unwanted warming and foaming are prevented.

$$Ca_3(PO4)_2 + 4\,HCl \longrightarrow 2\,CaCl_2 + Ca(H_2PO_4)_2$$

$$CaCO_3 + 2\,HCl \longrightarrow CaCl_2 + H_2O + CO_2$$

Fig. 11 At first glance, ossein particles still look like crushed bones.

Dicalcium phosphate is precipitated upon the addition of milk lime to the acid solution saturated in a separate apparatus, $Ca(H_2PO_4)_2 + Ca(OH)_2 \longrightarrow 2CaHPO_4 + 2H_2O$. After removal of water by centrifugation or filter presses followed by drying, this material is used as a fertilizer or as a mineral supplement for animal feed by the agricultural industry. [8]

Pressure Hydrolysis

Individual manufacturers have introduced in recent years, a process for the gelatin manufacture from raw bone that operates without the need for maceration.

This process, is an improved version of the traditional process for manufacturing bone glue and is known as ‘‘pressure hydrolysis’’. The material from the chip bone is treated in autoclaves, batchwise in a medium of an aqueous solution under extraction conditions over 20 min at a temperature of about 140 ˚C. The collagenous protein, during this process, is dissolved in a stepwise manner. The resultant is gelatine with a strong color, odor, and taste and the odor that shows a very low gelling power; these properties considerably limit its uses or application [8].

The extraction process follows a basic rule: the smaller the particle size, the shorter the process and this comes as a result of the process conditions of pressure hydrolysis.

Finely ground bone chips exhibiting good sensory properties within minutes and excellent gelling power and is well suited for continuous extraction processing can be converted into gelatin. Submission of appropriate systems have been made for patenting (Swedish Patent No. SE 9300912 and French No. FR 625412). However, no manufactural process has been developed so far.

2.4 Fish Gelatin

Fish skins can be used to produce food-grade gelatin and are rich in collagen. Films produced from fish skin gelatins can act as a barrier when applied to foods and are stable at room temperature. Lysozyme is an antimicrobial enzyme that is food safe, and can also produce films and gels. Fish-skin gelatin is increased with lysozyme when in cold-water, the resulting film has antimicrobial characteristics

Fish gelatin can be conditioned using both acid and alkali after extraction from fish skin. The skins can be obtained either from fishing vessels that carry out filleting at sea or from fish processors that work in conjunction with fish farms. However, cleaning of the fish skins thoroughly is encouraged in order to remove any fat that may still be present. The skins transported to the gelatine factory after they are frozen. There, they are defroze, several times washed , and treated over a period of 24 h with organic or mineral acids. A prior treatment stage with milk of lime is carried out first to stick any residual fat occasionally (European Patent No. EP 0436266 and US Patent No. US 6368656).

Fish taken from the cold water of the North Atlantic region contains significantly less hydroxyproline and proline and gelatine are produced from it. This reduces the gelling power of the gelatin produced even though of the high molecular weight. However, the exhibition

of good film formation and emulsifying properties are characterized by these gelatines. The main application areas as a result, are almost solely the submerging of oil-based vitamins using techniques such as spray-drying.

The fish gelatine produced from fish taken from warmer waters in contrast has good gelling properties and is frequently applied in the pharmaceutical and food industries.

However, it is significant to note that these gelatin types tend to have technological characteristics, e.g. the dependence of the stability of viscosity on the species of the fish from which the processing raw material is attained. As an outcome, the produced gelatin from a tuna fish skin has properties different from that of a Tilapia or Nile perch.

2.5 Poultry Gelatin

Fresh skin and bone material contributes to gelatin production from poultry. Acid process is used to preheat the material. The demineralization before conditioning is not normally done with the bone of the poultry, and the concentration of salt is high during the extraction. Thus, after extraction, a precipitation step after is necessary. Other steps that removes salts include deionization and ultrafiltration.

Chicken feet that were deboned were subjected to extraction with acetic acid and pepsin as described by Simões et al. (2014) and Shimokomaki et al. (1981), with modifications. To define the amount of acid, enzyme and hydrolysis time, a factorial design 23 was used, with three replications at the central point, totaling 11 assays.

The extraction process was started by immersing the chicken feet in acid solution for 24 hrs. The material was then homogenized for 10 min. In the hydrolysis, pepsin was added and the hydrolysis time was established, at 4 °C with continuous agitation. To stop the hydrolysis process, the pH was elevated to 7.5 and centrifuged for 30 min at 4 °C. The precipitate was discarded and the supernatant was precipitated with NaCl to a concentration of 3 mol/L and centrifuged for 30 minutes at 4 °C. The supernatant was discarded and the precipitate obtained was dialyzed with 0.5 mol/L acetic acid solution for 72 h with daily solution exchange. The dialyzed precipitate was lyophilized and thus the collagen isolate was obtained.

3 GELATIN PROPERTIES AND PROCESSING INTO FINAL PRODUCTS

Gelatine is a beguiling example of just how versatile nature can be. Protein is the most important component of gelatin and its content is between 85% and 92%, and mostly af-ter drying the remainder are mineral salts and. Gelatine is a product obtained by the par-tial hydrolysis of collagen derived from the skin, bones and other connective tissues of animals' i.e it is produced by the partial hydrolysis of native collagen. The most frequent-ly occurring protein class in both animals and humans is the Collagen. In difference to spherical globular proteins, collagen is made of linear, fiber-like structures.

Collagen is rather a family of proteins, but not a uniform substance. Up To date, there are about twenty-seven (27) different types of collagen that have been identified. The main types are the Type I, Type II and Type III. Type I collagen occurs widely, and predomi-nantly in connective tissue such as skin, bone, and tendons while Type II collagen occurs exclusively in cartilage tissue and Type III collagen strongly depends on the age: very young skin can contain up to 50%, but in the course of time this is reduced to 5 to 10%. The types of other collagen are mostly organ-specific and are found in very low amounts only.

3.1 Chemical composition and Molecular structure of collagen

Primary, secondary, and tertiary structural elements are exhibited by Collagen. Collagen also has a quaternary structure similar to other complex oligomeric proteins which are characterized by having multiple polypeptide chains or subunits.

The primary structure of type I animal collagen as used in the production of gelatine comprises some 1014 amino acids that are inter-connected in the form of a chain with a molecular weight of approximately 100 000 g mol-1. These alpha-chains contains 334 repetitive units of the general sequence glycine-X-Y.

Three alpha-chains consisting of two identical (alpha 1) and one slightly different (alpha 2) primary structures are composed in Type I collagen. With about three amino acids per turn (secondary structure), each alpha chain is coiled into a left-handed helix. Into a right-handed super-helix, the three alpha chains are then twisted around each other to form a rigid rope-like structure.

3.2 The condition process and its effect on the Amino acid composition of Gelatin

The solubility of collagen in warm water especially when they are obtained from very young animals is very high. However, with increasing age of the animal, these properties are lost. This is because of continuous conversion of labile structures into extremely stable, cross-linked ones. Its ability to bind water decreases at the same time; thereby, causing wrinkles in the skin of the elderly.

In Gelatine manufacture, partial splitting of these cross-links occurs as a result of the treatment of the animal raw material with dilute alkali or acid; the structure is dilapidated to that of a ‘‘warm water-soluble collagen’’, producing gelatin in the process. Through this chemical hydrolysis, the use of enzymes can be replaced or can be supplemented. As most of the protein-cleaving enzymes do not attack the water-insoluble collagen of skin and bone as they do in the case of gelatin, a special enzymes known as collagenases are required for such a process. Only highly particular protein-splitting collagenases have the ability of breaking down the native collagen structure. Within the gelatine industry, this type of chemical or biochemical denaturation and hydrolysis is known as ‘‘the conditioning process’’. In the next extraction step, that is the melting out of gelatine from the raw material, an additional thermal hydrolysis step takes place where the molecular weight is reduced.

The acidic amino acids glutamic and aspartic acids occur to the extent of about 35% in the amidated form of glutamine and asparagine respectively in native collagen. Both asparagine and glutamine are almost completely converted to aspartic and glutamic acids respectively in the case of alkaline-conditioned type B (basic) gelatine. The amino acid composition of collagen and an acid-conditioned type A (acid) gelatine are similar. This describes the different iso-electric points (IEP), typical of types A and B gelatine. The pH at which a gelatine molecule is neutral in charge is the IEP. For type A gelatine, this correlates to collagen at about pH 8–9 and for gelatin of type B, at pH 4.8–5.5. The primary raw materials used in gelatin production include porkskins, cattle bones, and cattle hides. Several alternative sources include fish and poultry. Other extra substances, such as fats and albuminoids (found in skin) and minerals (in the case of bone), are taken out by chemical and physical treatment to produced purified collagen. These pretreated materials are then hydrolyzed to gelatin which in hot water is soluble. Gelatin retrieved from bone is mainly used for pharmaceutical purposes. The bone obtained from a slaughtered cattle is cleaned, defatted, dried, sorted, and

crushed to a particle size of about 1-2 cm. Treatment of the pieces of bone with dilute hydrochloric acid helps to remove mineral salts which results to a sponge-like material called ossein. Both cattle hides and ossein receive similar treatment from this point on in the manufacture of Type B gelatin. Hides from cattle are available from trimming operations in the production of leather. Hairs from the hide pieces are usually removed chemically with a sulfide/lime solution followed by a mechanical loosening. Both ossein and hide pieces from cattle are subjected to a lengthy alkali (usually lime) treatment and water at ambient temperature for a Type B gelatin production. The material nature, the pieces size, and the exact temperature, where liming takes 5-20 weeks, usually 8-12 depending on previous treatment. The process is controlled by the alkalinity level of the lime liquor as determined by acid titration, or by making test extractions. More liming time is usually required by ossein than cattle hides. An additional lime is added, thereby compensating for any consumed to maintain an excess. Some deamination of the collagen occurs during liming, with ammonia being evolved. The raw material is meticulously washed with cold water to remove excess lime; acid is used to adjust the pH after conditioning; and in order to recover the soluble gelatin, hot water is used to extract the product. Porkskin is an important raw material source for an edible gelatin production in the United States. The short time required for pre-treatment prior to extraction, and the minimization of wastewater generated are significant economic factors in gelatin manufacture from this raw material. Porkskins from abattoirs and meat processing plants are already trimmed of fat, flesh and hair and is supplied as either fresh or frozen. Dehairing of porkskins are usually done by scalding with a hot dilute caustic soda solution.

Porkskin are washed with cold water and then soaked in cold dilute mineral acid for several hours until maximum swelling has occurred when they are utilized for production of Type A gelatin. Hydrochloric acid and sulfuric acid are most commonly engaged. The remaining acid is then taken out and the material is again washed several times with cold water. The porkskins are then set for extraction with hot water. Depending on product needs, type of equipment employed, timing of operations, and economics, the pH, time, temperature, and number of extractions varies from processor to processor. Procedure in extraction are closely controlled in both Type A and Type B gelatin manufacture since they affect both quality and quantity. Most methods still employ discrete batch fractions, although continuous extraction is used by some processors. Extraction is normally accomplished in stainless steel vessels equipped with provisions for heating and temperature control. The number of extractions differs, 3-6 is typical. The first extraction normally takes place at 50-60° C, next extractions

being made with consecutive increases in temperature of 5-10° C, with the final extraction being carried out close to the boiling point. To meet various customer specifications, extracts are kept separate, analyzed, and subsequently blended. The beginning extraction usually provides a higher product, compared with next extractions. Initial extractions have lighter color, higher gel strength, higher viscosity, and higher molecular weights. At increasingly higher temperatures, the later extractions are made; the final product has darker color, lower gel strength, and lower molecular weight. By crossflow membrane filtration and/or vacuum evaporation, the dilute gelatin solutions from the various hot water extractions are filtered, deionized, and concentrated. The gelatin solution is then froze and either gash into ribbons or extruded as noodles, and the gelled material is put down as a bed onto an endless, open weave, stainless steel belt. The belt is run through a drying chamber, which is divided into zones in each of which the temperature and humidity of the drying air is precisely managed. In the initial zone, typical temperatures range from about 30° C up to about 70° C in the final zone. The air is usually controlled by filtration, dehumidification and tempering. Drying involves gradual increases in air temperature, often with enervation of moist air and refilling with conditioned air. Depending on the concentration and quality of the material and the exact conditions employed, the drying time is 1-5 hours. Avoiding melting and case hardening comes as a result of carefully controlling the rate of drying. With a moisture content of approximately 10%, the gelatin leaves the dryer. The dried bed is then crushed into pieces that are ground to the needed size of particle. According to standard methods developed by the Gelatin Manufacturers Institute of America, dried gelatin is tested for quality and gel strength [9].

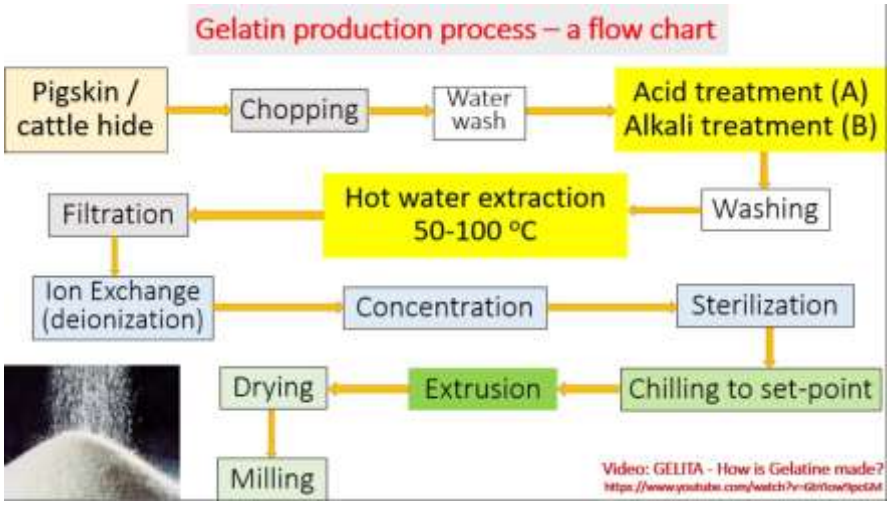


Fig. 12. Gelatin production process flow chart

3.3 Physical and chemical properties

Gelatin is almost odorless and tasteless. It is a vitreous, fragile solid faintly yellow in color. Gelatin has a relative density of 1.3-1.4 and contains 8-13% moisture. Gelatin granules hydrate into discrete, swollen particles when they are soaked in cold water. These swollen particles dissolve to form a solution when they are being warmed. Especially where high concentrations are desired, this method of preparing gelatin solutions is preferred. Behavior of gelatin solutions is influenced by pH, temperature, concentration, thermal history, method of manufacture and ash content.

In aqueous solutions of polyhydric alcohols such as glycerol and propylene glycol, gelatin is very soluble. Trifluoroethanol, acetic acid and formamide are examples of highly polar, hydrogen-bonding, organic solvents in which gelatin will dissolve. In less polar organic solvents such as benzene, acetone, primary alcohols and dimethylformamide, gelatin is insoluble [10].

For long periods of time, gelatin stored in air-tight containers at room temperature remains unchanged. It gradually loses its ability to swell and dissolve when dry gelatin is heated above 45° C in air at relatively high humidity (above 60% RH) [8]. Uncontaminated solutions of gelatin when stored cold are stable forever; but at elevated temperatures the solutions are prone to hydrolysis.

Gel strength and viscosity, two of gelatin's most useful properties, are gradually enfeebled on prolonged heating in solution above approximately 40° C. By extremes of pH and by proteolytic enzymes including those which may result from the presence of microorganisms, degradation may also be brought about [1-4]. Collagen may be considered an anhydride of gelatin. The hydrolytic collagen conversion to gelatin gives molecules of varying mass: each is a fragment of the collagen chain from which it was split. Therefore, gelatin is not a single chemical unit, but a mixture of fractions comprises entirely of amino acids joined by peptide linkages to produce polymers differing in molecular mass from 15,000 to 400,000 Da [11-17].

50.5% carbon, 6.8% hydrogen, 17% nitrogen and 25.2% oxygen [18] are the composition of gelatin in terms of basic elements. Gelatin is properly classified as a derived protein since it is derived from collagen. It gives typical reactions of protein and by most proteolytic enzymes, it is hydrolyzed to yield its peptide or amino acid components [4]. Gelatin functional

properties can be divided into two groups. The first has properties that are related with gelling e.g., gelling time, gel strength, setting and melting temperature and viscosity. The second group is associated to the gelatin surface behavior. These properties are, e.g., its adhesive properties, its dissolution behavior, and the formation and stabilization of foams and emulsions. The most significant properties – and these are typical of gelatine – are properties associated with gelling which include formation of gel, thickening, water binding and Surface effects which include formation of emulsion and stabilization, protective colloid function, formation of foam and stabilization, formation of film, Cohesion / Adhesion.

3.3.1 Gel formation, viscosity and texture

The Gel formation, viscosity, and texture is related to the determination mainly by the structure, molecular size and temperature. Gelatine is a mixture of chains of polymer of varying lengths. The difference between Colloidal solutions or sols and real solutions are that, colloidal solutions are mostly formed while real solutions are not formed. These sols are converted to gels on cooling and they revert to sols on warming. The most important technological property of gelatine is theoretically the limitless reversibility of the gelling process. Many other hydrocolloids that also gels include alginates, carrageenan, or pectin. However, this is by reaction by chemical means that may be irreversible or reversible to a limited extent only.

3.3.2 Gel strength and Bloom value

A test that is used to measure the strength of a gel or gelatin is called a Bloom. In 1925, the test was originally developed and patented by Oscar T. Bloom [19]. The test is used to determine the weight measured in grams required by a defined plunger to reduce the surface of the gel at a standard conditions without breaking it. Bloom value is the number of grams, and most gelatins are between a bloom value of 30 and 300 g Bloom. The higher the melting and gelling points of a gel, the shorter its gelling times, and the higher a Bloom value [20]. On soft gels, this method is most often used. To do the Bloom test on gelatin, a solution of 6.67% gelatin is kept for 17–18 hours at 10 °C before to tested.

Various gelatins are classified as "high Bloom", "medium Bloom", or, "low Bloom" but there are not generally determined specific values for these subranges. A biopolymer material composed of polypeptide chains of varying length can also be termed as Gelatin. The longer

the chain corresponds to a higher Bloom number [8]. The Bloom values of commercial types of gelatin are within the range 50–300 Bloom.

Table 6. Category of Bloom, their bloom number and average molecular mass

Sr No.	Category	Blood number	Average molecular mass (Da)
1	Low Bloom	50-125	20,000-25,000
2	Medium Bloom	175-225	40,000-50,000
3	High Blood	225-325	50,000-100,000

In the final product where gelatin material has a gelatin of high Bloom, it means that the gelatin has essentially higher melting and gelling points and shorter gelling times, and its colour is lighter and odorless and tasteless. Furthermore, a gelatin with stronger gelling power also means that smaller amounts of gelatin are required to bring about the desired gel firmness in the finished product.

3.3.3 The kinetics of gel formation

The molecular weight distribution mostly is depended on all the hydrodynamic gelatin properties. The temperature at which the system is observed is the only difference between gel formation and viscosity. That is, as the gel firmness primarily describes the structure formed by intermolecular hydrogen bonds, whereas the viscosity determines the mobility of the molecules in the liquid (can be seen in Fig. 11). The mobile molecules aggregate to small clusters if a solution of gelatine is cooled; these grow continuously and form a gel subsequently. In this situation, some influence is exerted by electrostatic and hydrophobic interactions. Thus, there is an uninterrupted transition between these two gelatin properties. No linear relationship exist between the Gel formation and viscosity. Bloom value and the standard viscosity as stated by the gelatin manufacturer are measurements of single-point which cannot be used to produce conclusions regarding the kinetics of formation of gel, mainly because the different fractions of molecular weight also play a role. Thus, the gel strength is primarily determined by the fractions proportion having a molecular weight of approximately 100 000 g mol-1, whereas those in the molecular weight range of 200 000 to over 400 000 g mol-1 is mainly a function of viscosity. For this reason, as a result of the dependence on the method of manufacture, gelatines of the same Bloom value can have quite varying viscosities. [8]

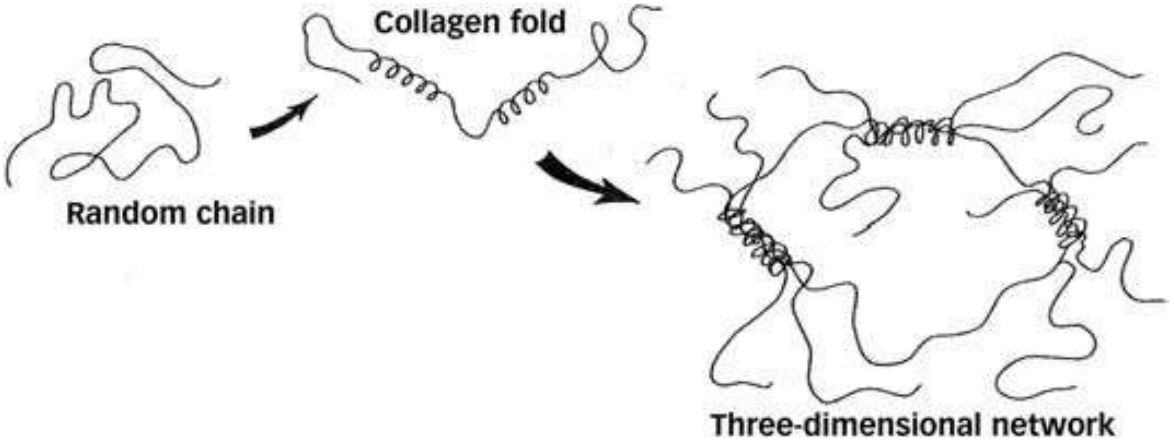


Fig. 13. Model of formation of gel (from sol to gel upon cooling).

3.3.4 Surface properties

The surface properties of gelatine are established from the facts that the gelatine side chains, like those of all proteins, have charged groups and that certain parts of the collagen sequence contain either hydrophilic or hydrophobic amino acids.

Both water-hating and water-loving parts tend to move toward surfaces, and this process tends to lessen the surface tension of aqueous solutions. At the same time, gelatine has numerous properties that stabilize and protect the surfaces formed. This gelatin multifunctional property is depleted in the production process and foams and emulsions stabilization.

The molecular structure of the substance in question is depended by the ability for the formation and stabilization of the foams. It must possess surface-active characteristics primarily, by reducing the surface tension at the liquid/air interface, for only in this way is it possible to cause a product to foam. The prevention of the rapid collapsing of such a foam is as a result of an increase in the viscosity of the continuous phase which is essential. The foaming agent will thicken the entire product, thus completely stabilizing the foam structure in the complete case. Emulsifiers are soluble substances that allow other substances that nature normally separate to be brought together. If such an emulsifier is added in low amounts to a dispersion of an oily phase in an aqueous phase, its molecules tend to meet at the interface between the two phases. The individual oil droplets with an extremely thin film is then covered by the emulsifier. They repulse each other, hence the formation of larger drops if these films have the same electrical charge are essentially prevented. However, high temperatures and high mechanical stress tend to destroy the fine film of emulsion and hence the emulsion itself. [8]

The stabilizer used should create an especially firm protective sheath around the droplets, prevent the latter from becoming electrically neutralized, or increase the viscosity of the emulsion. This last-named property is also termed as the ‘‘protective colloid effect’’ of the stabilizer. Gelatine reduces the aqueous systems surface tension and creates the required same charged film around the parts of the dispersed phase, which can be additionally strengthened by formation of gel. The distribution of charge and the gel firmness of the gelatine employed are important criteria in selecting a suitable gelatine type. This is because, the higher the gel firmness, and hence the Bloom value, the firmer is the gel-like protective sheath around the oil droplets or air bubbles at the same concentration and temperature.

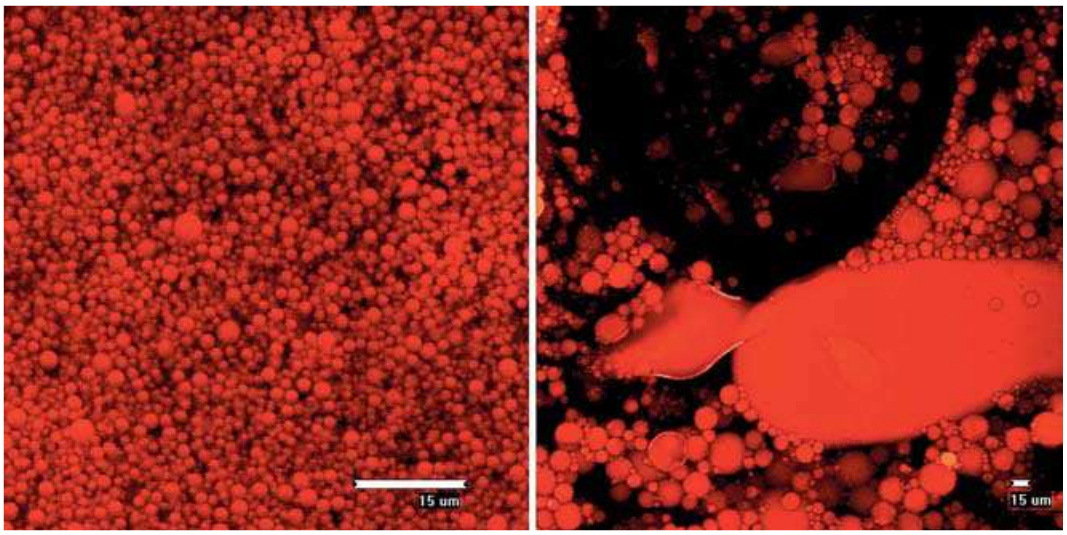


Fig.14. Stable emulsion with gelatine (left) and ‘‘broken’’ emulsion without gelatine (right). Source: DIL (Deutsches Institut fur Lebensmitteltechnik e. V.) Quakenbru ¨ ck, Germany

3.4 Application of Gelatin

3.4.1 Food industry

The stability over a specified temperature range (which depends on the quality of the gelatin and its concentration) comes as a result of the gel formed by gelatin in water. It disintegrates when it’s heated beyond the highest temperature in this range. When the gels are added to foods such as marshmallows, they break down at below body temperature. This means that when the food softens and collapses when eaten, thus creating a "melt in the mouth" sensation and releasing flavor confined in the gel.

Gelatin is also used to make gummy candies, gelatinous desserts, and some marshmallows as well as many yogurts. Also, to prevent some meats from drying out and to give them an

attractive glaze, they are added to certain meats. As Stocks and consommés are cool, they form gels. Stock is a liquid produced by gently boiling meat and vegetables in water for a long time. Flavour is provided to the stock by the solid and collagen provided by the meat. The solids are filtered out to leave the liquid stock hours after cooking. Consommé can be defined as a clarified stock. Addition of Egg whites to the stock is recommended and then heated. As the egg whites becomes solid, particles from the liquid are absorbed and create a raft on its surface. A clear consommé is found under the raft. Gelatin absorbs juices released from the meats as they are processed under pressure in canned meats. The substance helps to stabilize the emulsified fat in pâtés. Gelatin is functional for its thickening, binding, adhesive, and emulsifying properties. Additionally, it's able to allure impurities and clarify fruit juices, wines, and vinegar

Fig. 15 A: gammy bear candy made from gelatin and B: a desert made from gelatin

In order to provide the sensation of eating fat, gelatin is often added to low-fat dairy products such as yogurt, ice cream, and buttermilk. Addition may be to some full-fat products as well. The gelatin provides a creamy and smooth mouthfeel and the split of the gel in the mouth seem like fat melting. Gelatin in whipped toppings and creams help to stabilize their uniformity. Enzymes that stop a gelatin gel from forming are contained in fresh pineapple and papaya. The formation of the bonds needed to hold the amino acid chains together as the gel forms are prevented by the chemicals. Bromelain is the enzyme mixture in pineapple and that of papaya is papain. Since the heat applied during the canning process destroys the enzymes, canned pineapple doesn't contain bromelain. Other foods that include kiwi, figs, guava, and ginger root helps to prevent the gel from forming. During pasteurization or another process, any form of these foods that has been heated should allow the gelatin to set, since the essential enzyme or enzymes will have been destroyed.

3.4.2 Cosmetic industry

Gelatin may be applied in the production of skin creams and lotions, hair sprays, shampoos, lipsticks, face masks, hair conditioners, and nail polishes. They can be sometimes known as "hydrolyzed animal protein". The gelatin attracts moisture and stiffens the products, given them a creamy-like texture.

3.4.3 Pharmaceutical industry

Gelatin can also be applied in the pharmaceutical industry to make hard and soft capsules to confine medication and supplements. Hard capsules are made up of two parts and are made when into a warm gelatin solution, a stainless steel mold is dipped. The medications are mostly filled into the capsules. Soft capsules, also referred to as soft gels made up of one part and are made from gelatin sheets. As they are made, they are filled with a medication.

A B

Fig. 16. A: Soft Gelatin Capsules and B: Hard Gelatin Capsules

3.4.4 Other uses of gelatin

For both adults and children, gelatin is a versatile medium in arts and crafts. It's also used in some fun and unforeseen ways. Some known items that can be produced from the substance include printing plates, lenses, decorations of cake, sculptures, for school project in edible models, emulsions from Photographic application for film and paper, the shells of paintballs, gummy worms (straws filled with gelatin and allowing it to set) and air fresheners (after a scent is added). Gelatin is also applied in the binding of ingredients together in match heads, and it's added to some sandpaper types. It can also be used to produce glues.

II. EXPERIMENTAL PART

4 AIM OF THE EXPERIMENT

The head of laying hens is remarkably interesting case of study as it is a by-product of poultry processing.

For instance in Czech republic, with an increase in the consumption of poultry worldwide, approximately 25 kg a year per person make for by-product production from poultry slaughter, including feathers, blood, skin, bones, intestines, limbs, offal and various fat-like tissues keeps increasing. They create possible risks to human health and the environment because of the fact that these by-products are being generated. So there is the need for these risks to be removed either through safely disposing them or finding a different applications while keeping up with strict hygiene standards. Indeed, abattoirs for poultry would no doubt welcome any likelihood for utilizing such by-products, which could reduce the financial stress involved in disposing of the same at institutes for veterinary sanitation or landfill sites; the end result is associated with the potential release of unpleasant odours and contamination of groundwater [21]. As chicken head is made up of protein quantity (primarily collagen), the choice exists to change them into products with considerable added usefulness, e.g. hydrolysates or gelatin, [22]. This analysis is done by the use of enzyme protamex for conditioning of purified collagen from chicken head and finally aid into the gelatin production. It is hypothesized that an extracted collagen from chicken head using a petroleum ether ethanol solvent processed into gelatin by the help of a promatex enzyme can give varied results base on their temperature change affecting the quality of prepared gelatin. The main aim of this study to process laying hen's head as an untraditional source of collagen and further describe the type of gelatin recommended for its use. Mostly, chicken head are cut and discarded away in most of the regions in my country and there is the need to utilize these by-products into something beneficial to the humans, animals and the environment which is why the reason for this goal of study. Material fats and non-collagenous proteins are needed to be removed first. To produce gelatin from chicken head, pre-treatment techniques are required for gelatin extraction conditions. Experiment will be analyzed by ways of having better properties and prepared gelatin yields through factorial schemes. Furthermore, gelatins produced from the experiment will be analyzed based on their viscosity, ash content, dry matter content, gel strength, gel melting point etc, their chemical, thermal, rheological and physical properties will be compared.

5 MATERIALS, METHODS OF WORK FLOW

5.1 Raw material

5.1.1 Hen paws

The Hen by-product was supplied by Raciola, Ltd. (Uherský Brod, Czech Republic). Firstly, by conventional food methods, the by-product material analysis were performed. The composition of the starting raw material is showed on the table below

Table 7. showing the composition of the starting raw material

Dry matter content (%± SD)	Protein content (%± SD)	Collagen proportion in protein content (%± SD)	Fat content (%± SD)	Inorganic solid content (%± SD)
46.4±1.2%	47.9±1.1%	70.3±0.9%	33.8±1.1%	29.7±2.1%

5.1.2 Enzymes

Manufacturer: Novozymes (Copenhagen, Denmark)

Description: Promatex is a Bacillus protease complex with stated activity of 1.5 AU/g, used at mild processing conditions (pH 5.5-7.5 and at temperature <60 °C). For food-grade enzymes that have been issued by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex (FCC), the Enzyme satisfies with the approved purity specifications.

5.2 Gadgets, instruments and Chemicals

5.2.1 Gadgets and instruments

PE bottles 100ml volume (Czech Republic), Kitchen sieve 0.5mm size (Czech Republic), shaking machine LT 2 (Czech Republic), 1L measuring cylinder (Czech Republic), baking tray (Czech Republic), glass beaker (Czech Republic), petri dishes (Czech Republic), weighing balance scale BCBC 100, KERN 770 (Germany), Oven, BMT, MMM Group (Germany), milling machine (Czech Republic), desiccator (Czech Republic), souxhlet extraction apparatus (Czech Republic)

5.2.2 Chemicals

0.2 M NaCl, 0.03M NaOH. Petroether/ethanol

5.3 Methodology

The method of the work used in the experimental part of the thesis was by Factorial schemes. The actual type of factorial scheme used for this particular experiment was Tanguchi design. Tanguchi designs are statistical methods, also referred to as robust design methods, created by Genichi Taguchi to make the manufactured goods quality better, and more recently also applied to marketing and advertising [23][24], engineering, and biotechnology[25]. Statisticians who are professionals have accommodated the improvement and goals brought about by Taguchi methods [26], specifically by Taguchi's designs development for variation study, but have find fault with the inefficiency of some proposals of Taguchi [27]. Three principal contributions to statistics from Taguchi's work include a specific loss function, the off-line quality control philosophy and innovations in the design of experiment.

With this particular task, the levels of the factors with their possible combinations are investigated in each repeated step. This factorial schemes involved two studied factor on three (3) levels that is the minimum, maximum and the centre experiment (2 repetitions). The factorial schemes will be use to analyze the regression and also show which of the two (2) factors will have an effect on the extraction yield, gel strength, ash and viscosity.

5.4 Work flow of processing hen heads into collagenous product

5.4.1 Preparation of Purified Collagen (Non-collagenous parts and fat separation from the hen heads)

Approximately 700g of purified collagen was prepared. The hen paws were milled into smaller pieces, washed and stored in refrigerator.

a. Separation of albumins

When ready to be used, it was defrost and washed with tap water to aid in dirt removal and unwanted components by the help of a sieve for approximately 2 minutes. This was mainly done to ensure separation of albumins from the raw material.

b. Separation of globulins (Treatment in 0.2 M NaCl)

The sample was treated with 0.2 M NaCl in a ratio of 1:6 for 1.5 hours at room temperature while stirring. The sample was filtered out then rinsed with cold water for 1 minute. This was done to separate the globulins from the raw material.

c. Separation of glutelins (Treatment in 0.03 M NaOH)

The sample was further treated with 0.03 M NaOH in a ratio of 1:6 for 45mins whiles stirring. Then it was filtered and rinsed with cold water for 1 minute to separate glutelins from the raw material. Sample was repeated 3 times again. Finally, it was filtered with cold water for 5 minutes.

d. Drying

The sample was placed in a thin layer unto a metal plate and dried with air circulation at 35°C for approximately 24-36 hours.

e. Defatting of the raw material

The dried raw material was placed in a volumetric flask and 900ml of solvent (petrolether/ethanol) added to it. It was placed on a shaker at room temperature for a period of two (2) days for defatting the raw material. After defatting, the purified collagen was placed on a metal plated and left overnight.

f. Milling of Purified Collagen

Purified collagen is milled into small units approximately 3 mm and stored in a PE bottle in a dark

5.4.2 Preparation of Gelatin

For each experiment, 60.0g of purified collagen was used

a. Enzyme conditioning of purified collagen

Purified collagen dissolved in distilled water in a ratio of 1:10. The blend was shook for approximately 20 minutes and the pH adjusted at 6.5-7.0 with 10% HCl. 1.0% of Proteolytic enzyme Protamex was added after and the pH checked. The mixture was placed on a shaker at room temperature for 24 hours. The pH was checked if it was at 6.5-7.0. The liquid was filtered out through a fine sieve with 3 layers of clothing; the hydrolysate (liquid) was poured unto a metal plate covered with anti-adhesive plastic foil. It was dried at a temperature of

70°C for approximately 24 hours. The hydrolysate was scraped, weighed and put into a closed sack (stored in a dark).

Fig. 17 Enzyme conditioning of purified collagen

b. Rinsing of conditioned collagen

The collagen inside the sieve is thoroughly washed with cold water for approximately 5 minutes in order to remove remnants as possible

c. Extraction of 1st gelatin fraction

The rinsed collagen was dissolved in distilled water in a ratio of approximately 1:10. The mixture was extracted at the temperature according to Factor A; started with the heating set on a temperature 20°C higher than the prescribed extraction temperature. As soon as the temperature of the blend was 10°C lower than the prescribed extraction temperature, the temperature was set back to the prescribed extraction temperature. As soon as the extraction temperature was reached, the time according to Factor B was extracted; the mixture was stirred properly during extraction especially at the beginning of extraction. The liquid was filtered out through a fine sieve with 3 layers of clothing. Filtered liquid of solution of 1st gelatin was heated quickly unto a temperature of 85°C for 10 minutes. The gelatin was poured into a metal plate and placed in a refrigerator for 30 minutes; after, it was placed in a dryer with air circulation at 40°C and thirdly it was dried in a dryer with air circulation at 65°C overnight for 8-10 hours. The dried gelatin was scrapped, weighed and milled to a particle of approximately 1mm and put into a closed sack.

d. Extraction of 2nd gelatin

The undissolved collagen remained after the 1st gelatin fraction was blended with distilled water in a ratio of approximately 1:10. The mixture was extracted at 90°C; started with heating set on a temperature of 20°C higher than the prescribed temperature. The extraction temperature was lowered as soon as the temperature of the blend was 10°C lower than the prescribed temperature. As soon as the extraction temperature was reached, the mixture was extracted for a period of 90minutes; this was done by stirring the mixture properly especially at the beginning of the extraction. The liquid was filtered through fine sieve with 3 layers of clothing. The gelatin solution was poured unto a metal plate and firstly placed in a refrigerator for 30 minutes. After, it was a dried in a dryer with air circulation at 40°C overnight; finally, it was dried in a dryer with air circulation at 65°C overnight for 8-10 hours. The dried gelatin was scrapped, weighed and milled to a particle of approximately 1mm and put into a closed sack.

Fig. 18 Extraction of 2nd gelatin fraction

Fig. 19 Gelatin formed after drying of extraction of 3rd gelatin fraction

e. Extraction of 3rd gelatin fraction

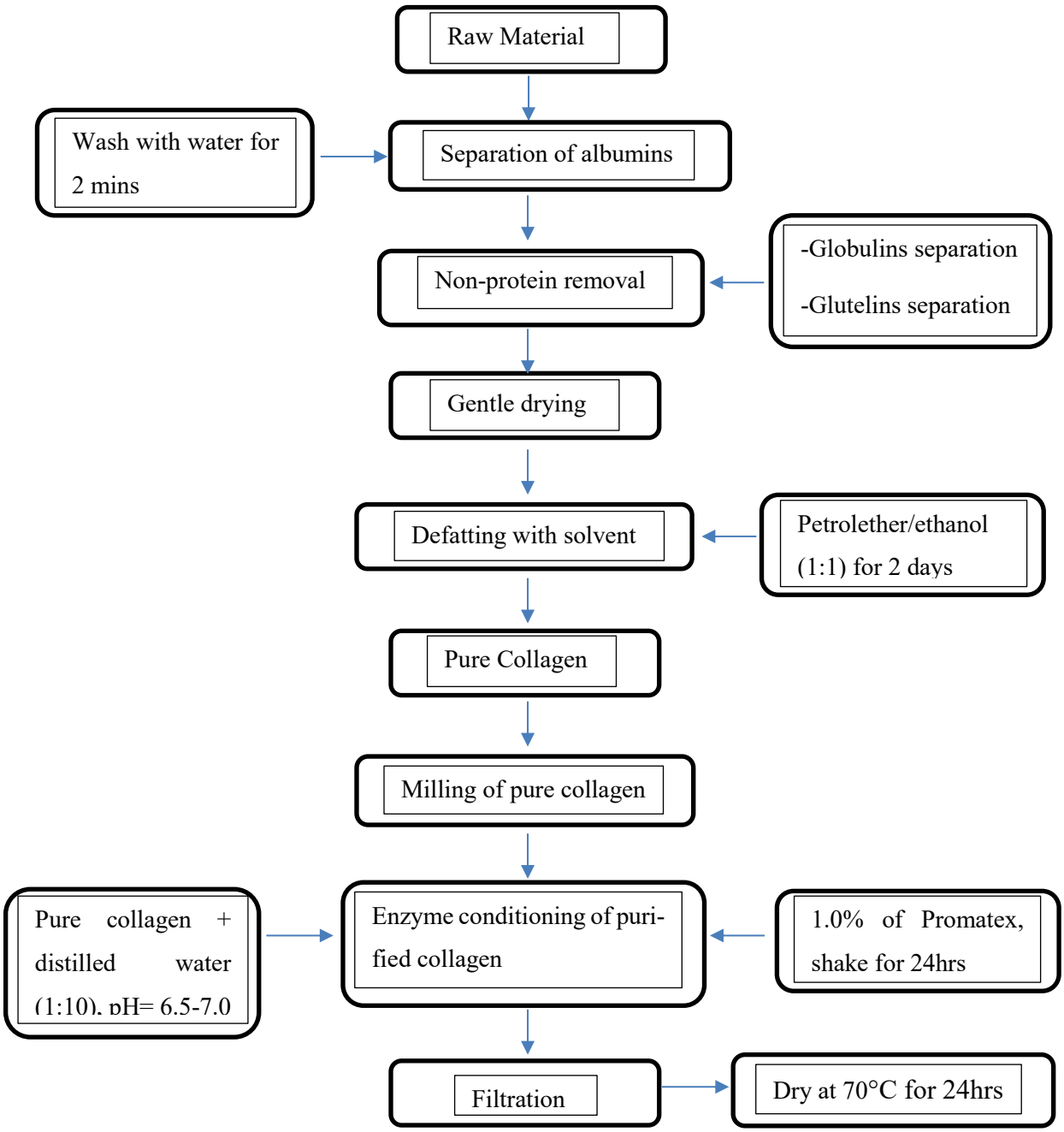
The undissolved collagen remained after the 2nd gelatin fraction was blended with distilled water in a ratio of approximately 1:10. The mixture was extracted at 100°C; started with heating set on a temperature of 20°C higher than the prescribed temperature. The extraction temperature was lowered as soon as the temperature of the blend was 10°C lower than the prescribed temperature. As soon as the extraction temperature was reached, the mixture was extracted for a period of 90 minutes; this was done by stirring the mixture properly especially at the beginning of the extraction. The liquid was filtered through fine sieve with 3 layers of

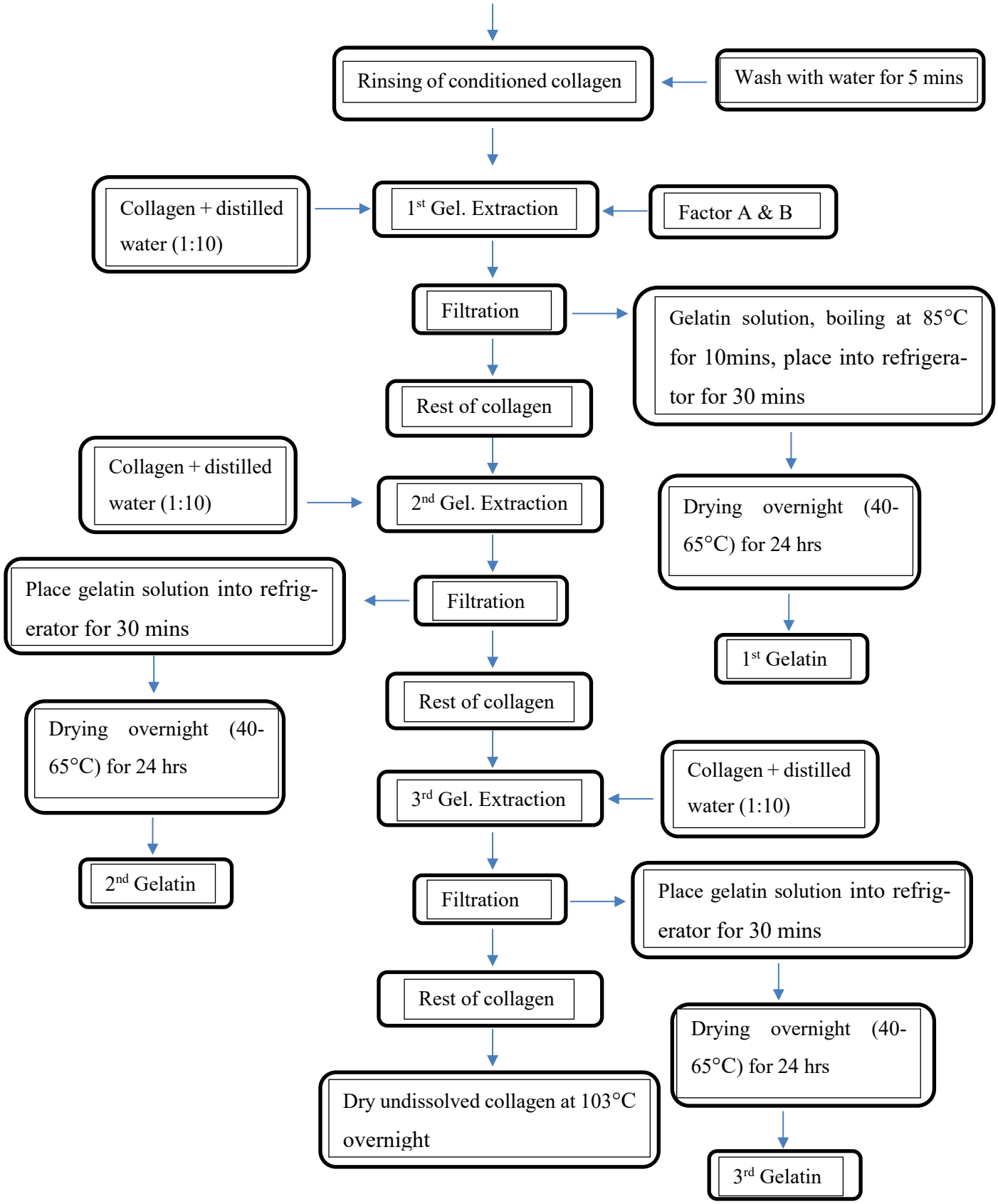
clothing. The gelatin solution was poured unto a metal plate and firstly placed in a refrigerator for 30 minutes. After, it was a dried in a dryer with air circulation at 40°C overnight; finally, it was dried in a dryer with air circulation at 65°C overnight for 8-10 hours. The dried gelatin was scrapped, weighed and milled to a particle of approximately 1mm and put into a closed sack.

f. Undissolved collagen

After the 3rd gelatin fraction was extracted, the remaining collagen (undissolved collagen) was dried at 103°C (overnight) and weighed.

5.4.3 Flow chart




Rinsing of conditioned collagen
Wash with water for 5 mins
Collagen + distilled water (1:10)
1st Gel. Extraction
Factor A & B
Filtration
Gelatin solution, boiling at 85°C for 10mins, place into refrigerator for 30 mins
Rest of collagen
Collagen + distilled water (1:10)
2nd Gel. Extraction
Drying overnight (40-65°C) for 24 hrs
Place gelatin solution into refrigerator for 30 mins
Filtration
1st Gelatin
Rest of collagen
Drying overnight (40-65°C) for 24 hrs
3rd Gel. Extraction
Collagen + distilled water (1:10)
2nd Gelatin
Filtration
Place gelatin solution into refrigerator for 30 mins
Rest of collagen
Drying overnight (40-65°C) for 24 hrs
Dry undissolved collagen at 103°C overnight
3rd Gelatin

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

5.5.1 Determination of gelatin strength [28]

As stated by the standard procedure, the determination of gelatin strength is done by using a Bloom test. The gelatin strength also known as the bloom value is the measure of the gel toughness and stiffness formed from a solution of 6.67% according to defined conditions.

Fig. 20 gelatin ready to be analyzed for gelatin strength

Procedure: 7.50g of gelatin was weighed into the bloom bottle using an analytical balance. 104.5ml of distilled water was added and it was allowed to settle for 30 minutes. The mixture was placed on heating plate and allowed to melt at a temperature of 45°C until it homogenizes using a magnetic stirrer. The sample was stored in a refrigerator for 16-18 hours.

Fig. 21 checking the gel strength of gelatin fraction

Table 8. The amount of gelatin, water and container size for different methods of determining gel strength

Method	Weight of gelatin	Weight of water [g]	Vessel
A	7,5	104,5	standard container
B	3	42	½ volume container
C	1,5	21	¼ volume container

* container dimensions: volume 150 ml, height 85 mm, inner diameter 59 mm, outer diameter 66 mm.

Final conversion for measuring the strength of gelatin gels in other than standard containers:

Method A measurement in a standard vessel

Method B measurement in the balance; diameter 50 mm (outer), 44 mm (inner), height 50 mm, the gel strength values are higher by a factor of: 1.2627

Method C measurement in the balance; diameter 40 mm (outer), 35 mm (inner), height 50 mm, the gel strength values are higher by a factor of: 1.6372

5.5.2 Determination of gelatin viscosity

The viscosity of the 6.67% gelatin solution was determined by measuring the flow time of 100 ml of solution with a standardized pipette at 60 ° C. The measurement was performed on an Ubbelohde viscometer and the measured flow time was converted to viscosity by fitting to the formula below.

$$v = K \cdot t - B/t \qquad (1)$$

Where
v is kinematic viscosity [mm^2 / s]
B anta constant for correction to kinetic energy determined from the dimensions of the viscometer (2.8)
K is constant of the viscometer determined by a validated calibration fluid (0.5)
t is the arithmetic mean of measured flow times [s]

The dynamic viscosity (mPa.s) was calculated by multiplying the density (1.005g/cm^3) by the kinematic viscosity.

$$\eta = v \cdot \rho \qquad (2)$$

Fig. 22 viscosity analysis of gelatin fraction

5.5.3 Determination of melting point of gelatin gel

The sample was dissolved in a water bath at 60 ° C. the solution was put into a capillary tube with diameter 2-4 mm; with a column height of gelatin 1.0-1.5 cm. the capillary was cooled in the refrigerator at a temperature of 10 ° C so that the gelatin hardens inside. The capillary can optionally be filled with solidified gelatin, when the end of the capillary is inserted into the solidified gel after determining the strength. The gelatin was nicely evenly distributed in the capillary. The capillary was inserted into a test tube. The beaker was heated to 55 ° C (at a rate of 3.5 ° C per minute). The heating rate was checked several times from the room temperature when measuring to the moment of melting temperature. During this period, the time was measured and the speed was calculated. As soon as the melting point was reached, the gelatin dissolved and pushed out of the capillary by the water pressure. At this point, the melting point of the gelatin solution was read.

Fig. 23 the melting point analysis of the gelatin fraction

5.5.4 Determination of the solidification temperature of gelatin gel

It is determined as the temperature at which a solidified gelatin solution retains a ball of defined weight on its surface (or in a layer) without sinking to the bottom.

Method:

The gelatin solution (after determining the viscosity) was poured into the test tube (approximately half the height of the test tube), the thermometer (sensor) was inserted; the assembly with the beaker was put in. When the solution in the test tube had a temperature of 30 ° C, water was poured into the beaker refrigerated to 4-6 ° C (the water must reach above the sample in the test tube, ie ideally up to 3/4 of the height of the test tube). Steel balls (weight 0.10 g) were dropped inside at a temperature of 1 ° C. When measuring, the cooling rate was checked several times: from 30 ° C to the moment of solidification temperature; during this period, the time was measured and the speed was then calculated.

5.5.5 Determination of ash content

It was determined traditionally, by burning, annealing; gravimetrically

5 - 50 ° C; 15 min at 5 ° C; 50 - 0 ° C; up 5 st/min; down 0.5 st/min; weight 7-35 mg 6.67% gelatin

5.5.6 Water holding capacity (WHC)

According to Nasrin et al. 2008 with fine adjustment, 1.00 g was dispersed in 25 ml of distilled water and mixed (ideally shake by hand in a stoppered test tube in which it will be centrifuged) for 5 min at 25 ° C. it was centrifuged at 5000 rpm for 30 min. The supernatant

(= liquid phase) was removed preferably with a pipette. The absorbed water, supernatant and counting were weighed respectively. WHC was determined as the weight of water absorbed per 1.0 g of sample.

Calculation of WHC in percent = $WHC_{\%} = \frac{m_1}{m_0} \times 100$ (3)

Where m_1 is total absorbed water

m_0 is the total original volume (25ml)

5.5.7 Fat binding capacity (FBC)

According to Li et al. 2009. 100 mg (= 0.1 g) was dispersed in 10 ml of soybean oil and mixed thoroughly (ideally shake by hand in a stoppered test tube in which it will be centrifuged). Sample was allowed to stand for 30 minutes at 25 ° C. It was centrifuged at 2500 rpm for 30 min. The supernatant (= liquid phase) was removed preferably with a pipette. The absorbed oil, the supernatant and counting were weighed respectively. FBC was determined as the weight of oil absorbed per g of sample - therefore the result must be multiplied by 10 to convert from 0.1 g to 1 g.

From $density(\rho)\ of\ oil = \frac{mass\ (m)\ of\ oil}{volume\ (v)\ of\ oil}$ (4) $but\ \rho\ of\ oil = 0.926$

Calculation of FBC in grams = $FBC = (Total\ masss\ of\ oil - Quantity\ of\ oil\ left) * 10$ (5)

5.5.8 Foaming capacity (FC) and Foaming stability (FS)

According to Sathe et al. 1982 (Effects of different drying methods on the rheological, functional and structural properties of chicken skin gelatin compared to bovine gelatin). 1.00 g of the sample was dissolved in 50 ml distilled water at 60 ° C. The measuring cylinder was attached to the stand with brackets and clamps. The stability of the cylinder was ensured by whipping with a shaft stirrer at high speed (mark) for 5 minutes; ideal for10000 rpm. The same cylinder or beaker and stirrer were used so that the parameters do not change and the results do not differ.

Calculation according to formulas:

$Foam\ Capacity, FC\ (\%) = \frac{v_1 - v_0}{v_0} * 100$ (6)

Where v_1 is the total volume after 5 minutes of whipping

v_0 is the original volume of the liquid (50ml)

$$Foam\ Stability, FS\ (\%) = \frac{v_2 - v_0}{v_0} * 100 \qquad (7)$$

Where v_2 is the total volume standing after 30mins (this comes after whipping the mixture)

v_0 is the original volume of the liquid (50ml)

5.5.9 Emulsification capacity (EC) and Emulsification stability (ES)

According to Net et al. 2001 (Effects of different drying methods on the rheological, functional and structural properties of chicken skin gelatin compared to bovine gelatin). 5.0 ml gelatin solution at a concentration of 10 mg / ml (= 0.01 g / ml) was ideally shaken by hand in a stoppered glass tube. It was homogenized with 5 ml of palm oil for 1 min. The oil was added to the gelatin solution and shake again. The emulsion was centrifuged at 1000 rpm for 5 min. The emulsion layer height (= oils) and total content were measured as emulsification capacity and the stability was determined by heating the gelatin solution to 55 ° C and centrifuging at 2000 rpm for 5 min; and then measured. The temperature was set 20 ° C higher than 55 ° C to heat up faster. Once 45 ° C was reached, the temperature was reduced to the required 55 ° C. The height was measured with a ruler

Calculation according to formulas:

$$Capacity, EC\ (\%) = \left(\frac{h_1}{h_0}\right) x\ 100 \qquad (8)$$

Where h_1 is the emulsion height

h_2 is the total height

$$Stability, ES\ (\%) = \left(\frac{h_2}{h_1}\right) x\ 100 \qquad (9)$$

Where h_2 is the emulsion layer height after heating

h_1 is the emulsion height before heating

Fig. 24 extracted and dried analysis ready for the functional properties of gelatin

6 RESULTS AND DISCUSSION

Exp No.	**Extraction of 1st gelatin fraction**		Yield of hydrolysate (%)	Yield of 1st gelatin fraction (%)	Yield of 2nd gelatin fraction (%)	Yield of 3rd gelatin fraction (%)	Total yield of gelatin (%)	Undissolved collagen (%)	Mass balance mistake (%)
	Factor A, extract. Tempt. (° C)	**Factor B extract. Time. (min)**							
1	60	60	4.3	15.4	2.2	0.7	18.3	31	2.89
2	60	90	3.9	6.3	3.5	2.5	12.3	36.4	4.71
3	60	120	3.6	7.9	3.5	3.0	14.4	34.6	4.71
4	70	60	2.2	13	3.4	1.9	18.3	33.5	2.17
5	70	90	3.1	6.4	3.7	3.1	13.2	35	7.07
6	70	120	3.7	8	3.9	3.5	15.4	33.7	4.35
7	80	60	3.8	7.7	3.4	2.5	13.6	34.9	5.25
8	80	90	3.9	7.9	4.5	2.7	15.1	33.5	4.89
9	80	120	7	14	2.8	0.8	17.6	29.3	2.36
	BLANK EXPERIMENT (WITHOUT THE ADDITION OF ENZYME)								
10	70	90	0.9	4.6	2.9	3.8	11.3	42.7	0.54

Table 9. Layout of experiments, evaluation of extraction process, characterization of gelatins

Table 10. Layout of experiments and evaluation of 1st gelatin fraction properties

Exp No.	Ash Content (%)	Dry Matter (%)	Gel Strength (Bloom)	Viscosity (mPa.s)	Gel melting temperature (° C)	Gel solidification temperature(° C)	Water holding capacity (g/1g)	Fat binding capacity (g/1g)	Foaming capacity (%)	Foaming stability (%)	Emuls. capacity (%)	Emuls. stability (%)
1	1.21	91.05	0	0	0	0	12.6	12.5	2	0	48.39	96.67
2	2.31	92.97	15.05[B]	1.347	28.5	13.5	15.2	11.0	6	0	43.55	98.15
3	1.86	91.61	27.3[A]	2.11	31.5	10.3	12.5	11.6	1	0	46.77	96.55
4	1.79	92.93	57[A]	1.64	31.9	17.0	24.9	9.8	10	2	46.77	93.10
5	2.02	92.86	103.75[B]	2.031	33.4	16.9	24.7	14.2	8	2	45.16	96.43
6	1.68	92.22	82.67[A]	2.311	29.8	16.7	11.1	15.1	6	0	46.77	96.55
7	1.83	93.41	73[A]	2.150	32.15	14.2	14.2	12.1	4	0	45.16	100
8	1.31	92.70	78[A]	2.169	31.5	17.2	18.4	11.0	2	0	46.77	93.10
9	1.24	93.18	0	0	0	0	13.8	14.3	6	0	48.39	93.33
	BLANK EXPERIMENT (WITHOUT THE ADDITION OF AN ENZYME)											
10	2.11	93.58	0	1.565	0	0	12.6	9.1	6	1	46.77	96.55

A, B and C shows the actual method of gel strength analysis used

6.1 Optimization of gelatin yield (1st gelatin fraction)

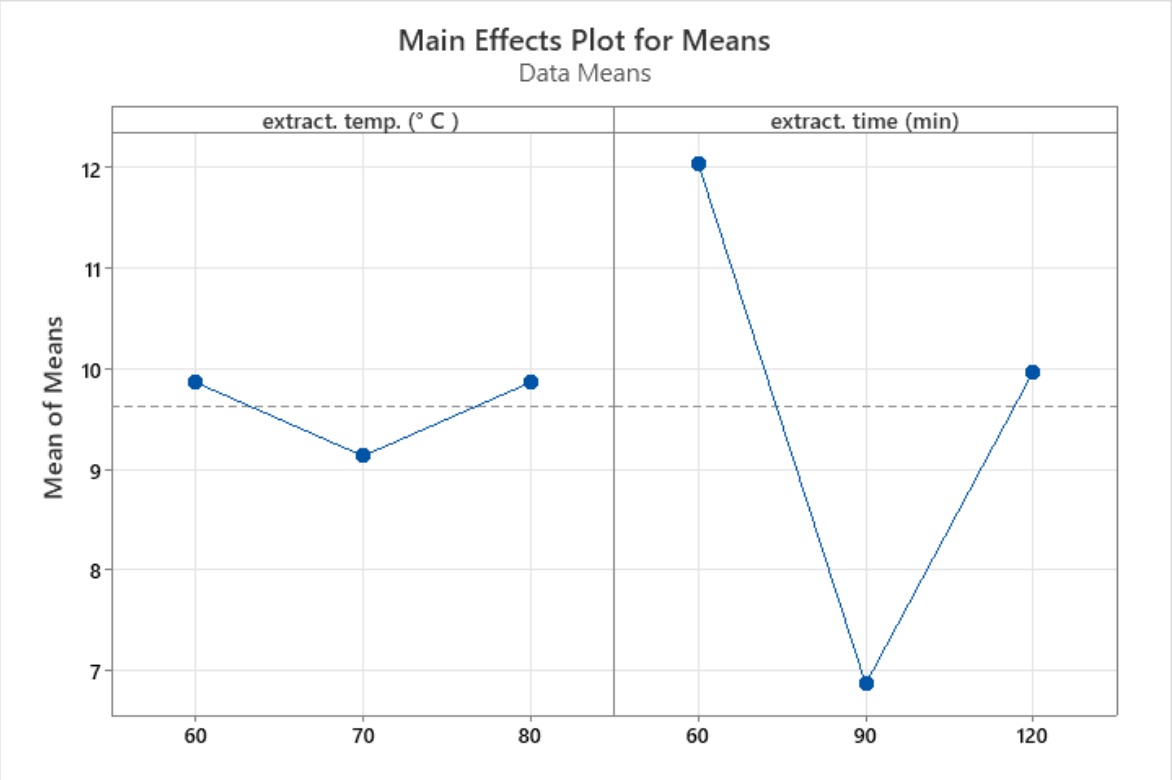


Fig. 25 Main effects of extraction temperature and time for 1st gelatin fraction

The optimum level for the 1st gelatin fraction of the extraction temperature was obtained to be 60° C and that of the extraction temperature was obtained to be 60 minutes. There could not be any relationship obtained between the processing conditions and the yield of the first gelatin fraction. Reasons for this results were as a result of the duration of samples stored before analysis were done due to the current situation (COVID crises). Furthermore, during the analysis, the heating plate used for the extraction process got broken down and in so doing, a different heating plate was used for analysis. An observation made in terms of the degree of heating by the temperature was that the earlier heating plate used was heating at a slower rate (between 7-12mins to attain the set processing temperature) as compared to the latter heating plate which was heating at a faster rate (between 3-5mins). These observation (s) could be the reason for the disparities in the result in the first gelatin fraction

Regression Equation

$$Yield\ of\ 1st\ gel\ fract. = 12.7 - 0.000\ extract.temp - 0.0344\ extract\ time \quad (10)$$

Table 11. Parameters estimating the percent yield of 1st gelatin fraction of hens head obtained by regression analysis

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	12.7	12.2	1.07	0.337	
Extract temp.	-0.000	0.159	-0.00	1.000	1.00
Extract time	-0.0344	0.0531	-0.65	0.540	1.00

From the figure below, the P-value is greater than the significant value (3.89934), hence the data does not favors the hypothesis hence changes in the processing conditions are not associated with changes in the gelatin yield and in so doing, the processing conditions does not have an effect on the yield of gelatin in the first fraction.

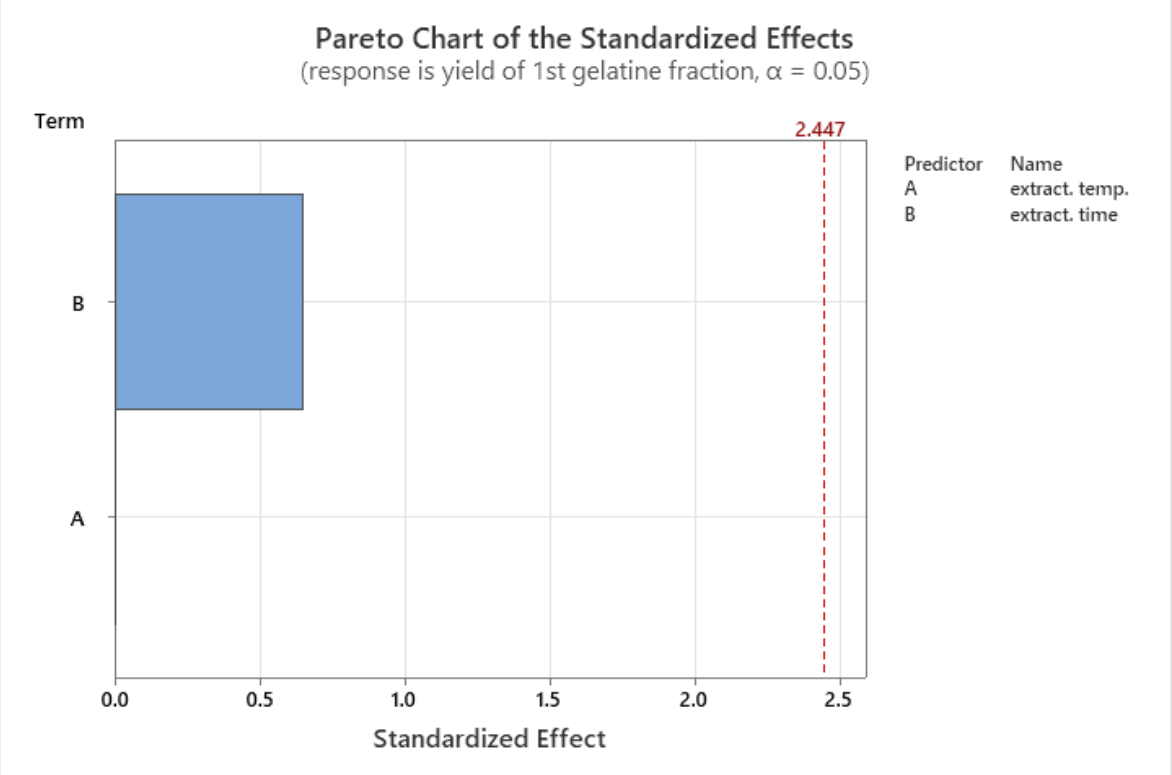


Fig. 26 Pareto chart of the standardized effects on yield of gelatin in the first fraction.

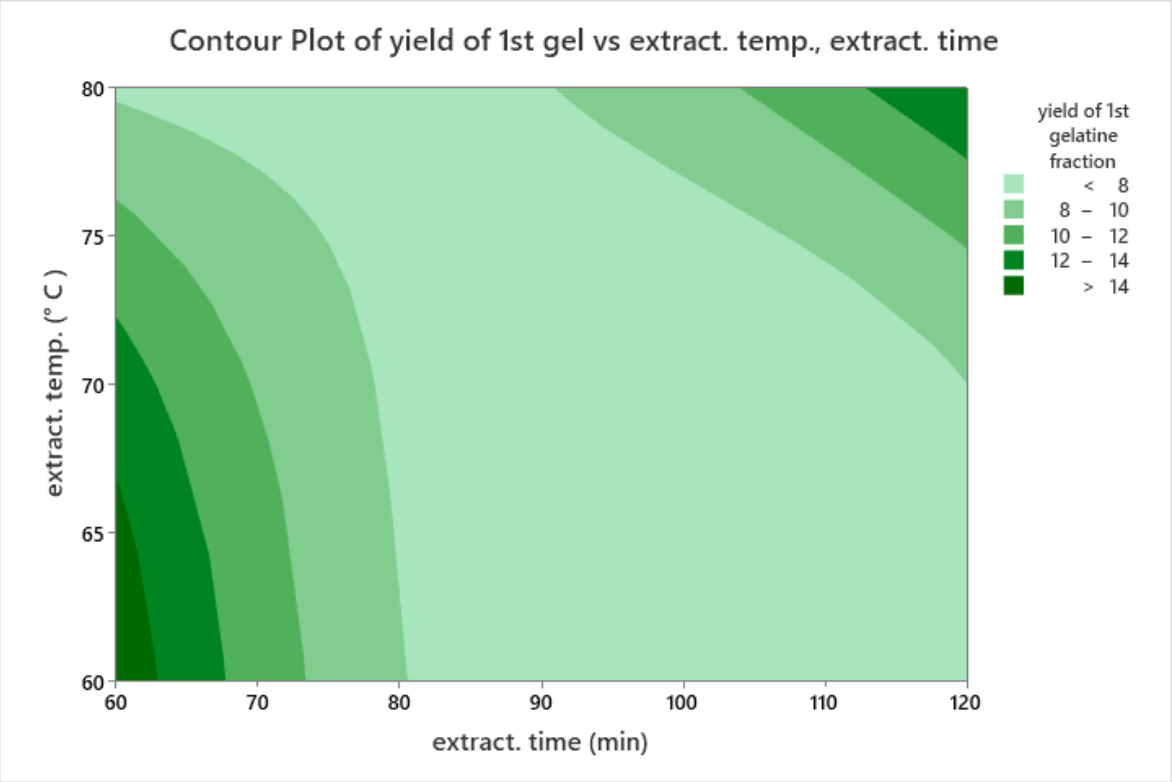


Fig. 27 contour plot of yield of 1st gelatin fraction under processing time & temperature

As explained earlier, the results of the statistical evaluation does not show any statistical significance for the yield of the first gelatin fraction. This represented by the contour graph in Fig. 27. It is evident that the addition of enzymes played a major role in the yield of gelatin. Furthermore, the extraction time and extraction temperature had an influence in the yield of the gelatin. This is to say, increasing the extraction time during the extraction process decreases at an increased temperature decreases the gelatin yield. A further increase of the extraction time will no longer affect or have an influence with the yield of gelatin. Yield of 1st gelatin fractions are compared better to the results that are accessible mostly which are derived from poultry tissues that are processed into gelatin. Acid extraction procedure mostly used indicates after extraction very low yield of gelatins (4% and 17%) from chicken feets are reported [28]. Almeida, Calarge and Santana by pressure extraction in water (120˚C for 20mins) processed chicken feet obtaining 36% yield of gelatin with low gel strength [29]. Du et al, processed the heads of chicken and turkey in an acidic condition, extracting them in two temperature stages (50˚C and 60˚C) obtaining high-quality gelatin yield of 21% and 31% of chicken heads and 25% and 35% turkey heads [30]. Using a combination of alkaline and enzymes to process chicken skin, Sarbon, Badii, and Howell processed the raw material to produce high-quality gelatin from chicken skin, with a gelatine yield of 16% [31].

6.1.1 Optimization of gel strength (1st gelatin fraction)

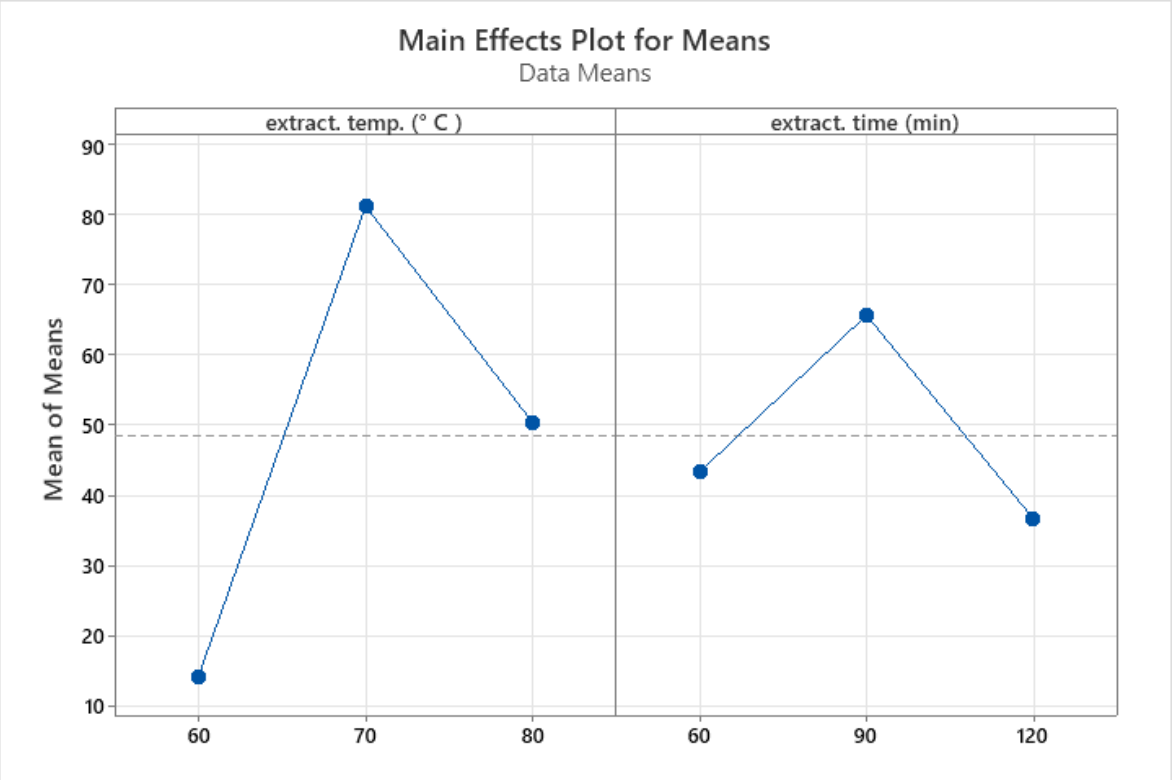


Fig. 28 Main effects of extraction temperature and time for the gel strength

The optimum level for the gel strength of 1st gelatin fraction of the extraction temperature was obtained to be 70° C, at the extraction time was obtained to be 90 minutes

Regression Equation

$$gel\ strength\ (bloom) = -68 + 1.81\ extract.temp - 0.111\ extract\ time \qquad (11)$$

Table 12. Parameters estimating the gel strength of 1st gelatin fraction of hens head obtained by regression analysis

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-68	128	-0.53	0.613	
Extract temp.	1.81	1.67	1.09	0.319	1.00
Extract time	0.111	0.556	-0.20	0.848	1.00

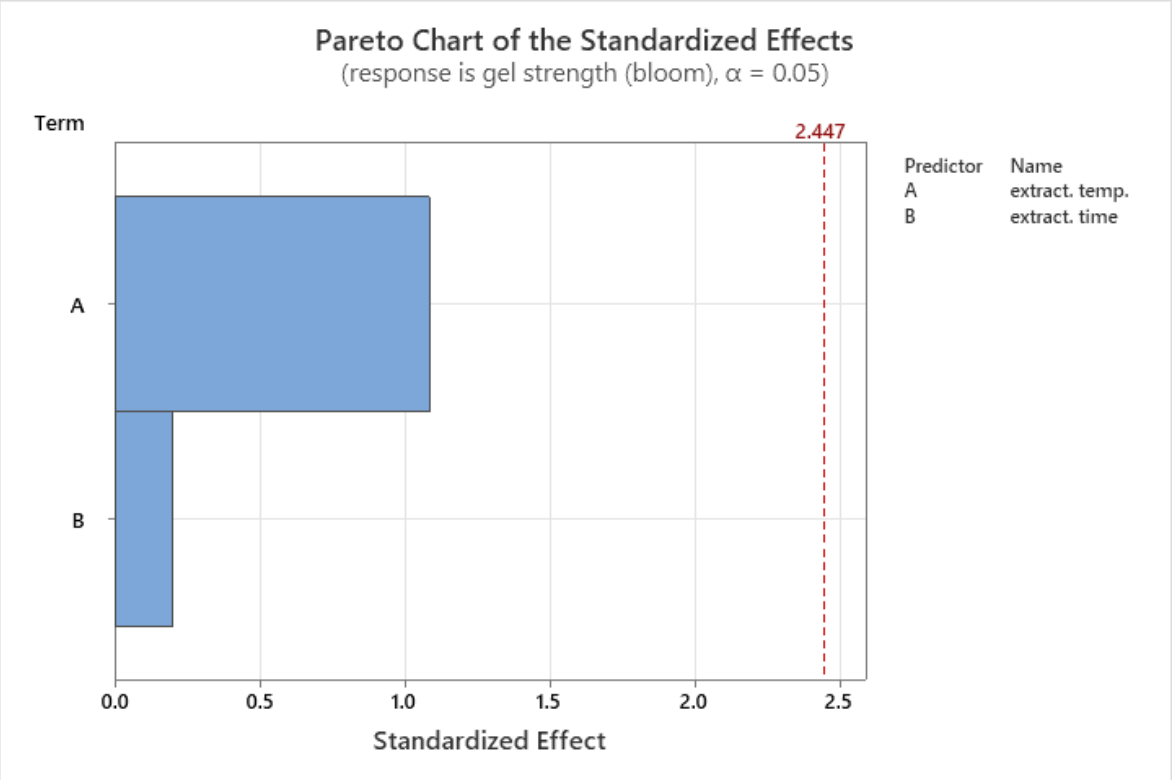


Fig. 29 Pareto chart of the standardized effects on gel strength in the first gelatin fraction

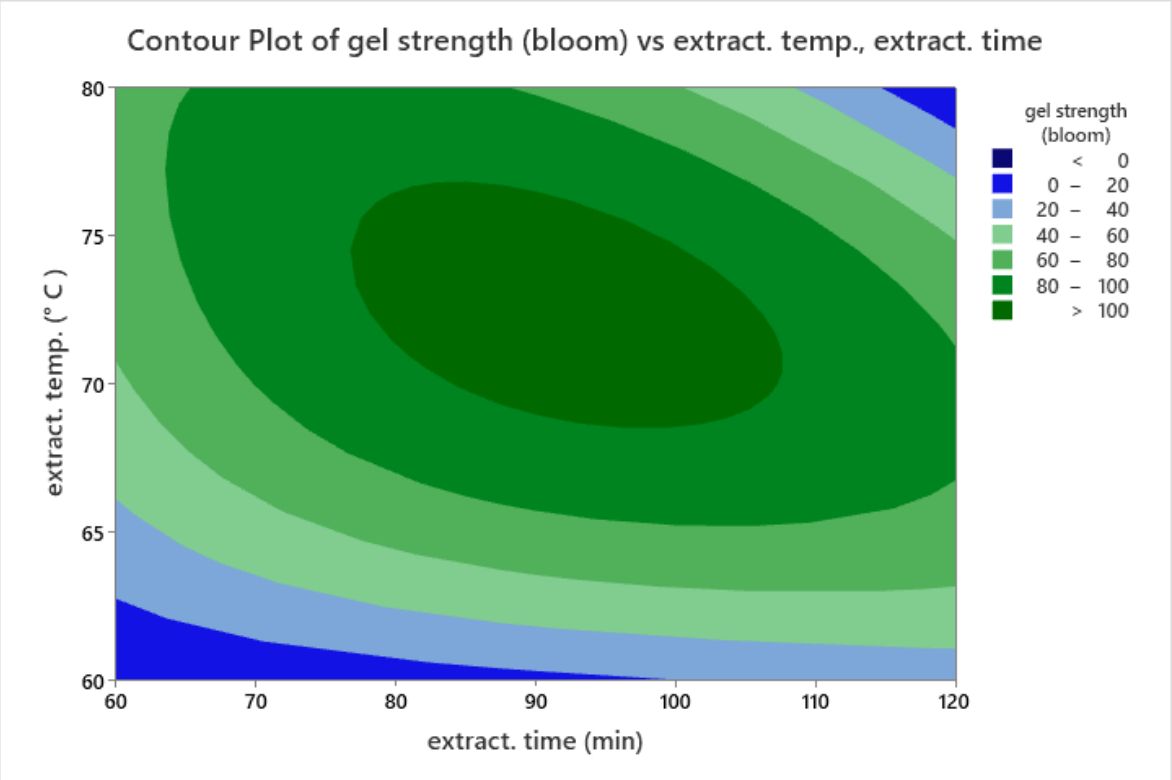


Fig. 30 contour plot for gel strength of 1st gelatin fraction under processing time & temperature

Fig. 30 represents the contour graph highlighting the influence of Factor A (temperature) and Factor B (time) have on the strength of the gelatin of the first fraction. It can be seen from the figure that within the limits of the processing factors (70˚C - 80˚C) and time (60-120 mins), gelatins of low quality (F = 50 – 103.8 Bloom) can be prepared. Gelatin strength below the low-quality with F< 50 was recorded between the process factor with temperature (60˚C) and time (90-120 mins). The standard requirements for gelatins in the food industry specifies F = 103.8 Bloom which specifies low bloom subjected to the method of application. For the production of gelatin used as clarifying agent in the food industry, F = 50-125 Bloom is prescribed.

Under various processing conditions, the gelatin strength of gelatin prepared is somehow similar to that of the commercial high strength gelatins (F>200 Bloom) from pork hides/skins, bovine, bones and fish. The gelatin prepared in my case study have a very low gelatin strength compared to gelatins processed from other poultry by-products by other authors. Gelatin processed from the feet of chicken by treating with acid of the starting material had a gel strength of 295 Bloom [29]. Reports made by Du et al also shows that gelatins processed from chicken heads produced F = 200 – 248 Bloom; however, in relation to turkey heads, higher gelatin strength were recorded (F = 333 – 368 Bloom) [30] and likewise to that of the chicken skin which is also of high-quality gelatin (F = 335 Bloom). [31]

6.1.2 Optimization of viscosity (1st gelatin fraction)

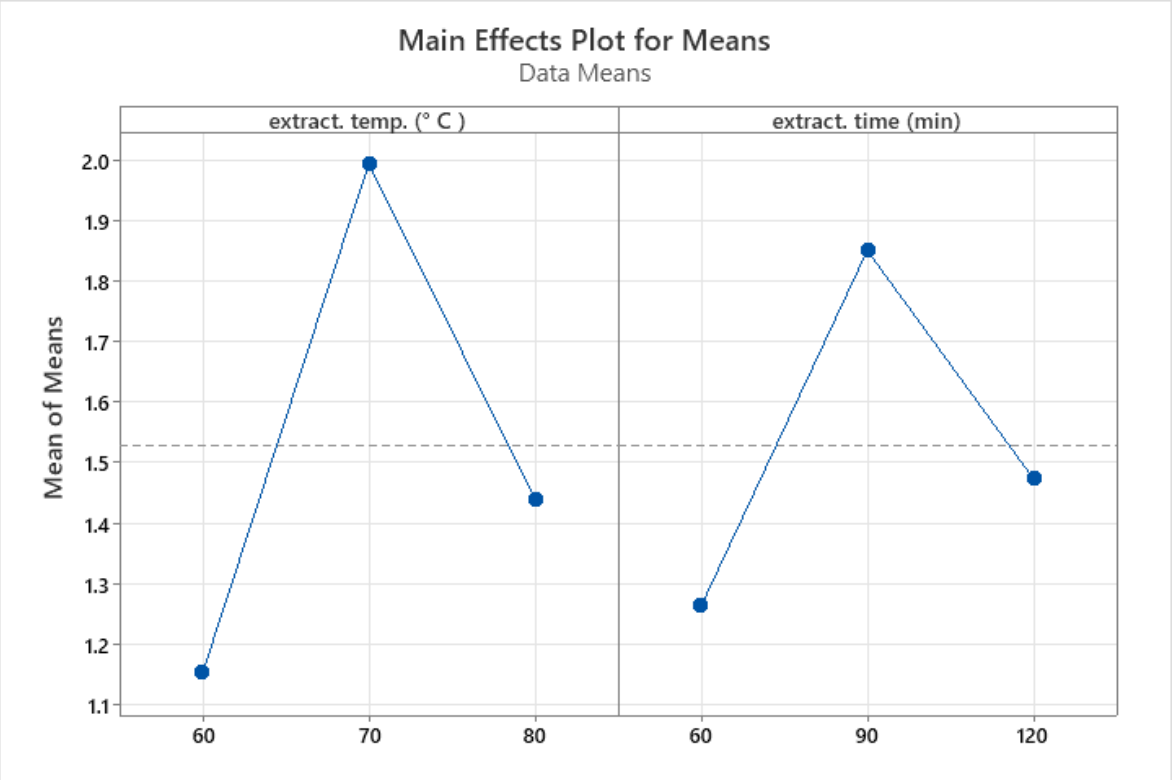


Fig. 31 main effects of extraction temperature and time for the viscosity

The optimum level for the viscosity of 1st gelatin fraction of the extraction temperature was obtained to be 70° C, at the extraction time was obtained to be 90 minutes

Regression Equation

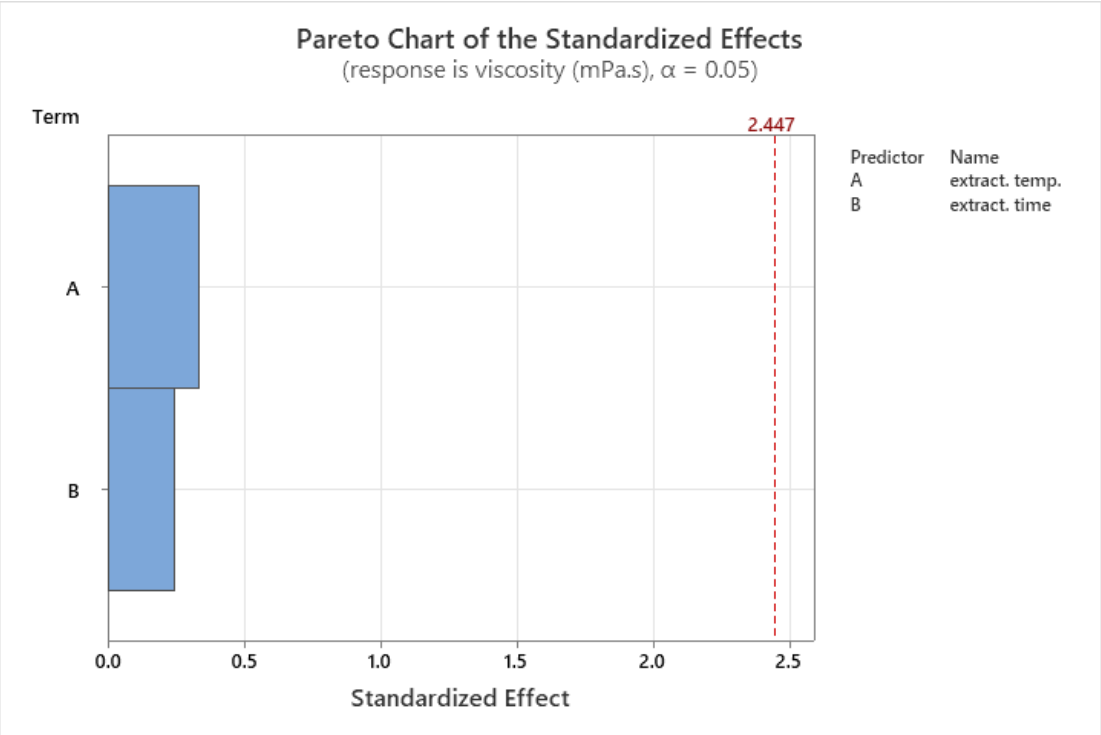
$$viscosity\ (mPa.s) = 0.21 + 0.0143\ extract.temp + 0.0035\ extract\ time \quad (12)$$

Table 13. Parameters estimating the viscosity of 1st gelatin fraction of hens head obtained by regression analysis

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.21	3.26	0.06	0.951	
Extract temp.	0.0143	0.0426	0.34	0.748	1.00
Extract time	0.0035	0.0142	0.25	0.813	1.00

Fig. 32 Pareto chart of the standardized effects on viscosity in the first gelatin fraction



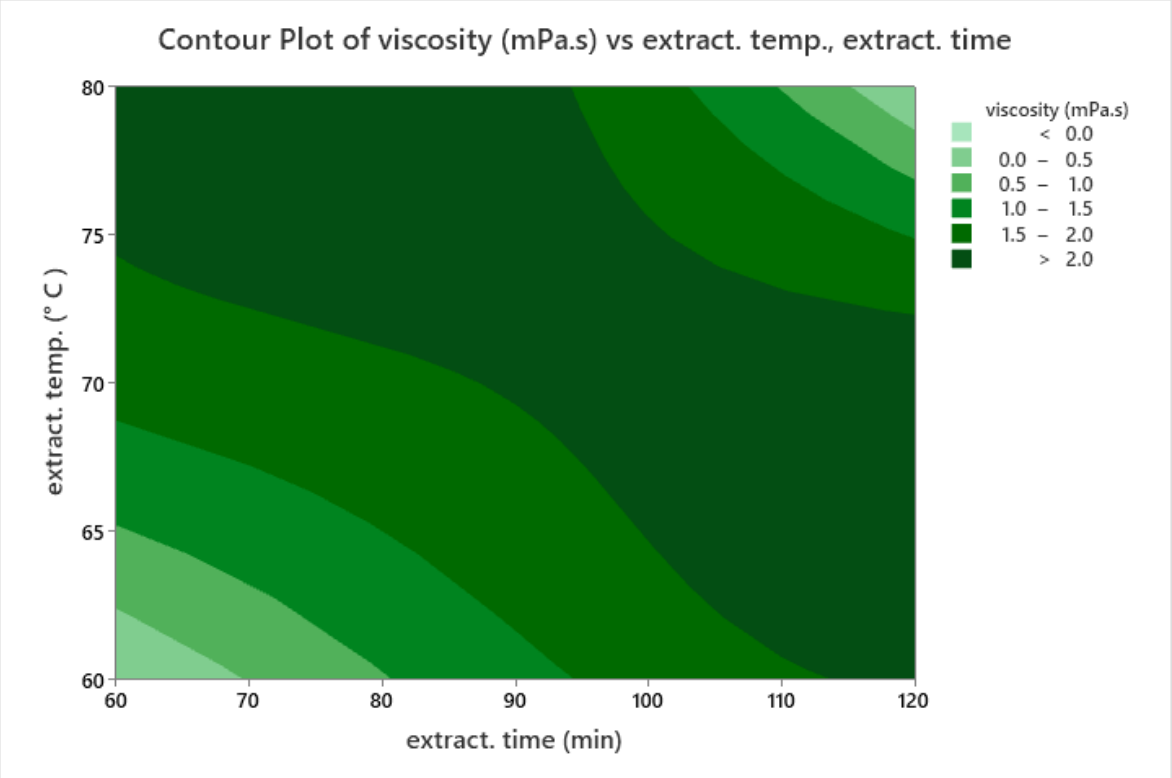


Fig. 33 contour plot for viscosity of 1st gelatin fraction under processing time & temperature

The influence in the processing conditions (time and temperature) on the viscosity is shown in Fig. 33. From the figure, it is evident that the viscosity is between the ranges (1.3mPas –

2.3mPa.s) where the highest ranges were recorded (> 2.0mPa.s). The higher gelatin viscosity within the earlier range was recorded between the processing conditions with temperature (70˚C - 80˚C) and time (60-120 mins). Furthermore, for clarifying agents used in the food industry, gel viscosity within this range id mostly applied. The gel viscosity of gelatin used for clarifying agents in the food industry is about 2mPa.s. It can be seen that from the measurement results, gelatin process under these conditions meet the specifications of gelatin for the production of clarifying agents in the food industry

From a similar project that was worked on by other authors, specifically in gelatin produced from chicken feet, a gel viscosity (> 6.5mPa.s) was recorded [32] which signifies a high viscosity in comparable to my case study. Gel viscosity produce from my case study was between 1.3mPa.s – 2.3mPa.s where the average was about 2.1mPa.s. Furthermore, a similar work was done but in this case from chicken skin as alternative to pork and beef solutions where the gel viscosity (3mPa.s – 5.7mPa.s) was recorded [33]

6.1.3 Optimization of Ash Content (1st gelatin fraction)

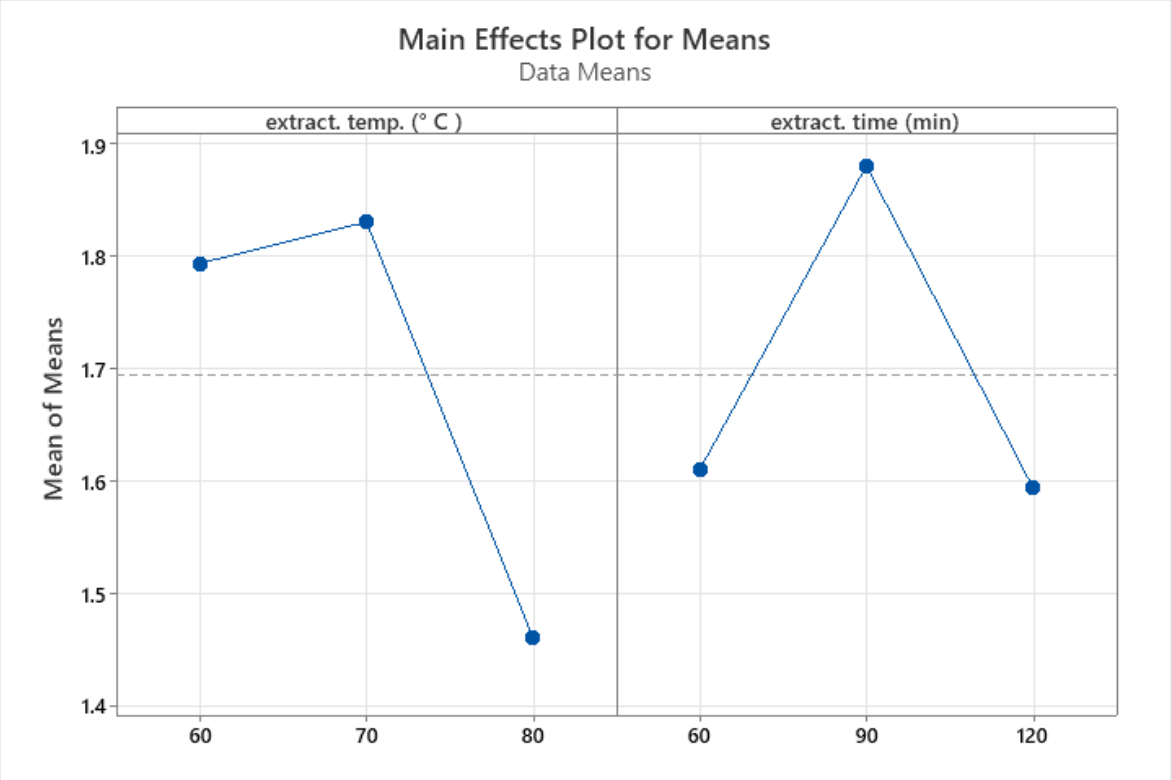


Fig. 34 main effects of extraction temperature and time for the ash content

The optimum level for the ash content of 1st gelatin fraction of the extraction temperature was obtained to be 70° C, at the extraction time was obtained to be 90 minutes

Regression Equation

$$Ash\ content\ (\%) = 2.89 - 0.0167\ extract.temp - 0.00028\ extract\ time \quad (13)$$

Table 14. Parameters estimating the ash content of 1st gelatin fraction of hens head obtained by regression analysis

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	2.89	1.25	2.31	0.061	
Extract temp.	-0.0167	0.0163	-1.02	0.347	1.00
Extract time	-0.00028	0.00545	-0.05	0.961	1.00

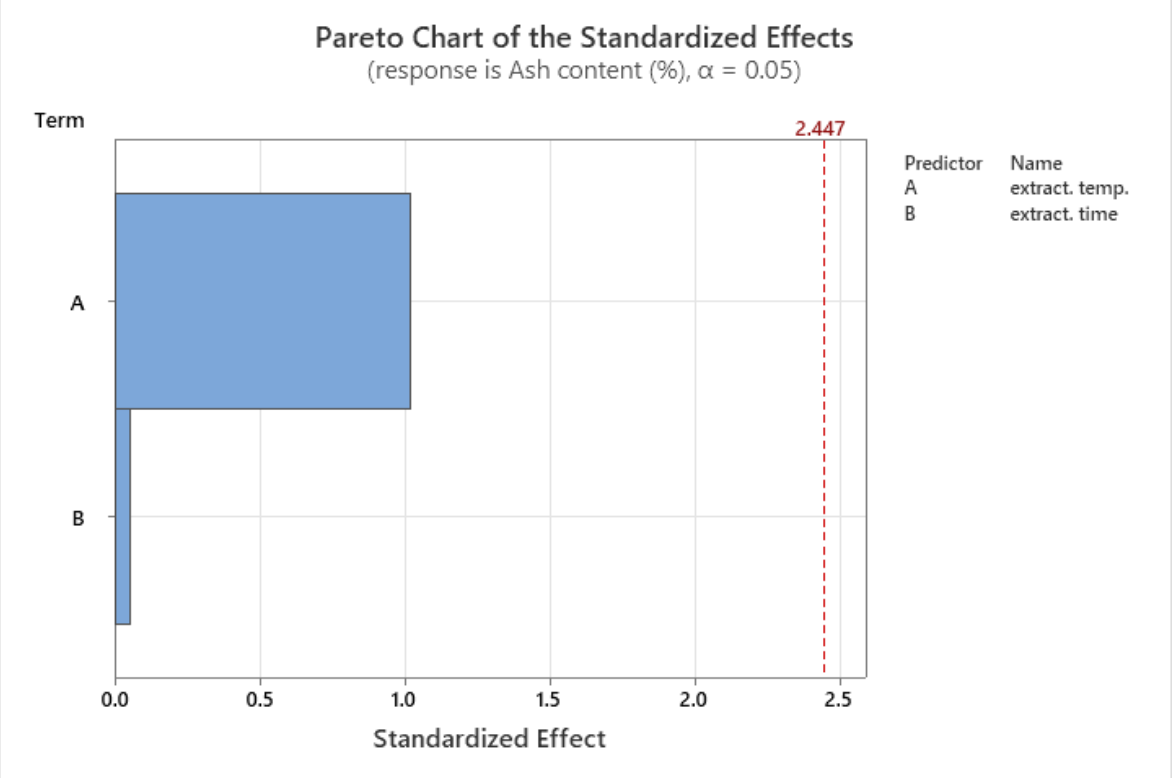


Fig. 35 Pareto chart of the standardized effects on ash content in the first gelatin fraction

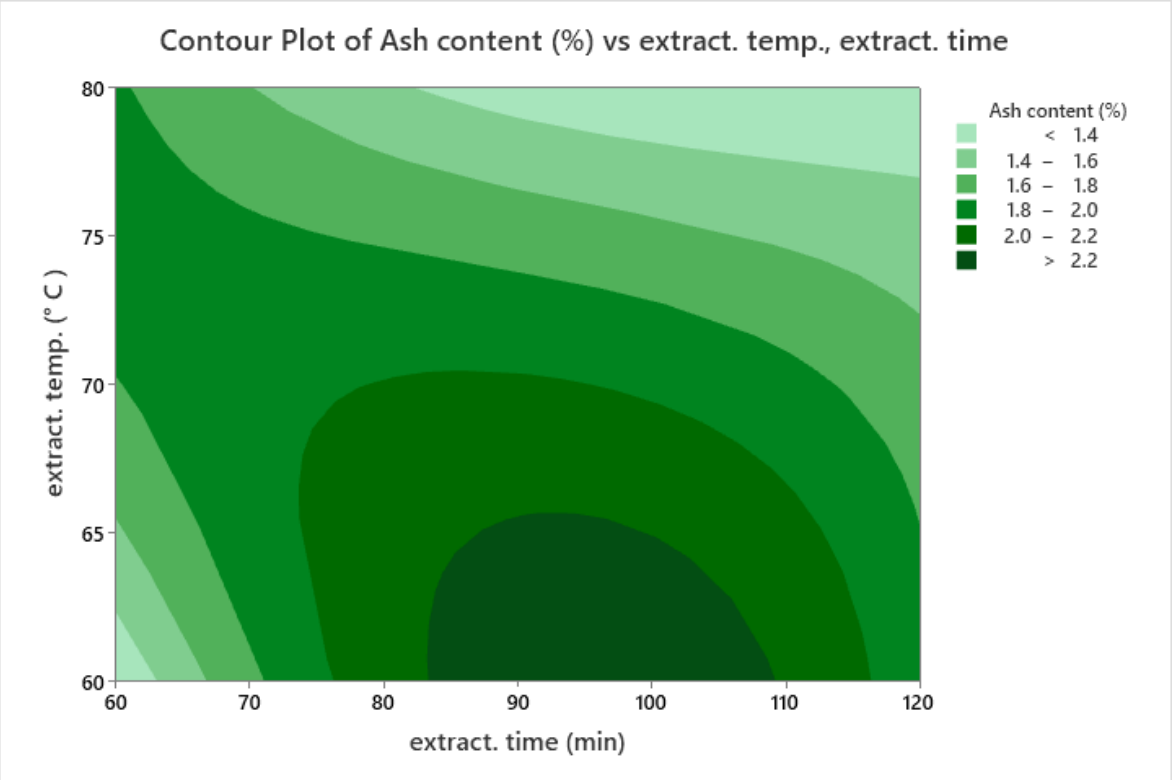


Fig. 36 contour plot for ash content of 1st gelatin fraction under processing time & temperature

Fig. 36 also represents the contour graph highlighting the influence of Factor A (temperature) and Factor B (time) have on the ash content of the gelatin in the first fraction. It can be seen from the figure that the ash content from was between 1.2% - 2.0% constituting to a lower ash content which in this case is actually better. This shows that effective demineralization process was ensured and hence there are few traces of minerals in the gelatin processed. Furthermore it can also be seen that the gelatins with the highest amount of yield recorded the lowest ash content (1.21%, 1.24%, 1.31% and 1.79%).

Under various processing conditions, the ash content from the prepared gelatin of the first fraction is somehow similar to that of the commercial high strength gelatins (from deboned chicken meat residue). The gelatin prepared in my case study have a very low ash content compared to gelatins processed from other residue of deboned chicken meat by other authors. Gelatin processed from the residue of a deboned chicken meat by treating with a combination of alkaline-acid extraction process of the starting material had an ash content of 10.10±0.17% [34]. Also, gelatin extracted from goat skin by pre-treating with HCl and extracted with distilled water at 60˚C for 9hours of the starting material had a low ash content of 0.11% [35] which is much lower than that of my thesis.

6.1.4 Optimization of dry matter (1st gelatin fraction)

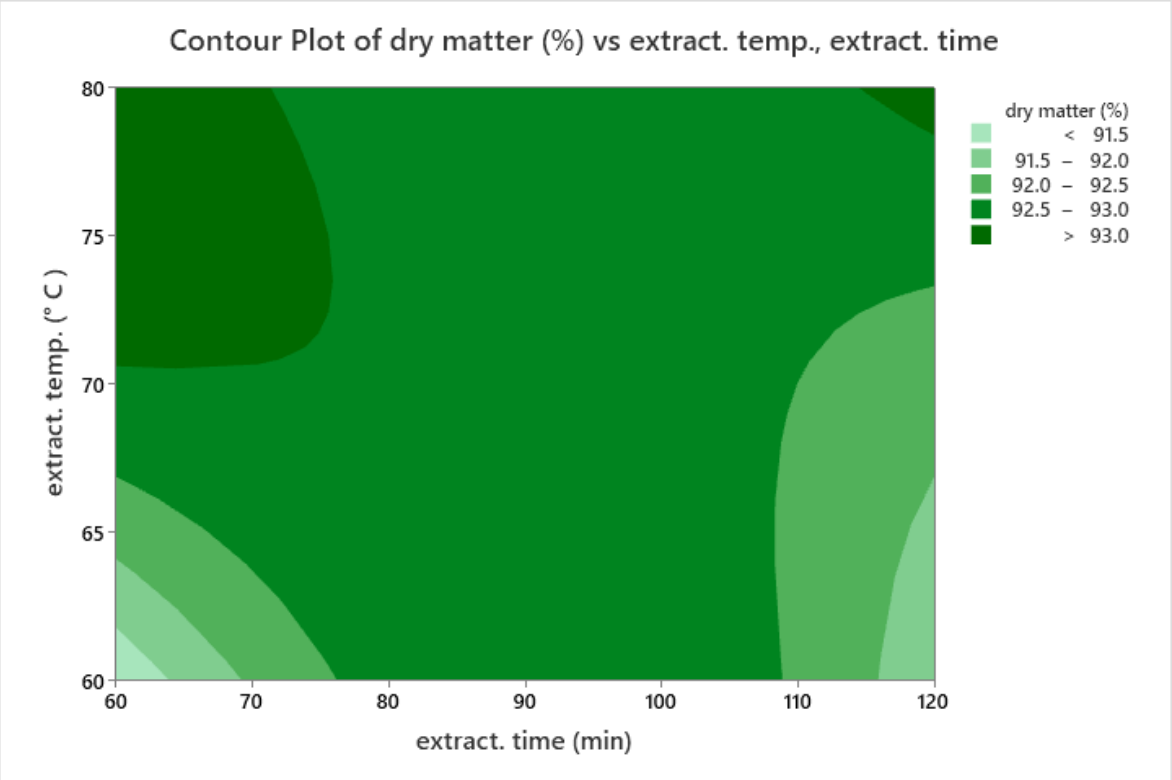


Fig. 37 contour plot for dry matter of 1st gelatin fraction under processing time & temperature

In fig. 37, it can be observed that the dry matter of the 1st gelatin fraction was between 91-94%. Comparing this to starting material (46.4±1.2%) shows that an effective drying process hence better processing condition.

6.1.5 Optimization of WHC (1st gelatin fraction)

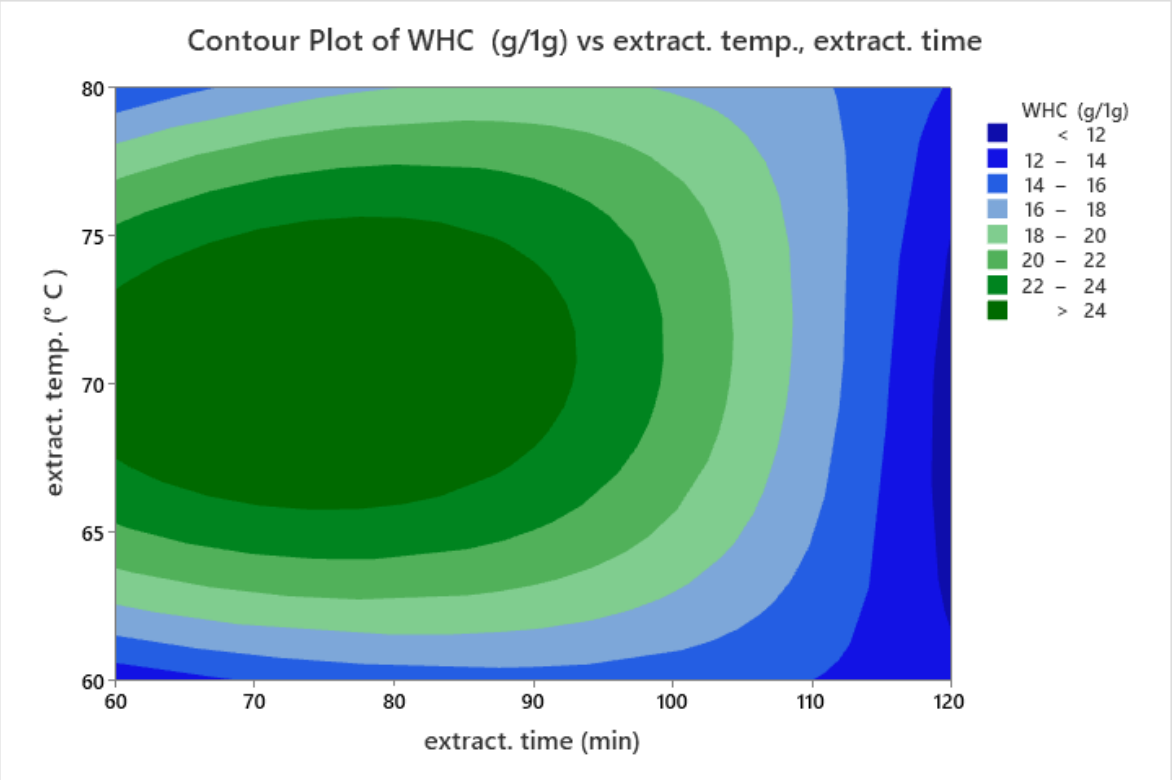


Fig. 38 contour plot for WHC of 1st gelatin fraction under processing time & temperature

6.1.6 Optimization of FBC (1st gelatin fraction)

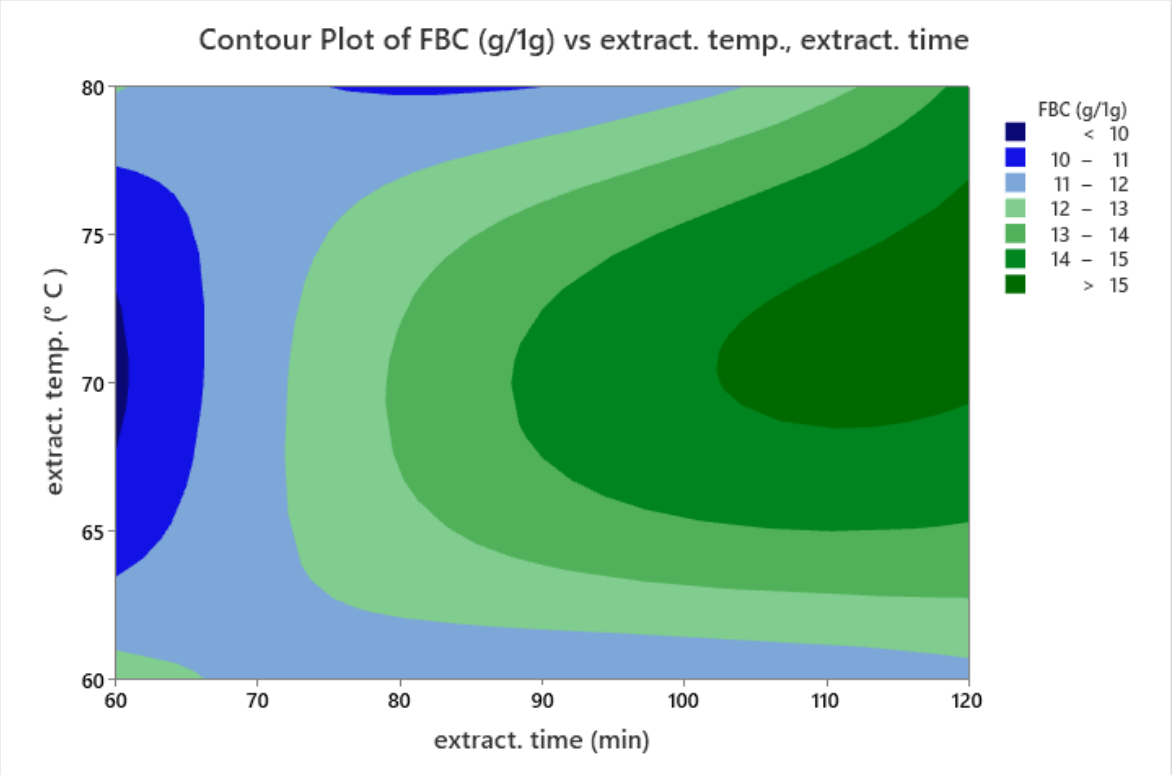


Fig. 39 contour plot for FBC of 1st gelatin fraction under processing time & temperature

Fig 38 and 39 shows contour plot for WHC and FBC of 1st gelatin fraction under processing time & temperature respectively. According to a similar case study in relation to the WHC and FBC shows that the gelatin produced had a WHC between 3.8 – 5.6 and that of FBC was between 0.9 – 1.3. This result was in relation to a work from chicken skin as alternative to pork and beef solutions [33]. The WHC and FBC recorded in my case study was 11.1g/1g – 24.9g/1g and 9.8g/1g – 15.1g/g respectively. One key observation made in this result is that gelatin with high yields recorded high WBC and FBC.

6.1.7 Optimization of Gel Melting Temperature, ˚C (1st gelatin fraction)

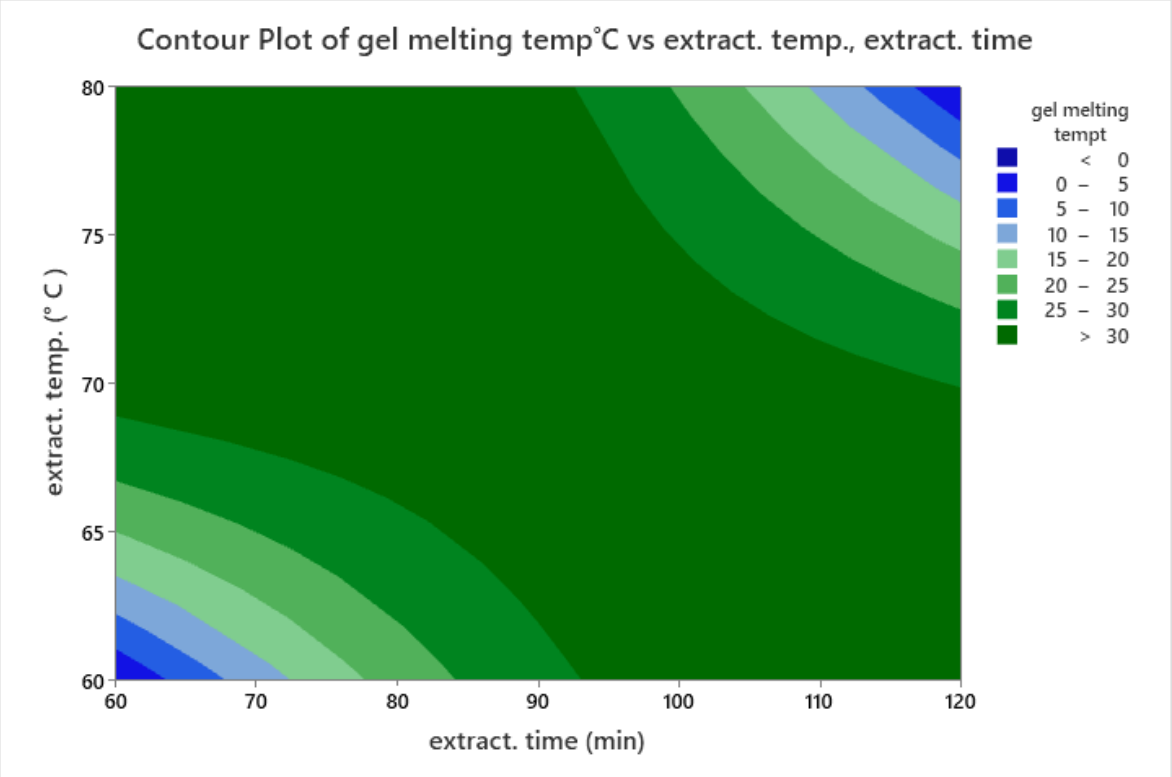


Fig.40 contour plot for gel melting temperature of 1st gelatin fraction under processing time & temperature

6.1.8 Optimization of Gel Solidification Temperature, ˚C (1st gelatin fraction)

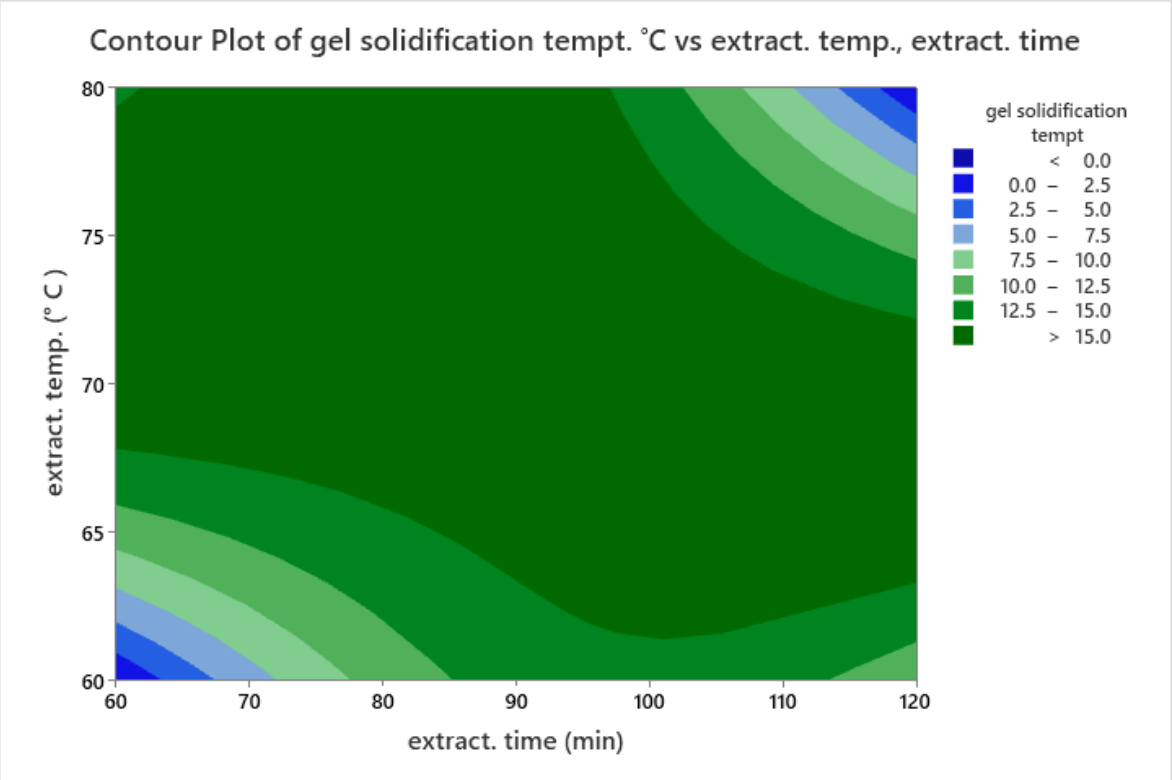


Fig.41 contour plot for gel solidification temperature of 1st gelatin fraction under processing time & temperature

Fig 39 and 40 shows contour plot for the gel melting temperature and gel solidification temperature of 1st gelatin fraction under processing time & temperature respectively. The gel melting temperature for the first gelatin fraction was observed to be in the range from 28.5˚C – 33.4˚C while that of the solidification temperature was observed to be in range from 10.3˚C – 17.2˚C. Gelatin melting temperature properties from a sprayed dried-skin goat gelatin and a freeze-dried counterpart shows the gelling and melting temperature range from 25.14˚C – 25.23˚C and 34.09˚C – 34.18˚C respectively [36].

From the values in table 11, it can be observed the bloom value obtained from the gelatin in the range between 50 - 125 blooms, hence signifying a gelatin from the low bloom category. Gelatins in this category has an average molecular mass between 20,000 - 25,000 Da

From the figures above, it can be observed that in figure 23, the gel strength was optimized between the extraction time 80 - 100 mins at an extraction temperature between 70° C - 75° C. From the table 10, it can be deduced that the processing conditions for the extraction of 1st gelatin fraction had an effect on the gel strength. From the early stage of extraction (at a

temperature of 60° C for an extraction time of 60mins), the gel strength was 0. This further increased from 0 to 15.05 bloom and further increased to 27.3 bloom as the time for the extraction increased. At the extraction of 70° C for a time of 60mins, the gel strength was recorded to be 57 bloom and increased to its optimum at a time of 90mins. At its maximum peak, the gel strength starts to decrease as we further increased the extraction temperature. With this observation, the optimum conditions for the gel strength for the 1st gelatin fraction was between 70° C - 75° C at an extraction between 80 - 100mins as shown in figure 23. Also in figure 26, we can observe a direct relationship between the gel strength and the viscosity. An increased in the gel strength increases also the viscosity of the gelatin yield. The processing conditions for the optimum gel strength was observed at an extraction temperature of 70° C and at an extraction time of 90mins. The Water holding capacity, WHC (in fig 27) increased between the extraction time 60mins-90mins and at temperatures between 65° C - 75° C. Finally, the Fat binding capacity, FBC (in fig 28) attained its optimization conditions between the extraction time 90mins - 120mins and extraction temperature between 65° C-80° C. It can also be observed that the use of enzymes also played a major role in the extraction process since gelatin that were utilized effectively by the enzymes produced good results. Experimental results with low yield of gelatin fractions extracted gave high bloom value than those with high gelatin fractions. This can be based on the utilization of the enzymes. Temperature and time can be another source for the enzyme utilization hence producing better results. Furthermore, due low gelatin yield, this could be as a result of low protein level contained in the head of poultry (Hen). Base on the Bloom value which is actually a low Bloom value, it is recommended that gelatin of this nature can be used in the food/beverage industry as clarifying agent

6.2 Yield of 2nd gelatin Fraction

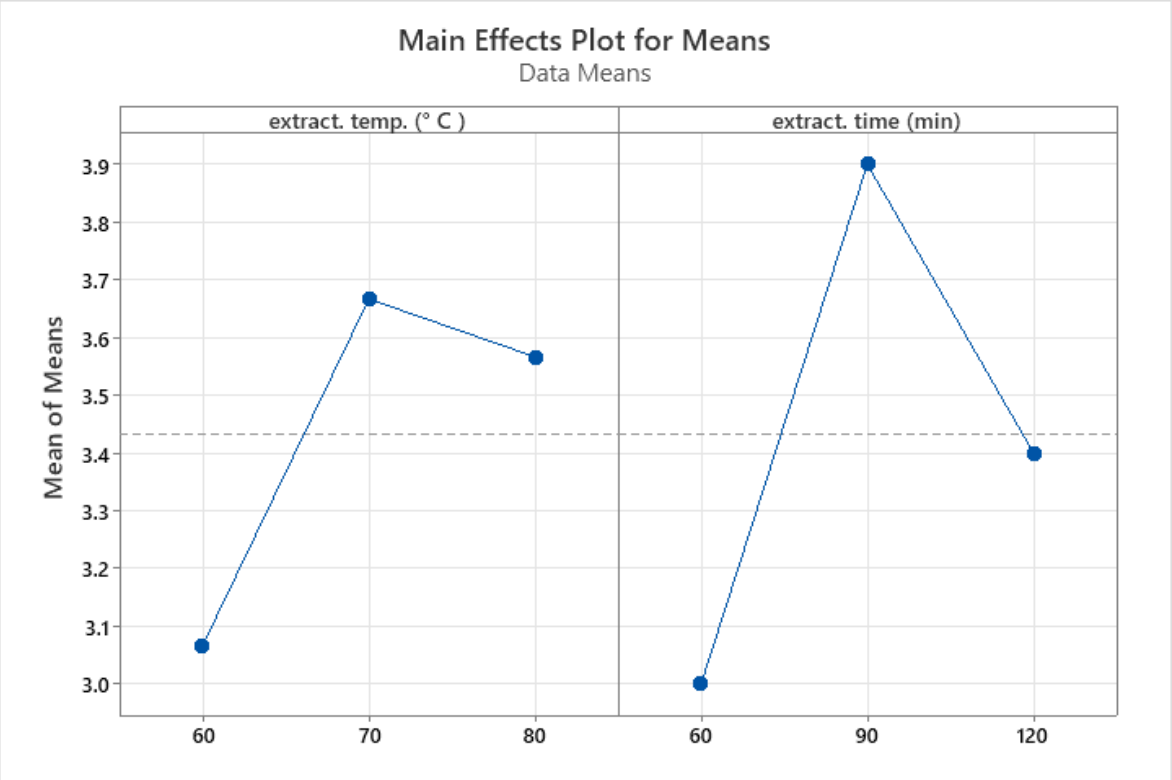


Fig. 42. Main effects of extraction temperature and time for 2nd gelatin fraction

The optimum level for the yield of 2nd gelatin fraction of the extraction temperature was obtained to be 70° C, at the extraction time was obtained to be 90 minutes

Regression Equation

$$2nd\ gel.fract.(\%) = 1.08 + 0.0250\ extract.temp + 0.00667\ extract\ time \quad (14)$$

Table 15 Parameters estimating the yield of 2nd gelatin fraction of hens head obtained by regression analysis

Coefficient

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	1.08	2.12	0.51	0.627	
Extract temp.	0.0250	0.0276	0.91	0.400	1.00
Extract time	0.00667	0.00920	0.72	0.496	1.00

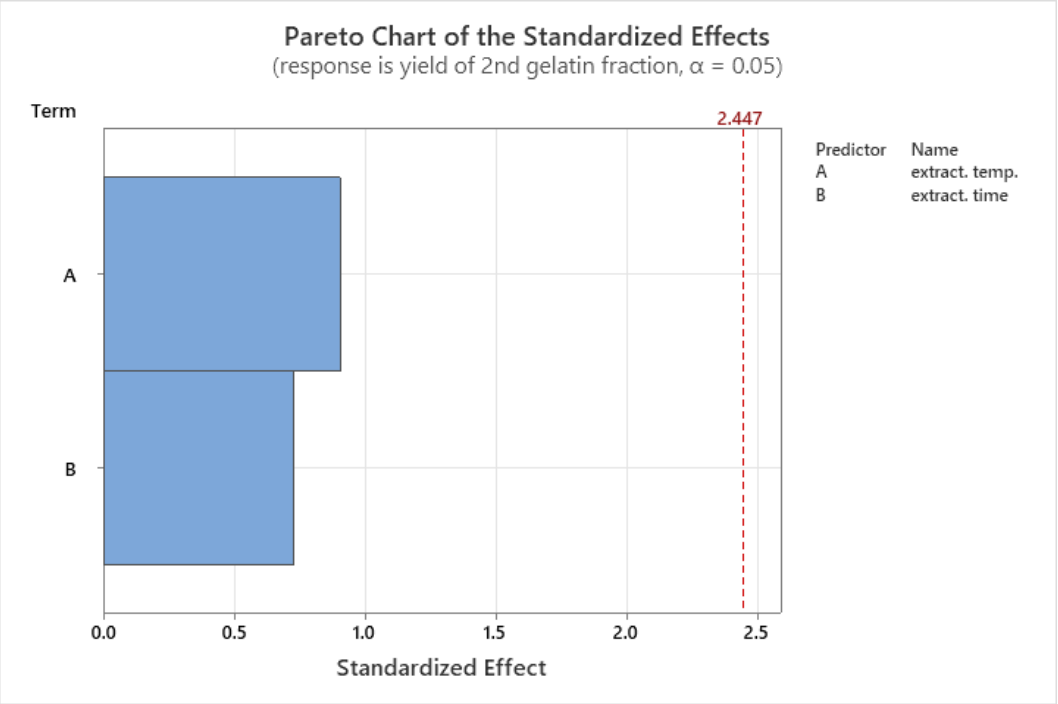


Fig. 43 Pareto chart of the standardized effects on yield in the second gelatin fraction

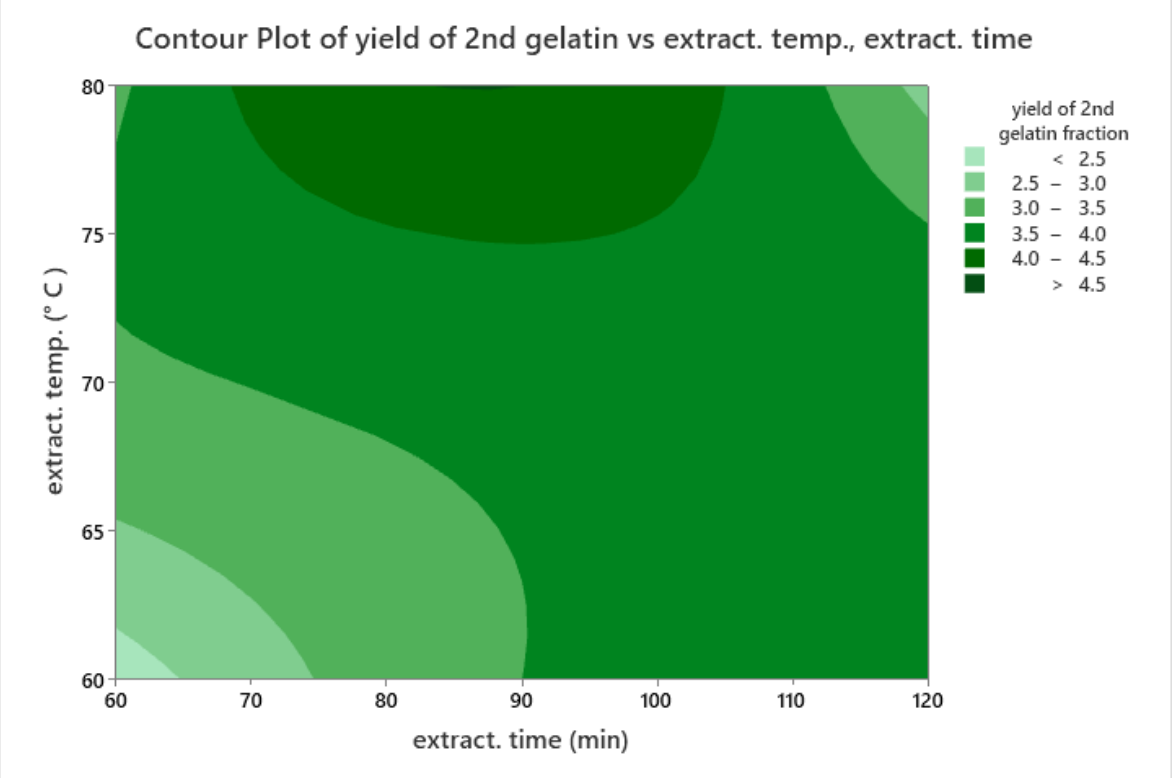


Fig.44 contour plot for yield of 2nd gelatin fraction under processing time & temperature

Fig. 41 shows the main effects of extraction temperature and time of 2nd gelatin fraction. The optimum level for the yield of 2nd gelatin fraction of the extraction temperature was obtained to be 70° C, at the extraction time was obtained to be 90 minutes. The represented contour graph in Fig. 43 shows the plot for yield of 2nd gelatin fraction. It is evident that the yield of the 2nd gelatin fraction increases with increasing extraction temperature and time. This may be as a result of the action or the influence of enzyme during the extraction process. The optimum processing conditions for the yield of the 2nd gelatin fraction occurred at the 5th experiment which was at the extraction temperature of 70˚C at a time of 90 mins. This can be said for that of the processing conditions (temperature and time) for the 1st gelatin yield fraction also occurring at the same extraction temperature and time

Table 16. Layout of experiments and evaluation of 2nd gelatin fraction properties

Exp No.	Gel strength (Bloom)	Viscosity (mPa.s)	Water Holding Capacity, WHC (g/1g)
1	0	0	11.5
2	24.43[C]	2.27	12.0
3	5.5[B]	2.169	11.4
4	27.5[C]	-	24.0
5	17.7[C]	-	6.4
6	60.45[B]	2.7135	11.3
7	68.11[B]	2.941	11.1
8	0	0	10.7
9	0	0	8.3
BLANK EXPERIMENT (WITHOUT THE ADDITION OF AN ENZYME)			
10	131.3[C]	3.935	35.4

A, B and C shows the actual method of gel strength analysis used

Base on the result of the 2nd gelatin fraction of the 10th experiment, the melting and solidifying temperature were done. The melting temperature was 33.3° C and the solidifying temperature was 14.8°

With respect to the gelatin strength of the 2nd gelatin fraction, the extraction temperature and time for the optimum processing conditions occurred at a temperature of 70˚C-80˚C. The viscosity of the second gelatin fraction was recorded to be higher than the first fraction (2.0mPa.s – 3.0mPa.s). Interestingly, in certain experiment, gels were not able to form during the first fraction but upon further investigations in the second fractions, gels formed in the process. This could mean that the processing conditions were not favourable in some of the experiment in the first fraction but was better in the second. Experiment number 10 in particular. In respect to the WHC, the result in the first gelatin fraction were higher than that of the second gel fraction. This can be said that this is caused as a result of the high amount of gelatin yield in the first fraction as compared to the second fraction

6.3 Yield of 3rd gelatin Fraction

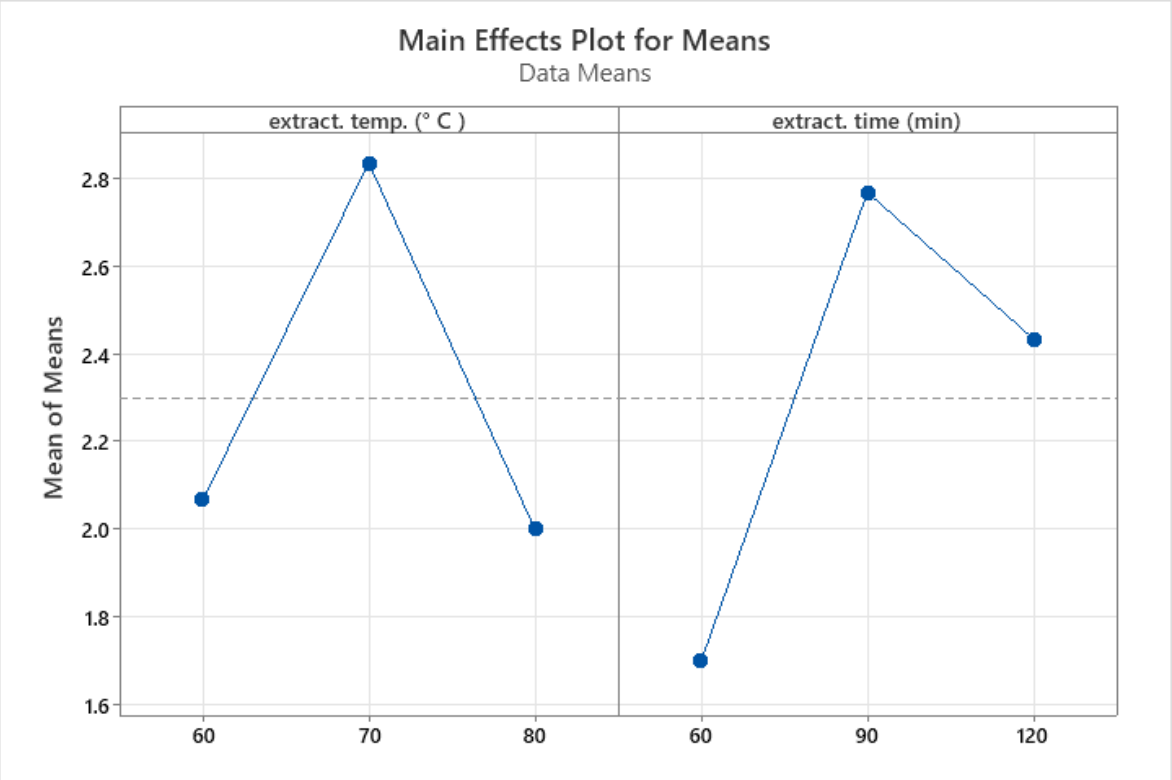


Fig. 45. Main effects of extraction temperature and time for 3rd gelatin fraction

The optimum level for the yield of 3rd gelatin fraction of the extraction temperature was obtained to be 70° C, at the extraction time was obtained to be 90 minutes

Regression Equation

$$3rd\ gel.fract.(\%) = 1.43 - 0.0033\ extract.temp + 0.0122\ extract\ time \quad (15)$$

Table 17 Parameters estimating the yield of 2nd gelatin fraction of hens head obtained by regression analysis

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	1.43	3.37	0.43	0.685	
Extract temp.	-0.0033	0.0440	-0.08	0.942	1.00
Extract time	0.0122	0.0147	0.83	0.436	1.00

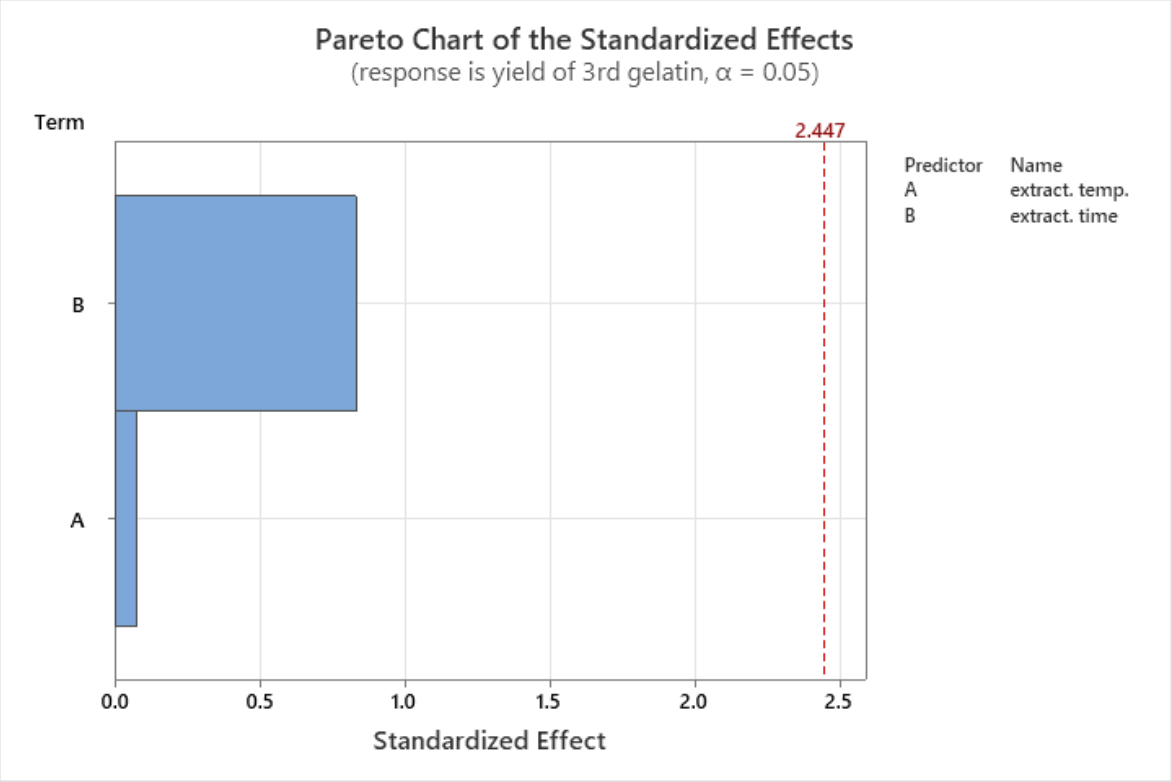


Fig. 46 Pareto chart of the standardized effects on yield in the third gelatin fraction

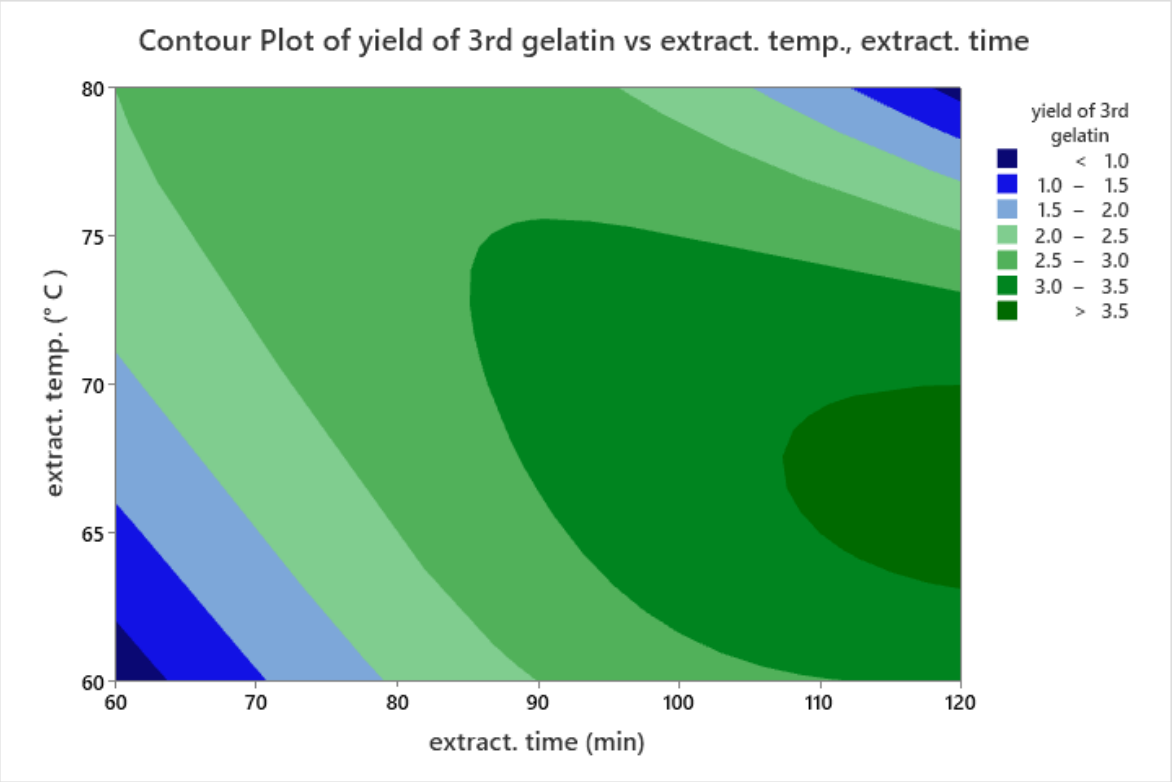


Fig.47 contour plot for yield of 3rd gelatin fraction under processing time & temperature

From the contour plot for the yield of 3rd gelatin fraction shown in fig. 46, it can be seen that yield of the gelatin fraction also increases with increasing processing condition and time. The total yield of the 3rd gelatin fraction was between 0.7 - 3.5%. The yield of gelatin produced in the 3rd fraction was small as compared to the 2nd and 3rd fraction. This confirms then effective use of the enzymes in all these fraction. The utilization of the enzymes with respect to the amount of gelatin yield in their respective fractions decreased. Further analysis on gelatin properties were not done due to the smaller amount of yield produced at the end of the extraction process.

6.4 Comparing of results to literature reference

Table 18 Comparing results to literature reference according to their optimum processing conditions and time

Articles	Gelatin Yield (%)	Gelatin Strength (Bloom)	Gel Viscosity (mPa.s)	WHC (g/g)	FBC (g/g)	FC (%)	FS (%)	EC (%)	ES (%)
[33]			3-5.7	3.8-5.6	0.9-1.3	18-61	4-39	35-50	73-88
[35]	20	-	-	-	-	-	-	-	-
[41]	20-32	-	-	3.9-47	16-21	-	-	-	-
[42]	19.8-22.4	426	-	-	-	-	-	-	-
[30]	52-63	200-380	-	-	-	-	-	-	-
MT	23.7	50-130	2.0-4.0	11.1-25.0	11.0-15.20	1-10	0-2	40-47	90-100

[33] Chicken skin gelatin as alternative to pork and beef solutions, Petr Mrázek, Pavel Mokrejš, Robert Gál, Jana Orsavová : chicken skin, enzyme conditioning (24hr, extraction at 40° C for 60 mins)

[35] Characterization and Antioxidant activity of collagen, gelatin and the derived peptides from yellow fin tuna (Thunnus albacares) skin: Tuna skin, acid conditioning (4° C, extraction at 65° C)

[41] Effect of preparation method on physiochemical scavenging and proliferative properties of Gelatin from Yak skin: Yak skin, freeze-dried enzymatic conditioning (24hrs at 37° C, extraction at 90° C for 6hr)

[42] Valorization of discarded industrial fish processing wastes for the extraction of gelatin to use as biodegradable fish bait matrix using RSM: fish waste (head, skin and fins), acid conditioning (30mins, extraction at 55° C for 18hr)

[30] Physiochemical and functional properties of gelains extracted from turkey and chicken heads: turkey and chicken heads, acid conditioning (18hrs at 4° C, extraction at 50° C for 18hrs and 60° C for 6hrs)

MT is my thesis study

From the first case study which was Chicken skin gelatin as alternative to pork and beef solutions. Considerably large amounts of by-products such as chicken heads, feathers, skin, legs, bones and even legs containing important volumes or proteins, specifically collage are produced by poultry meat-processing industries. Utilization of these under used by-products can be one of the possibilities of an advantage in respect to their application as a raw material rich in collagen for gelatin preparation. In this particular study, chicken skin was the main source of raw material obtained from the chicken-breast. This processing were purified from protein non-collagenous part, fats and pigments. The collagen was treated with a proteolytic enzyme and the extraction of gelatin performed after treatment with the enzyme. This extraction was done in distilled water at varying temperatures 40, 50, 60, 70 and 80˚C during a fixed extraction time 60 min. The effect of the processing conditions on the functional properties of gelatin which includes the clarity, viscosity, water holding capacity and fat binding capacity, foaming and emulsifying properties were analyzed. Some of the gelatin functional properties prepared were importantly influenced by the extraction temperature while other properties had no significant effect caused by the extraction temperature. The viscosity obtained from the chicken gelatin was in the range of 3 to 5.7 mPa.s. Comparing this result to my thesis shows that this study had a high gelatin viscosity. WHC obtained in this study was 3.8 to 5.6 ml/g and FBC was 0.9 to 1.3ml/g. My case study had high WHC and FBC hence gelatin produced from hens head has high WHC and FBC than gelatin produced chicken skin. The emulsion capacity and stability were 35 to 50% and 73 to 88% respectively while that of the foaming capacity and foaming stability were 18 to 61% and 4 to 39%. Comparing these values to my case study shows that the foaming capacity and stability and emulsifying capacity had higher results than hens head. The emulsification stability on the other hand was lower as compared to my thesis.

In the second case study which was also Characterization and Antioxidant activity of collagen, gelatin and the derived peptides from yellow fin tuna (Thunnus albacares) skin. Utilization of skin waste from tuna processing came into effect by extracting its collagen and preparing gelatin. The collagen functional properties and gelatin were improved by enzymatic hydrolysis for peptides conversion. The aim of this particular study were the characteristics and antioxidant activity of collagen, gelatin examination and peptide derived from yellowfin tuna fish. Extraction of collagen was ensured by treating with 0.75 M acetic acid at 4˚C, and treating with 0.25% citric acid to prepare gelatin and extracting it at 65˚C. With 2% Alcalase, hydrolysis was carried out which was followed by the division with a molecular weight cut-off sieve for collagen and gelatin. The yield of collagen obtained was 22.6% with pH of 6.63. The yield of gelatin was 20.0% with a pH of 4.94. The gelatin yield obtained in my case study was 23.7% which 3.7% above this study. The pH value in my thesis study was 6.90 to 7.10. Hence the pH value of my thesis is considered neutral hence can be used for consumption and not acidic or acidic. This one way or the other makes my thesis much better since I used mainly the use of enzymes for my extraction process.

Considering the fifth case study, which was the Physiochemical and functional properties of gelains extracted from turkey and chicken heads, Gelatins were prepared from heads of turkey and chicken and this was done in a series of batch extractions at two different temperature, 50 and 60˚C, and where their composition and gelatin functional properties evaluated. The starting raw materials were treated by acid conditioning for 18 hours at a temperature of 4˚C. Extraction of the collagen into gelatin was done at firstly a temperature of 50˚C for 18 hrs and secondly an extraction temperature of 60˚C for 6 hrs. The yield of Gelatin from chicken and turkey heads was obtained as 55.29 and 62.76% respectively. The gelatin yield in this study case was much higher than that worked in my study case. The gelatin strength on the other hand that was evaluated in this case study was recorded from 332.7 to 368.4g.

Bothe Gelatin from the head of chicken and turkey had high solubility at acidic and alkaline values of pH. Comparing this case to my thesis study shows that the gelatin strength in this case study was very high and hence have higher bloom value. On the other side it is not environmentally safe since acid was used for conditioning treatment

6.5 Importance of my case study

Considering the demand of gelatin for its application either in the food industry, pharmaceutical industry, cosmetic industry, photographic industry or other industries. These have placed so much demand gelatin production hence shortages of raw materials in the meat processing industries. Per statistics, it can be observed that there have been a decline of gelatin production from the main sources and this has one way or the other affected production. Also, people mostly discard away the by-products of animals without necessarily finding an alternative use of it. In this regard, using my case study as an example, it can help cut down the cost and burden on the processing industry and channel so much focus on the use of by-product for gelatin production. This is just saying ‘‘using one solution to solve two questions or problem’’. Hence one advantage of my case study as compared to other case studies

The use of chemicals (either acid or alkaline) can have an effect on the environment if not properly treated. These chemicals when they find their ways into the water bodies can have an adverse effect on the environment, humans and animals. Furthermore the use of chemicals for conditioning treatment of raw materials make of so much water. This is say so much amount of water is mostly needed to wash away these chemicals before the extraction process. The question one can ask is why waste so much water when ideally countries in other part of the world needs this to survive. The amount of water that is mostly lost during the use of chemicals as treatment for conditioning of the raw materials is something that needs to be paid attention to. As the saying goes water is Life and as an individual coming from the shores of Africa and realizing water as a basic need or necessity, I value so much about the usage of water. This is of course one of the main reason why my case study is much better than other case studies where chemicals (acid and alkaline) were used for the conditioning process. On the other hand, I used enzymes which is environmentally friendly and poses no harm to the environment, humans and animals in general. Furthermore, only small amount of water is needed to wash away the enzymes before the extraction process occurs

6.6 Application of Gelatin produced in my case study

Since the bloom value in my case study is from 50-130 Bloom, this represents a low bloom gelatin strength. Hence this can be used in the pharmaceutical industry for making soft gelatin capsules [37]. The solutions of this gelatin can be used as binder in the process to form larger agglomerates containing active, diluents, glidants and binder [38]. Furthermore, it can be used as a carrier for potential use in retinal sheet encapsulation and transplantation [39]. Traditionally, gelatin has been used to clarify wine, beer and fruit juices. Gelatin of low bloom strength specifically 100-200 Bloom gelatin. Gelatin of this nature are introduced into the top of the tank and before filtration, it is allowed to settle [40].

CONCLUSION

Animals are reared and killed to provide nutrition from meat for humans, and few of what we consider 'meat' animals would be allowed to exist except as examples of species in zoos without this utilization. There is the demand by the economics of the world's meat industry that in order for the livestock industry stay economically competitive with vegetable protein sources, there is the need for animal by-products to be utilized. A valuable source of potential revenue is lost if animal by-products are not effectively utilized, and the increasing and added cost of disposal of these products is sustained by the industry. The cost of the live animal of-ten surpass the selling price of its carcass currently; therefore, to generate the profit for the meat slaughtering operation, the value of the by-products must pay the expense of slaughter.

The main aim of this study to process laying hen's feet as an untraditional source of collagen and further evaluate the gelatin functional properties. Mostly, chicken head are cut and discarded away in most of the regions in my country. Material fats and non-collagenous proteins are needed to be removed first. To produce gelatin from chicken feet, pre-treatment techniques are required for gelatin extraction conditions. Experiment was analyzed by ways of having better properties and prepared gelatin yields through factorial schemes based on their viscosity, ash content, dry matter content, gel strength, gel melting point etc

Method used for this experiment was by enzyme conditioning. Collagen produced after separation process was treated with enzymes under varying temperature conditions and time to further prepare gelatin in the process.

Results obtained from this results shows that low yield of gelatin was obtain (about 24%). Furthermore the gelatin bloom strength was also low (50-130 Bloom) with gel viscosity (2.0-4.0mPa.s). The WHC and FBC were recorded as 11.1 to 25.0g/1g and 11.0 to 15.20g/1g respectively. The foaming capacity and foaming stability was recorded as 1 to 10% and 0 to 2% respectively and finally the emulsification capacity and stability obtained as 40 to 47% and 90 to 100% respectively.

During the cause of the experiment, issues and challenges were encountered and hence some remedies or recommendations have been made. The COVID-19 had a major influence in my result because the collagen was stored for a very long time before extraction was performed. Furthermore, during the process of extraction, the heating plate got broken and hence a different heating plate was used. The heating plate had a different (faster) degree of heating

which was about three times faster than the earlier one hence caused some disparities in the final results. So base on these reasons, it is recommended to immediately work on the extraction process after the obtaining the collagen and not store it for too long. Also, it is better to work on one type or the same device to have some consistencies in the results.

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LIST OF ABBREVIATIONS

ES	Emulsification Stability
EC	Emulsification Capacity
FAO	Food Agriculture Organization
FBC	Fat Binding Capacity
FC	Foaming Capacity
FCC	Food Chemical Codex
FS	Foaming Stability
GMIA	Gelatin Manufacturers Institute of America
JECFA	Joint FAO/WHO Expert Committee on Food
PE	Polyethylene
WHC	Water Holding Capacity
WHO	World Health Organization

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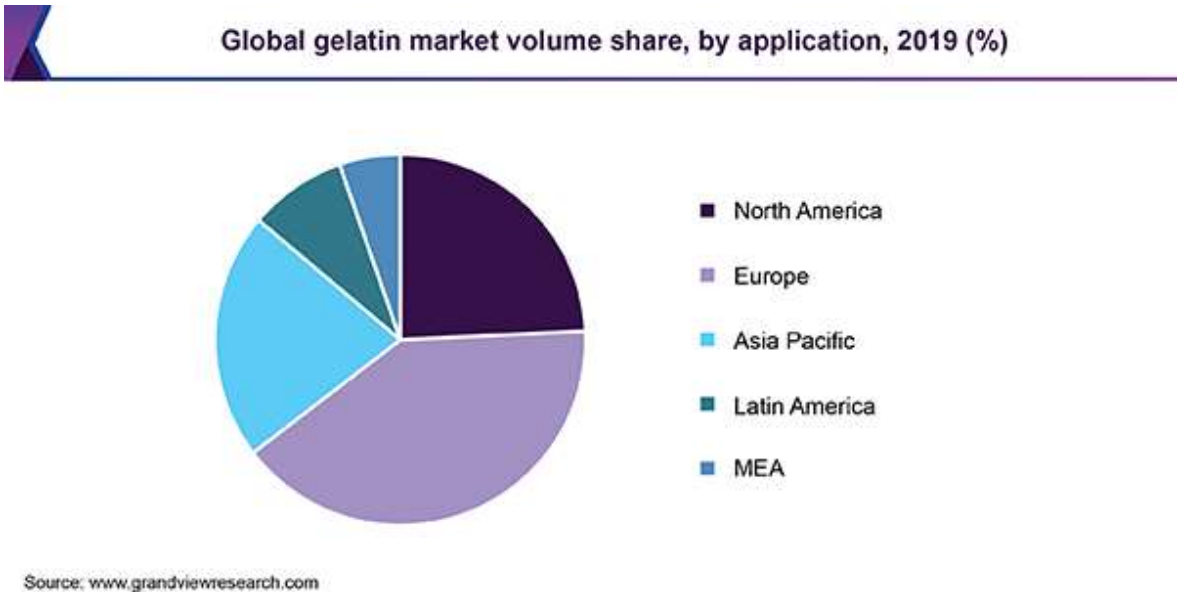
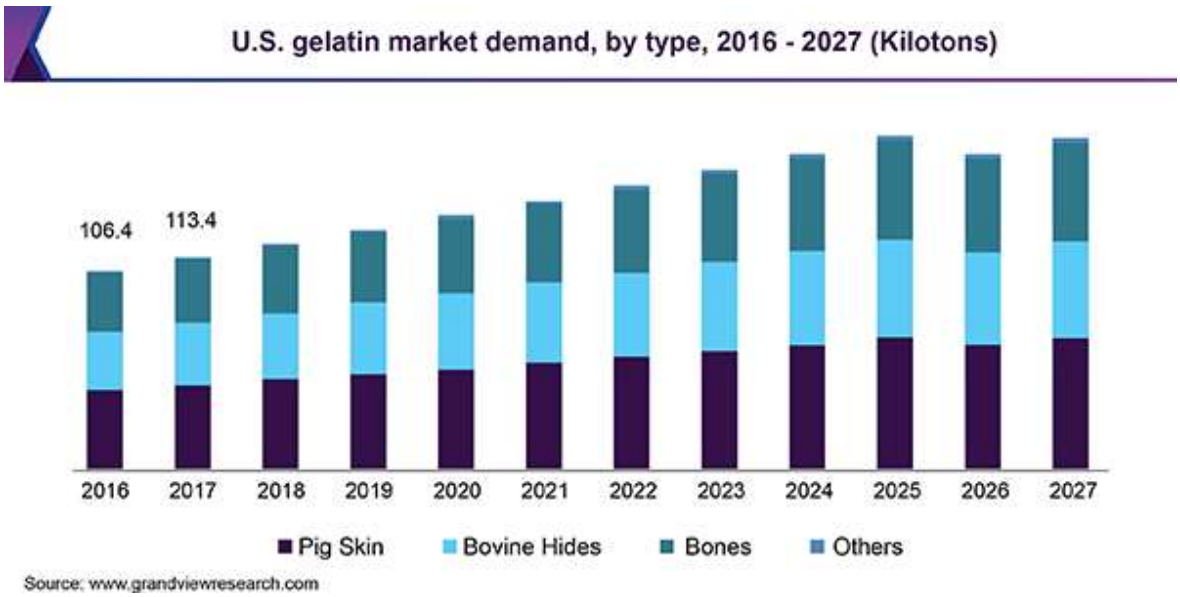
APPENDICES

Appendices 1: Global gelatin market share by application, 2019 (%)



Appendices 2: The demand of Gelatin in the U.S



Appendices 3: Market Analysis and Insights; Global Gelatin Market