Extraction of gelatines from untraditional sources of chicken/hen collagen

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R. Schrieber, H. Gareis: Gelatine Handbook: Theory and Industrial Pradice. Weinheim: **Wiley-VCH Verlag, 2007.**

H.W. Ockerman, C.L.**Hansen: Animal By-Product processing & Utilization. CRC Press: Boca Raton, 2000.**

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ABSTRAKT

Czech abstract

Cílem diplomové práce byla extrakce želatin ze slepičích běháků za použití enzymu; přečištěný kolagen byl připraven z rozemleté suroviny opracováním v několika krocích. Ke studiu vlivu vybraných 3 procesních faktorů byla použita faktorová schémata 2^3 s centrálním experimentem. Studovanými faktory byly: přídavek enzymu, doba enzymového opracování a doba extrakce želatiny. Data byla vyhodnocena statistickým softwarem Minitab 17; byly stanoveny optimální podmínky extrakce vysoce jakostní želatiny a želatiny s vysokým výtěžkem (vyjádřená stupněm konverse výchozí suroviny na želatinu): 0,8 % přídavek enzymu, 72 h doba enzymového opracování a 120 min doba extrakce želatiny; za těchto podmínek je při 28,01 % výtěžku připravena želatina s vysokou pevností želatinového gelu (413 g). Doba enzymového opracm ování má výrazný vliv na výtěžek želatiny; ani jeden ze sledovaných faktorů nemá statistickou významnost na pevnost želatinového gelu. Enzymové opracování suroviny má rovněž vliv na obsah popelovin v připravených želatinách; za optimálních podmínek extrakce je obsah popelovin v želatině nízký; připravené želatiny splňují přísné potravinářské standardy. Doba enzymového opracování má vliv rovněž na viskositu želatinových roztoků. U připravených želatin byla hodnocena rovněž čirost a pH; studované procesní parametry mají minimální vliv na tyto vlastnosti želatin.

Klíčová slova: želatina, pevnost gelu, slepičí běháky, extrakce, enzymové opracování

ABSTRACT

The aim of this master thesis is to extract gelatine from hen paws by using enzyme, pure collagen was made from raw material (hen paws which was cut into small pieces) by several steps pre-treatment. A three factors and two levels central composite rotatable design was used to optimise the process. Three factors were namely amount of enzyme treatment, time of enzyme treatment and main time extraction, their properties were evaluated by following to standard with some modifications. The data were analysed by using Minitab software to bdetermine the optimum conditions which has the highest yield of gelatine and gel strength, the plots responded that at conditions 0.8% amount of enzyme treatment, 72h time of enzyme treatment and 120 minutes of main time extraction we did obtain the highest yield of gelatine (28.01%), similarly, at the conditions 0.8% amount of enzyme treatment, 72h time of en-

zyme treatment and 30 minutes of main time extraction the gel strength of gelatine was maximum (413g). The enzyme treatment had an effect on the yield of gelatine and there is no factor impacting on gel strength. We also obtained at the same optimal yield of gelatine the ash was about 1.7% which was suitable for standard of content of the ash in gelatine (< 2%) according to Gelatine Manufacturers Institute of America, 2012 so an amount of enzyme treatment also effects on the presence of the ash in gelatine and the viscosity were influenced by time of enzyme treatment. The properties of gelatines such as clarify, dry matter, pH were also evaluated and after extraction its properties were not much different with commercial gelatine.

Keywords: : Gelatine, gel strength, hen paws, extraction, enzyme treatment

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

Zlín 14th May, 2019 ………………………..

NGO THI HONG LINH

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INTRODUCTION

Gelatine is a soluble protein compound obtained by partial hydrolysis of collagen which is the main fibrous protein components in cartilages bones and skins; in some study cases of scientist they found that age of the animal, the source, and type of collagen are main factors which influence the properties of the gelatines. Insoluble native collagen must be spent a pre-treated before converting it into a form suitable for extraction, it is done by heating in water at temperatures higher than 45ºC. A chemical pre-treatment will break non-covalent bonds in order to disorganize the protein structure and making collagen be swelled and soluble. The efficiency of extracting collagen into gelatine is affected by both of the pre-treatment and the temperature water extraction process, as a function of enzyme pre-treatment, pH, temperature, and extraction time. There are two types of gelatine which are obtained by the pre-treatment procedure and are known commercially as type-A gelatine (isoelectric point at pH from 8 to 9) by acid pre-treatment and type -B gelatine (isoelectric point at pH from 4 to 5) obtained by alkaline pre-treatment conditions. The application of Gelatine depends largely on its rheological properties. Apart from basic physicochemical properties, such as solubility, composition parameters, colour, transparency, odour and taste, the main attributes that best define the overall commercial quality of gelatine are gel strength and thermal stability (gelling and melting temperatures). The development of gelatine alternatives has gained importance in recent years as the demand for non-bovine and non-porcine gelatine has increased due to the bovine spongiform encephalopathy crisis and for religious and social reasons. Since then, there has been much concern about using gelatine derived from possibly infected animal parts. Pig skin gelatine is not acceptable for Judaism and Islam and beef gelatine is acceptable only if it has been prepared according to religious requirements. Therefore, the development of gelatine alternatives is highly desirable to food processors as the global market for food certified halal is growing rapidly [1]. The manner for converting chicken feet into hydrolysates and gelatine by proteases is absent from the literature, although offering numerous benefits (low temperature mild reaction conditions, and crucially neutral pH). Inside the laboratory of the University, researchers have previously studied the enzymatic treatment of some solid by-products from animal slaughter processes, poultry feathers notably skin, tendons and sheep wool. Of these, tendons have potential ability to convert into high-quality gelatine, while deeper hydrolysis yields both collagen and elastin hydrolysates. [2]. The classical food, cosmetic, photographic and pharmaceutical ap-

plication of gelatine is depended mainly on its viscoelastic properties and gel-forming. Recently, and especially in the food industry, an increasing number of new applications have been found for gelatine in products such as emulsifiers, foaming agents, colloid stabilizers, fining agents, biodegradable packaging materials and micro-encapsulating agents, in line with the growing trend to replace synthetic agents with more natural ones. Over the last decade, people knew how to extract gelatine from available sources and applied in daily meal or deserts. Recently, industry have been producing gelatine which has higher properties from waste or by-products to save the money, high efficiency and especially friendly environmentally, in many cases, they used sources from mammalian to produce gelatine such as big, cow, castle, but in some religious, they are banned to use all mammalian products so that is why some scientist found gelatine from poultry which is use to alter these things. Hen paw is a new and uncommon source nowadays and there are not much cases study about it, industry wasted by-products every day with a huge amount of hen paws which contains potential value nutrients that is the reason we chose this topic to study about extracting gelatine from hen paws and evaluate the optimum conditions of extraction even so its properties in industry.

I. THEORY

1 BY PRODUCTS FROM ANIMAL PROCESSING PLANTS

1.1 Slaughter houses

1.1.1 Pig slaughter

The applied slaughterhouse processes and techniques often depend on the size of the installations. In general, and in the larger installations, trucks deliver pigs to the warehouse of the slaughterhouse and kept for up to 2 hours, and according to Danish Meat Research Institute 1995 that excrements and urine are sent to anaerobic co-digestion (biogas & sludge production). However, in some cases pigs will be spent the night in the warehouse areas, as it is then possible to have an early processing start at the following day. Finnish Environment Institute 2002 said that from the warehouse area, the pigs then are moved to chambers where they are stunned with CO2. After that, they are hung in the above rail by two back legs then they are stuck in the throat. From there, employer will obtain 4kg of blood and used in other purposes. Subsequently, and after being removed from the rail, the pig hair is loosened by scalding in approx. 60 °C hot water or by steam After that, about one kilogram of hair per pig can be removed in a de-hairing machine that applies rotating rubbers and pressurized water jets. After being hung by two their back legs on the moving rail, using the burning process to remove the residual hair and bacteria The burning process makes it more soft and changes the skin. the skin is scraped to obtain a light uniform colour. The process will go to the cleaner stage of the slaughterhouse, the pigs will be eviscerated, and the stomach and bowel set are taken out and being cleaned in the another place. At here, then the by-products such as bone, heart, liver and lungs) are taken out and further processed in the "pluck area" of the slaughterhouse. The pig then weighs around 85 kg. by- products (heart, liver, kidneys and washed intestines) are commonly sold for people or market where it will be used to produce to foods or use it to animal feed or composted for other agricultural purposes. Trimmings, lungs, and washed stomachs and intestines are used for human consumption or also use for biogas & sludge production. Then meat is split into two and chilled from approx. 35 to 7 °C. The subsequent stage will produce trimmings and bones, which are sent to produce animal feed. Around 14–16 kg/pig transfer to animal feed or pet food, while ca. 11 kg/pig goes to rendering. The mass that goes to biogas & sludge production is approx. 8–10 kg/pig, and includes stomach and intestine contents and wastewater treatment residues [3] [4].

1.1.2 Cattle slaughter

The cattle are delivered to the slaughterhouse by trucks and stay in the storage area, then they are driven to the stunning area one at a time to fix their body and heads. After stunning, they are hung by the hind legs on an overhead rail, and then they are cut on the throat leading bleeding can occur. For one we can collect approx. 10– 22 kg blood /head and then it is used for fur animal feed and human consumption. Afterwards, hides are washed and salted before being sent to tanneries and further processing into leather goods. in the next processing, the head, forelegs, tail, udder or testicles are removed. In the clean part of the slaughterhouse, the chest is cut open and removing some unwanted part such stomach, udder, lungs and used it for pet food for animal feed production beside that heart, liver and kidneys are used for human consumption. In that fact that cattle slaughter house contains huge of harmful effects on human environment and life, removed stomach content (approx. 60–80 kg/head) is sent to composting. If slaughtered cattle is older than 6 months than approx. 40–50 kg/head is considered specified risk material and is incinerated, so there are some solutions to reduce by product from slaughter house, co-incinerated or used for fuel is one of the best way such as meat-bone meal is incinerated and fat is used as fuel according to Finnish Environment Institute 2002. In the end, the cow carcass weight is approx. 250– 260 kg/head, and the calf carcass weight approximately half of that [3] [4].

1.1.3 Sheep slaughter

Sheep and lambs are delivered to slaughterhouses by trucks and led to the storage area. Hereafter they are killed by electricity. After that they are hung and cut out the head to lead bleeding can occur. According to Danish Meat Research Institute 1995 Approximately 1–2 kg of blood/head are achieved, which corresponds to 60% of the total blood mass per animal. Afterwards their hides are removed and using for leather goods, stomach, intestines are transfer for animal feed, and plucks are mainly for human consumption, all of these by products are removed. In the end, the approx. 21 kg/head slaughtered weight is showered with water to remove blood and fat residues and sent for cooling. Later they can be sold as an entire carcass, divided into half's or smaller parts [3].

1.1.4 Poultry slaughter

Chickens are transported to slaughterhouses in storage area and are then manually hung on the conveyer by their feet. Then they are moved to an area where they are electrically

stunned, and after that the neck will be cut out of the body according to Danish Meat Research Institute 1995. In Finland, CO2-stunning is also applied – these chickens are hung after stunning. Amount of blood collecting is 40 g/chicken and used in animal feed according to Finnish Environment Institute 2002. Then they are burned in order to remove unwanted hair by dipping 2–3 minutes in approx. 50–52 °C hot water (if later sold as fresh) or for ca. 1 minute in approx. 60 °C hot water (if later sold as frozen). De-feathering, or plucking is the step to remove hair approx. 180 g/chicken of feathers. These are further processed, e.g. into animal feed. The next step, heads are cut out of the body around 80 g/chicken and the feet around 120 g/chicken. The feet can continue to produce some animal feed or in some Asian countries they use it to make a special food because it contains nutrients and further processing such as making gelatine, collagen or hydrolyse. The rest of the chicken is transfer to another part, now the legs will be held on, where the bowel is pulled out and gizzard, removing lever and heart with the purpose of being used for human consumption. Then the trachea and esophagus are removed and the chicken is showered by water, followed by an inside-out water flushing of the chicken. The chicken, or more precisely broiler is then weighted approx. 1.4 kg and is preserved on below 4 °C. Further processing produces trimmings and bones of approx. 150–160 g/broiler. These by- products are used for making animal product or composite as well according to Danish Meat Research Institute 1995 and Finnish Environment Institute 2002 [3] [4].

1.2 By products from animal slaughter houses

Irshad (2015) points out that slaughtering and processing of meat animal, only one third is meat while the rest comprise of by-products and waste, which need to be adequately processed and utilized. Traditional markets for edible meat by-products have gradually been disappearing because of concerns about health and economic returns. In response to these problems, meat processors have found out the way to use that by-product which directly effect on the economy and environmental pollution of the country [5].

Using by product depends on the traditions, culture and religion of the countries which are often important when apply by- product into the food. In some countries using by-product is also important because many countries restrict the use of meat by-products for reasons of food safety and quality. By-products such as blood, liver, brains, lung, spleen, kidney and tripe has good nutritive value. By-product even use in medicinal and pharmaceutical which are also highlighted in this review. Waste products is one of the issue that need to solve from

the poultry processing and egg production, so waste management is ideal and efficiently approach of industries to make waste and by product become more and more valuable. Beside that treated fish waste has become popular in many applications among with which the most important are animal feed, dietetic products (chitosan), biodiesel/biogas, and cosmetics (collagen) and natural pigments (after extraction).

According to Irshad (2015), in general, the total by-products range from 10% to 30% of the live weight for beef, pork and lamb and from 5% to 6% of the live weight of chickens. The yield of edible by-products including blood and organs in cattle averages 12%, in sheep 14%, and, if pork rinds are also included, 14% for hogs. The consumption of offal is also influenced by disease outbreaks and government policies e.g. brain and spinal cord were out of the list for consumption since the outbreak of BSE (Mad cow disease). On the basis of live weight of an animal the by-products account for almost 60% and out of this 40% are edible and 20% are inedible [5].

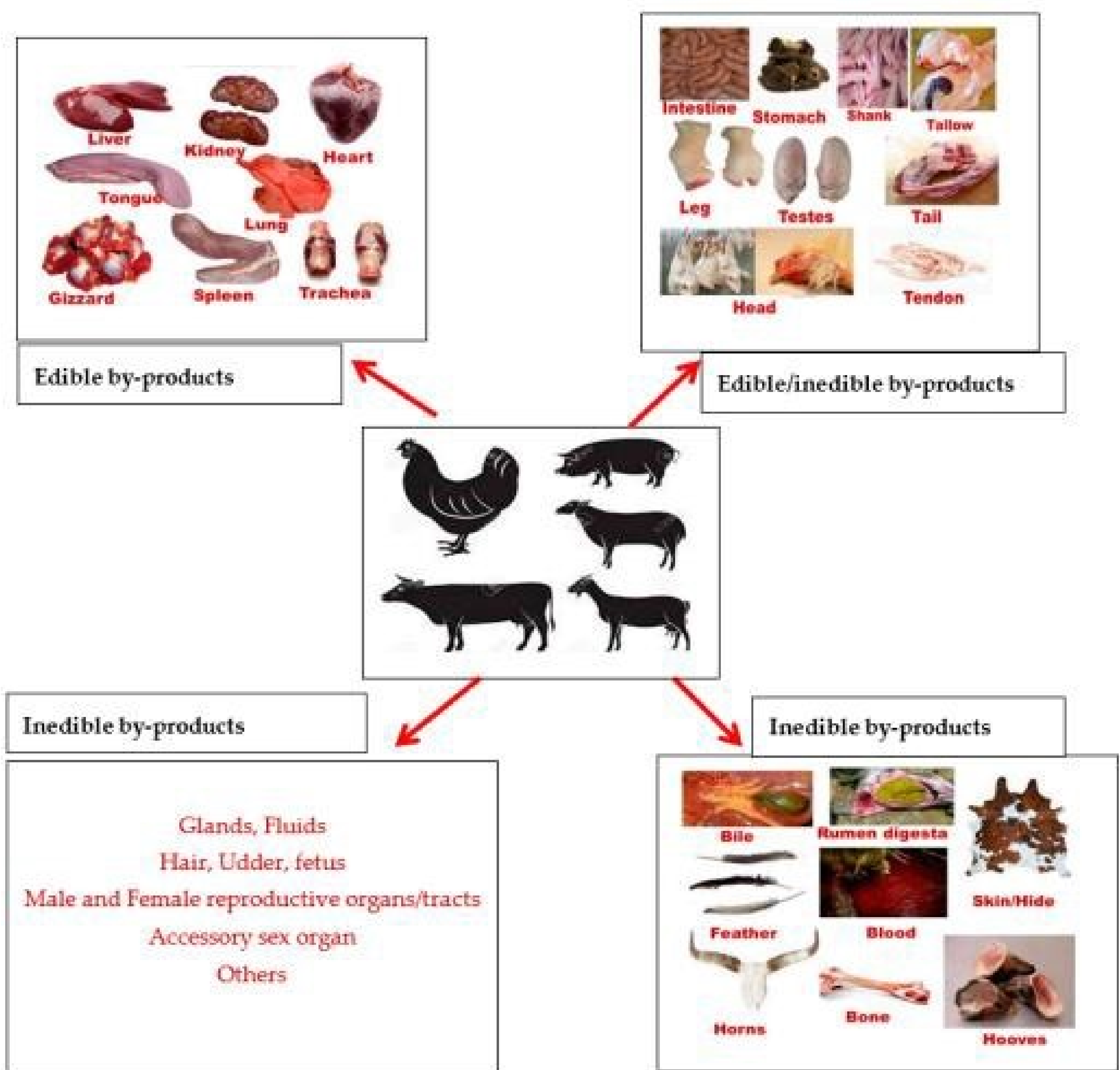


Figure 1: Edible and inedible by-products of animal [6]

1.2.1 Kinds of by-products and the amount of by-products from animal slaughter houses.

There are many slaughter by-products from the animal industries which are used in some applications. These include:

- Blood meal- Whole blood meal is produced by spray-drying at low temperatures
- Feather meal- Feather meal is produced by hydrolysing clean, non-decomposed feathers from slaughtered poultry. Hydrolysis is done with steam and pressure, which breaks the keratinous bond and increases the digestibility of the protein in the feathers.
- Meat meal- Meat meal is produced by cooking by-products from the animal slaughter industry and includes the edible parts such as organs and certain condemned carcasses.
- Meat and bone meal- When bones are added to meat meal it becomes meat and bone meal.
- Poultry by-product- Similar to meat meal, but derived specifically from the poultry industry

Table 1: By products from poultry slaughter houses [7]

Type of by-product	% of live weight	Uses
Poultry litter and manure	–	Recycled feed, surface dressing of agricultural land
	Hatchery by-products	
Egg shells, infertile eggs, unhatched 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 dressing plant		
Feathers	7–8	Bedding material, decorative purpose, sporting equipment, manure or fertilizers, 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 chitinolytic enzyme.
Feet	3.5–4.0	Soup, technical fat/poultry grease
Intestines and glands	8.5–9.0	Meat meal, poultry grease and active principles (hormones and enzymes)

The literature indicates that by-products (including organs, fat or lard, skin, feet, abdominal and intestinal contents, bone and blood) of cattle, pigs and lambs represents 66.0, 52.0 and 68.0% of the live weight respectively.

The yield of animal by-products ranges between 50-60% of the live weight. According to various statistics 107 million livestock and more than 650 million poultry birds were slaughtered annually in India leading to production of 6.3 million tonnes meat. It leaves huge loads of by- products [8].

According to EMEAT, poultry production volume in Russia amounted to 1,192.100 tons in slaughter weight with 1,622.0 tons in live weight for the period from January to March 2018, which is 4.0 percent (by 46.4 thousand tons in slaughter weight) more than the same period 2017.In Viet Nam: From Poultry farming: It is estimated that by June 2018, total poultry production in the whole country will increase 5.2% over the same period of 2017; Poultry meat production reached 608.4 thousand tons, up 6.1%, poultry egg production reached 6.27 billion fruit increased 11.3% over the same period in 2017 [9].

According to the Food and Agriculture Organization 2012, global poultry meat production and consumption are growing steadily with a projected annual growth of 3.6%. In 2010, around 78 million tone of poultry meat were produced worldwide. It is estimated that around 22 to 30% of the global poultry production is considered to be by-products such as head, feet, viscera, blood, and feathers. In developed countries, animal by-products are rendered into animal feed or composted for other agricultural purposes according to Bolan et al., in 2010, whereas in many developing countries, burial in landfills is a common disposal method to manage waste products and The disposal of poultry by-products in landfills is not only costly but also risky because of human and environmental contamination [10].

In a recent survey conducted among 1030 United Kingdom public members, 43% of the respondents indicated that they eat chicken livers. The study also revealed that chicken liver dishes are eaten by people of ages ranging from 18 years and above. Brazil is the third largest producer of chicken meat in the world with 13.058 million tons in 2011, behind only China and United States, generating with this a great amount of by-products, including chicken feet which in turn are collagen-rich and can be used to extract gelatine [10].

1.2.2 Economic of by-products from animal slaughter houses

Because of their unusual physical and chemical characteristics so more than half the animal by-products are not using for normal consumption. As a result, the cost of disposing of these products is increasing while a valuable source of potential revenue is lost. The United States Dept. of Agriculture Economic Research Service has found that 11.4% of the gross income from beef is from the by-products. The figure for pork is 7.5%. In addition to economic is decreased, unused meat products lead to the serious environmental pollution. However in recent year improved utilization of meat by-products can give a good profit to meat processors [7].

This contribution would have been much greater if the animal by-products have been also utilized efficiently. Utilization of by-products has directly effect on the economy and environmental pollution of the country. Non-utilization or waste of by-products not only lead to loss of potential revenues but also lead to the added and increasing cost of disposal of these products. Non-utilization of animal by-products has huge impact on the environment and human health. But besides pollution and hazard aspects, in many cases meat, poultry and fish processing wastes have a potential if they are recycled from raw materials or conversion into useful products of higher value.

The intensive and large scale production of food animals and animal products has generated an enormous disposal problem for the animal industry. These wastes, including animal excreta, mortalities, hair, feathers and processing wastes are convertible to useful resources. An efficient thermophilic anaerobic digester system that converts animal manure to methane for an energy source. Properties of a feather degrading bacterium, Bacillus licheniformis, which can ferment and convert feathers to feather lysate, a digestible protein source for feed use. An enzyme, keratinase, secreted by this bacterium, was purified and characterized. The keratinase is a potent proteinase that hydrolyses collagen, elastin and feather keratin [7].

2 POSSIBILITIES OF PROCESSING ANIMALBY-PRODUCTS

2.1 Possibilities of by products in utilization

2.1.1 Asian countries

Viet Nam is one of the world's largest organ transplants market, so Viet Nam has imported products from Europe and China massively. Imported organs are cheaper approximately 2-3 times than Vietnamese ones. OEC statistics show that the United States, Australia and Germany are the major Western nations in the export of animal organs, Asian is potential market of organ which are exported from those countries. Vietnam is the 4th largest importer of organ, with 4.9% of the world's total, nearly double that of South Korea. Led by China, it accounted for 29% - nearly one third of global organ imports [11].

China: Chicken feet and head are used in several regional Chinese as specific cuisines, they can be cooked as a beer snack, soup cold, dish or main dish, Like Vietnamese, In Guangdong and Hong Kong, they are typically deep fried and steamed first to make them swell before being stewed and simmered in a sauce flavour with some ingredient such as bean paste, black fermented beans, and sugar; or in abalone sauce. Today, packaged chicken feet are also sold in most supermarkets, grocery stores in China as a snack, often seasoned with rice vinegar and chili. Another popular cuisine, which is marinated in a sauce of rice vinegar, rice wine flavoured with sugar, salt, and minced ginger for an extended period of time and served as a cold dish. In southern China, soup is made by combination of chicken feet and raw peanuts. The huge demand in China raises the price of chicken feet, which are often used as fodder in other countries. As of June 2011, the price of 1 kg of raw chicken feet costs around 12 to 16 yuan (1.6 to 2.1 euro) in China it is even greater than compared to 1.6 euro for 1 kg of frozen chicken breast. In 2000, Hong Kong is strong by shipping chicken feet from over 30 countries, traded a total of 420,000 tons of chicken feet and get back around US$230 million. Two years after joining the WTO in 2001, China approved the direct import of American chicken feet, and since then China has been the major destination of chicken feet from around the world. Beside benefits from chicken feet, they also get high income from duck feet. Duck feet with mustard, which is often served with vinegar, fresh green pepper and crushed garlic, is a popular salad appetizer [12].

In Indonesian cuisine, chicken feet are known as *ceker*, and it is a common popular dish in Indonesia, especially in Java. In Indonesia usually use it in spicy traditional soup

called *soto*, *Soto ceker* is chicken foot which is in rather clear yellowish spicy *soto* broth soup, which uses spices including some ingredient such as ground shallot, garlic, galangal, ginger, candlenut, bruised lemongrass, *daun salam* which is Indonesian bay leaf and turmeric that add the yellowish color, served with cabbage, celery, rice noodles, and garnished to taste with *sambal*, lime and soy. *Soto ceker* is one of the popular street food in Jakarta, Bali, Surabaya, Bandung, and almost cities in Indonesia. A popular *soto ceker* stall such as Soto Ceker Pak Ali in Gandaria street, South Jakarta, eating and cooking up to 40 kilograms of chicken feet every day, and can get 5 million (US$360) in sales each day and Another specific dish is preparation of chicken feet as simple soup called *sop ceker*, which mainly contains chicken broth, shallot, chicken feet, vegetables especially potato and carrot garlic and black pepper. Chicken feet can also deep fried, which has been separated from its bones, might be served as a crispy snack treat as chicken feet crackers) [12].

Chicken feet are also known as *ceker* in Malaysia and are traditionally popular mostly among Malays of Javanese, Chinese and Siamese descent. Many traditional Malay restaurants in the state of Johor offer chicken feet that are cooked together with Malay-style curry and eaten with roti canai. In the state of Selangor, chicken feet are either boiled in soup until the bones are soft with vegetables and spices or deep fried in palm oil. Chicken feet are also eaten by Malaysian Chinese in traditional Chinese cooking style [12].

2.1.2 Eastern Europe

In Russia, Ukraine, Romania and Moldo, vachicken feet are cleaned, seasoned, and boiled, often with vegetables, and then cooled, to make an aspic called "kholodets" in Russian and Ukrainian, and piftie in Romanian. The legs are not always eaten, they cook chicken including feet to make food more healthy as it contain a high amount of gelatine [12].

2.1.3 America

In Jamaican cuisine, chicken feet are mainly used to make chicken foot soup. The soup contains yams, potatoes, green/yellow banana, dumplings and special spices in addition to the chicken feet, and is slow cooked for a minimum of two hours. Chicken feet are also curried or stewed and served as a main part of a meal [10].

Chicken feet are a popular ingredient across Mexico, particularly in stews and soups. They are often steamed to become part of a main dish with rice, vegetables and most likely another part of the chicken, such as the breast or thighs. The feet can be seasoned with mole sauce.

On occasion, they are breaded and fried. Many people will also take the chicken feet in hand as a snack and chew the soft outer skin. The inner bone structure is left uneaten [10].

2.1.4 South Africa

In South Africa, chicken feet are mainly eaten in townships in all nine provinces, where they are known as "walkie talkies" (together with the head, intestine, hearts and giblets), "runaways" and "chicken dust" .The feet are submerged in hot water, so the outer layer of the skin can be removed by peeling it off, and then covered in seasonings and grilled. The name "chicken dust" derives from the dust chickens create when scratching the ground with their feet. They are often eaten as snack [10].

2.2 Extraction hydrolysate, collagen and gelatines from variety sources

In several recently years, there have been many studies about by-products from poultry slaughter, by-products were not wasted and throw in the streams or landfills anymore, They use it to produce some foods for eating such as fried chicken feet, heads, livers chicken, or extract collagen, gelatines, or hydrolysates from skin, feet, head, organs...,and applying in many fields such as food additive industry, pharmacy, photography, other technical .., besides that waste from factories are increasing day to day, pollution is one of the issue that need to be solved. So, waste products from the poultry processing and egg production industries must be efficiently dealt with as the growth of these industries depends largely on waste management. Animal and poultry waste management centre, at North Carolina State University, North Carolina, USA is engaged in conversion of wastes to valuable products and the work being supported by various organization, agencies, companies etc [13]. Studies of extraction by-products to make gelatine, collagen … is not only the best way to decrease the harmful effects of by-products but also convert waste into valuable product which has important roles either in science or industries.

2.2.1. Extraction collagen and gelatine from poultry

To achieve gelatine from poultry, there are many researches of scientists about by products of poultry such as head, feet, paws, skin, tendons… have been studied. In some study cases they found that before extraction of by product into gelatine or collagen, it should be pre-treatment to increase the efficient of extracted gelatine or collagen, two popular methods that usually use are alkaline treatment and acid treatment. After extraction of gelatine, collagen from poultry by products, yield extraction, physical properties, gel strength, amino

acids composition… are evaluated and make a conclusion that which technological condition is better for objection which they want and also found out which material is the best for extraction and an alternative to mammalian gelatine.

According to Hashim (2014): In this study they used using papain and pepsin enzymes in acetic acid solution at 4°C for 24h with a yield of 18.16% and 22.94% by dry weight, respectively to isolate Collagen from chicken feet. Collagen is a one of main protein present in animal connective tissues, it occupies 30% of total animal protein. Collagen is a polymer which is built by the composition of the repetitious aggregation of tropo-collagen monomers. Tropo-collagen is a transitional molecular species introduced by procollagen. The chicken feet collagen contains the amino acids glycine, glutamic acid, proline and hydroxyproline. This study using two enzymes which are papain and pepsin to isolate collagen of chicken feet. Electrophoresis pattern demonstrated two distinct αchains (α1 and α2) and β chain, indicating that type I collagen is a major component of chicken feet collagen. The thermal stability of collagen isolated by papain and pepsin revealed stable denaturation temperatures of 48.40 and 53.35°C, respectively. The FTIR spectra of both collagens were similar with amide regions in A, B, I, II and III. The study demonstrated that chicken feet collagen using papain isolation method is possible as commercial alternative ingredient [14].

There is also another study of Liu (2001) indicates that Optimum Condition of Extracting Collagen from Chicken Feet and its characteristics by evaluating alternative treatments for the best extraction condition for collagen from chicken feet. Various properties such as chemical composition, amino acid, pH, swelling percentage, yield and pure collagen, collagen loss, colour and electrophoresis of collagen from chicken feet treated by 5% acids (acetic acid, citric acid. hydrochloric acid and lactic acid) and soaking times (12, 24, 36 and 48 h) were evaluated. The crude protein, fat, ash and moisture contents of chicken feet was 17.42, 12.04, 5.98 and 62.05%, respectively. Amino acid composition of collagen from chicken feet indicated that the protein of collagen was markedly hydrolysed by the hydrochloric acid treatment. The result of electrophoresis also supported this phenomenon. Both the swelling percentage of lactic acid and citric acid treatments were significantly higher than that of acetic acid and HCl treatment. The pH of the acid treatments ranged from 2.43-3.62. According to the result of yield, pure collagen and loss of collagen, the best condition of extracting collagen from chicken feet was soaked in 5% lactic acid for 36 h. However, a brighter yellow colour of collagen from all treatments was observed with a longer soaking time [15]. Regarding to extraction of collagen by acid pre-treatment, according to Poliana

(2013), the acetic acid is the most popular solvent used in collagen preparation because one of the most important role of acetic acid in extraction is the high extraction ability and the solubility of collagen in this acid, study used 4% acid acetic for 16h the result found that the yield of collagen was extracted from chicken feet around 6-9% while according to Liu with using the same acid with 5% at 12h, 24h, 36h, 48h had an yield of extracted collagen are 30.69 %, 30.86%, 29.95% and 31.23% respectively [15], so it means with using of different concentration acid and different levels of time will reveal different result and at this case the higher concentration acid and higher temperature will get the higher yield of collagen [16].

Table 2: Yield of collagen extracted of chicken feet using acid acetic at the different conditions of pre-treatment of extraction

Concentration of acid acetic	Time for pre-treatment	Yield of collagen(%)
4%	16h	6-9
5%	12h	30.69
	24h	30.86
	36h	29.95
	48h	31.23

And comparison with study of Hashim [14] we can see that in this study they used using papain and pepsin enzymes in acetic acid solution at 4°C for 24h with a yield of 18.16% and 22.94% by dry weight while study used 4% acid acetic for 16h the result found that the yield of collagen was extracted from chicken feet around 6-9% Poliana [16].

Table 3: Yield of collagen extracted of chicken feet using acid acetic 4% at the different conditions of pre-treatment of extraction:

Pre-treatment time	Added Enzymes	Yield of collagen extraction(%)
16h	-	6-9
24h	Papain	18.16
	Pepsin	22.94

Norizah (2012) found that chicken skin gelatine can use as an alternative to mammalian gelatine therefore the comparison of extraction and physical-chemical properties of chicken skin gelatine and bovine gelatine. The result points out that extracted chicken skin

gelatine 6.67% (w/v) had a higher bloom value (355 ± 1.48 g) compare to bovine gelatine (259 ± 0.71 g) it seems to be that much better to use chicken skin gelatine. The dynamic viscoelastic profile which are viscous and elastic modulus values of chicken gelatine are greater than bovine gelatine for a range of concentrations and frequencies. They used differential scanning calorimetry (DSC) to get the thermal properties of gelatine and studies showed that the melting temperature of 6.67%, chicken skin gelatine was significantly higher ($p < 0.05$) than the bovine gelatine, though that result bovine gelatine had lower stability compared to chicken skin gelatine. Results obtained in this study showed that Gly (33.70%), Pro (13.42%), H.Pro (12.13%) and Ala (10.08%) were the most dominant amino acids in chicken skin gelatine which contributed to the higher stability and gel strength. Raman spectra of chicken skin was similar to bovine gelatine and displayed typical protein spectra. Chicken gelatine showed strong hydrogen bonding compared to bovine gelatine as the tyrosine doublet ratio (I855/I830) of chicken gelatine was significantly lower than that of bovine gelatine. Significantly, the alpha helix and b-sheet type structures were higher for chicken skin gelatine compared with bovine gelatine [17]. Relating to skin of chicken Poliana [16] did study further about skin and tendons of chicken feet and also points out that Chicken gelatine from skin waste by-product can provide an alternative source of gelatine as it shows similar chemical composition to bovine gelatine and better physicochemical properties compared with reported fish gelatines, they said that Physical and chemical properties of gelatine gels extracted from skins and tendons of chicken feet were characterized as an alternative to mammalian gelatine commonly used commercially. After extraction, the preparation of gels for analysis was made by dissolving the dry gelatine with two concentrations: 3.33 and 6.67% (w/w) in distilled water. The determination of gel strength and texture profile analysis was performed using texture analyser. Gelatine showed high protein content and the value observed for gel strength was higher for the gelatine with concentration of 6.67% (294.78 g), classified as high bloom, while for the gelatine with concentration of 3.33% (96.47 g) was lower, low bloom. The hardness and chewiness increased significantly with increasing concentrations of gelatine and the gelatine with concentration (6.67%) behaved as a solid and it can be used in the preparation of gelatine gums, marshmallows, gelatine dessert among others that requiring high bloom. Norizah used the method of pre-treatment by using alkaline soaking, to extract gelatine, 14 g defatted dried chicken skin was mixed with 200 ml sodium hydroxide (0.15% w/v) [17]. The mixture was shaken well and slowly stirred at room temperature (22ºC) for 40 min before centrifuging at 3500 g for 10 min. This step was repeated

three times. The alkaline solution was changed every 40 min to remove non-collagenous proteins and pigments. The final extraction was carried out in distilled water at a controlled temperature (45ºC) overnight without stirring. About Poliana did use different pre-treatment, they used acid pre-treatment with 4.0% acetic acid for 16 h for soaking of 200 g chicken feet were cut into small pieces [16]. After two results of two studies indicate that the protein obtained from skin and tendons of chicken feet and from skin chicken are 84.96% and 80.78% respectively, but the strength of gelatine at 6.67% is 294.78 g and 355g so using of method alkaline soaked pre-treatment looks better than using of acid pre-treatment.

Table 4: Comparison between to pre-treatment method of extraction gelatine from skin chicken

Methods	Protein (%)	Strength gel at 6.67%	Reference
Acid pre-treatment (4% acetic)	84.96	294,78	Poliana fernandes almeida,2013
Alkaline pre-treatment (NaOH 0,15%)	80.78	355	Norizah Mhd Sarbon,2012

L.Du (2013) also found that the different gelatines yield between to different temperature if extraction, using of chicken and turkey heads in a series of batch extractions at 2 different temperatures (50 and 60°C) to extract gelatine, and their composition and functional properties were evaluated. Gelatine yield from chicken was 52.29 and turkey heads was 62.76%, after the result showed that the gel strength of chicken gelatines was lower than turkey gelatines varied from 332.7 to 368.4 g, which was significantly ($P < 0.05$). Both turkey and chicken head gelatines had high solubility at acidic and alkaline pH values. However, turkey head gelatines had the better emulsifying and foaming properties than chicken gelatines. This study used the pre-treatment is treated with 0.1 M NaOH at a ratio of 1:10 (wt/vol) for 6 h at 4°C and the alkaline solution was changed every 2 h but Gelatine from the pre-treated poultry heads was extracted in 2 stages at 2 different temperatures. [18]

According to Huda (2013), Study indicated that duck feet collagen was extracted using 5% lactic acid soaked for 24 hours at 4-7°C and examined for their physicochemical properties Chemical composition of duck feet collagen such as moisture, protein, fat and ash content was 5.85, 29.11, 35.43 and 28.60%, respectively. 17 amino acids were detected in duck feet collagen and included 20.46% glysine, 7.73% hydroxyproline and 10.24% proline. The yield

of collagen obtained from this treatment was 28.37%. The collagen extracted was light in colour with a pH 2.67 (soaking period) and the swelling percentage was 240.50%. Duck feet collagen (DC) possessed similar bands with commercial Fish Collagen (FC) and commercial Cow Collagen (CC) for the FTIR comparison to lactic acid 5% pre-treatment of extraction of gelatine from chicken feet. According to the result of Liu, at condition of extracting collagen from chicken feet was soaked in 5% lactic acid for 24 h we can see that although the yield of collagen and swelling percentage of chicken feet are higher than duck feet and pH of duct feet is slightly higher than chicken feet but there is not different between collagen properties of chicken and duck feet which were extraction at the same soaked conditions. [19]

Table 5: Comparison between two different resource collagen extraction at the same soaked conditions (5% lactic acid for 24h)

Collagen resource	Yield of colla-gen(%)	pH	swelling percent age (%)
Chicken feet	30.74	2.54	245,85
Duck feet	28.37	2.67	240.50

2.2.2. Extraction gelatine from fish skin

Fish gelatine can be obtained from the skin and bones of fish. In the fact that amount of waste from fish around 75% of the total catch weight. Among them about 30% of such waste consists of skin and bones which contains high collagen content so in industry they use it to produce fish gelatine. Extraction of gelatines from fish skins may provide an alternative source that is acceptable for kosher (Jewish) and halal (Muslim) products and serve as an alternative for markets concerned about bovine spongiform encephalopathy. The yield and quality of gelatine are influenced not only by the species or tissue from which it is extracted but also by the extraction process, which may depend on pH, temperature, and time during both pre-treatment and extraction'. Extraction of gelatine from fish is use both of type A and type B which are acid treatment and alkyline treatment, he also uses hydrotic enzymes to produce of gelatine from fish skin [20]. Fish gelatine extraction from wastes of fish Herring species (Tenualosa ilisha) was carried out by a series of pre-treatment with 0.2 M Ca(OH)2 followed by 0.1 M citric acid and final water extraction at 50 C for 3 h. The resulting fish gelatine preparation was evaluated for its dynamic viscoelastic properties, gelling

and melting temperatures and gel strength. The gelling and melting temperatures of gelatine samples (at 6.67%, w/v) were obtained from differential scanning calorimetry and rheological studies. The melting temperature of extracted fish gelatine (EFG) obtained ranged from 16.2 to 16.7 C compared to that of commercial fish gelatine gel (CFG), from 23.7 to 25.6 C and halal bovine gelatine (HBG), from 26.5 to 28.7 C. On the other hand, gelling temperatures of EFG, CFG and HBG ranged from 5.1 to 5.2 C, 11.9 to 17.46 C, and 12.6 to 19.33 C, respectively. EFG gave gels with a considerably lower G0 values than CFG and HBG. The bloom strength of EFG gels at 6.67% (w/v) was 69.03 g which was much lower than HBG (336.2 g) and CFG (435.9 g). Enzyme transglutaminase was added in the amounts of 0.5, 1.0, 3.0 and 5.0 mg/g gelatine to modify the gel properties of the extracted fish gelatine. The modified EFG gels obtained had higher gel strengths of 101.1 g and 90.56 g with added transglutaminase of 1.0 and 3.0 mg/g, respectively. However with addition of 5.0 mg/g enzyme the gel strength increased only up to 75.06 g. SDS-PAGE of extracted gelatine gel showed protein band intensities for a1-chains and 53 kDa but in gels added with higher concentration of transglutaminase, these protein band intensities seemed to disappear. Thermal properties of EFG from fish wastes, commercial fish gelatine gels and commercial halal bovine gelatine gels were investigated in this study. The effects of transglutaminase on EFG were evaluated in terms of melting, gelling temperature, gel strength and pH. The enzyme has significant effect on gel strength when used in appropriate amount. It caused an increase in G0 and G00 values compared to untreated gels. The results indicated that fish gelatine gel from Tenualosa ilisha fish waste had low melting and gelling temperatures and gel strength even when transglutaminase was added compared to both commercial fish and commercial halal bovine gelatine gels. However, the untreated EFG gels as well as modified gels with transglutaminase have potential uses in food applications such as edible films and gelling agents. Furthermore, it would be interesting to extend the current understanding achieved for EFG, CFG and HBG to a high solid regime, where molecular dynamics of the interaction between porcine gelatine and sugar co-solute have been discussed on the basis of the theory of cooperativity [20] [22].

2.2.3. Extraction gelatine from insects

Insect gelatine may provide an alternative source that is acceptable for Muslims products, in Sudan many edible insects consumed and desert locust considered the most famous one in many parts of the country beside sorghum and melon bugs. Aspongopus viduatus (melon bug) and Agonoscelis pubescens (sorghum bug), commonly known in Sudan as Um-

bugga and Dura andat, respectively. In some areas of Sudan the collected bugs were extracted and the obtained oil was used for cooking and some medicinal uses. The reported that the crude oil and the phenolic compounds-free oil of melon bug showed high antibacterial activities against some test species. The two bugs' protein contained 16 known amino acids, including all of the essential amino acids [21].

2.2.4. Extraction gelatine from mammalian

There is also raw material from Mammalian gelatine which is derived from collagen which is the principal constituent of connective tissues and bones of vertebrate animals. In the Study of two different mammalian gelatines, i.e. from bovine (type B) and porcine (type A) sources points out that both sources contained components of different molecular weights with wide distribution ranging from 10 to 400. The study also indicates that strong correlation between gel strength and average molecular weight of gelatine, with high isoelectric and melting points. Mammalian gelatines of porcine and bovine, being the most popular and widely used are subject regarding to concerns of consumers due to socio-cultural and health. The gelatine which derived from mammalian can be prepared in both ways of the acid process (type A gelatine), or by the alkaline process (type B gelatine) [20].

3 GELATINES AND THEIR APPLICATIONS

3.1 Gelatine

Gelatine is a unique protein due to both its ability to form thermo-reversible gel with a melting temperature close to body temperature and its solubility in water. Gelatine is produced by partial hydrolysis of collagen. Despite gelatine's unique properties, a wide range of potential functionality is due to its specific structure consisting of twenty amino acids [22]. Gelatine is a water soluble proteinaceous substance prepared by processes, gelatine involve the destruction of the tertiary, secondary and to some extent the primary structure of native collagens. It is a high molecular weight polypeptide and an important hydrocolloid, which has proved popular with the general public and finds use in a wide range of food products largely because of its gelling and thickening properties. Gelatines is different with other hydrocolloids because gelatine is a digestible protein which contains all the essential amino acids except tryptophan otherwise most of hydrocolloids are polysaccharides, whereas. It has a relatively low melting point in comparison to polysaccharides and thus it is not used very much [20].

Gelatine has gelling, foaming and emulsifying properties that contribute to a wide range of applications in the food, pharmaceutical, photographic and cosmetic industries [17], It can be obtained by the thermal denaturation of collagen, which is the main structure and most common proteins organize the organism of almost animals. In recently years we can obtain gelatine from Cattle bones, hides, pig skins, fish sources or in some studies of scientists they even can obtain gelatine from insects [21]. Many foods use gelatine as source for texture and binding agent, gelatine from insect can be used to produce ice cream by using 0.5% insect's gelatine and compared with that made using 0.5% commercial gelatine as stabilizing agent. The properties of the obtained ice cream produced using insect gelatines were found to be acceptable for the panelists, and no significant differences between ice cream made using insect gelatine when compared with that made using commercial gelatine in their general preferences. Thus the current study was carried out to review gelatine methods of extraction of its main sources and uses, as well as its industrial applications [23].

3.1.1 Primary structure

The primary structure and composition of gelatine resembles the parent collagen. This similarity has been substantiated for several tissues and species. Slight differences are due

to the source of raw material in combination with the pre-treatment and extraction procedures used [21].

3.1.2 Secondary structure

Johnston-Banks, 1990 indicates that various aspects of gelatine behaviour in solution and gels have been explained regarding to its molecular weight. And gelatine is not poly dispersed completely, but has a definite molecular weight distribution pattern, which corresponds to the α-chain and its oligomers [16]. One to eight oligomers may be found or detected in solution, but the possibility of higher numbers being present cannot be ruled out. Oligomers of three α -chains will maintain mainly as intact triple helices, whilst a certain proportion will exist as extended α -polymers bonded randomly by end-to-end or side-to side bonds [20].

The presence of oligomers with increasing numbers of α -chains becomes more complex and difficult to read. Addition to it becomes necessary to separate these molecular weight fractions. In order to obtain highly accurate molecular weight spectra of both commercial and laboratory gelatines using Polyacrylamide gel electrophoresis (PAGE) is needed, giving quantitative separation [20].

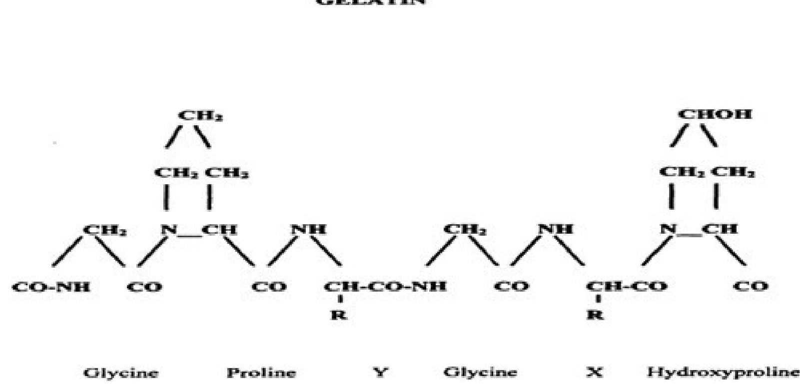


Figure 2: Configuration of gelatine

3.2 Physical and chemical properties

Gelatine is nearly tasteless and odourless. It is a vitreous, brittle solid faintly yellow in colour. Gelatine contains 8- 13% moisture and has a relative density of 1.3-1.4. When gelatine granules are soaked in cold water they will be dissolve, swollen particles. On being

warmed, these swollen particles dissolve to form a solution. This method of preparing gelatine solutions is preferred, especially where high concentrations are desired. Temperature, pH, ash content, method of manufacture, thermal history and concentration are the factors influencing on the behaviour of gelatine solution. Gelatine is soluble in liquid of polyhydric alcohols such as glycerol and propylene glycol. Examples of highly polar, hydrogen-bonding, organic solvents in which gelatine will dissolve are acetic acid, trifluoro ethanol, and formamide. Gelatine is insoluble in less polar organic solvents such as benzene, acetone, primary alcohols and dimethyl formamide. To make sure gelatine unchanged their properties for long periods of time it should be stored in air-tight containers at room temperature. When dry gelatine is heated above 45° C in air at relatively high humidity (above 60% RH) the loses its ability will be occurred to swell and dissolve. Sterile solutions of gelatine when stored cold are stable indefinitely; but at elevated temperatures the solutions are easy to hydrolysis. Two of gelatine's most useful properties, gel strength and viscosity, are gradually weakened on prolonged heating in solution above approximately 40°C. Degradation may also be happened by extremes of pH and by proteolytic enzymes including those which may result from the presence of microorganisms. Collagen may be considered an anhydride of gelatine. The hydrolytic conversion of collagen to gelatine yields molecules into varying mass: each is a fragment of collagen chain from that it will be divided. Therefore, gelatine is not a single chemical entity, but gelatine is a mixture of fractions consist of amino acids which connect with each other by peptide linkages to form polymers varying in molecular mass from 15,000 to 400,000. The basic elements of gelatine is composed of 50.5% carbon, 6.8% hydrogen, 17% nitrogen and 25.2% oxygen. Since it is derived from collagen, gelatine is properly classified as a derived protein. It gives typical protein reactions and is hydrolysed by most proteolytic enzymes to yield its peptide or amino acid components. The various amino acids obtainable from some gelatines by complete hydrolysis, in grams per 100 grams of dry gelatine [23].

Table 6: Amino Acid Composition of Gelatine

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

3.2.1 Amphoteric Properties

'Amphoteric Properties (24)– Gelatine in solution is amphoteric, it can present as both acid and as a base. In alkaline solutions gelatine is negatively charged and migrates as an anion. The pH of the intermediate point, where the net charge is zero and no movement occurs, is known as the Isoelectric Point (IEP). In acidic solutions gelatine is positively charged and migrates as a cation in an electric field. Type A gelatine has a broad isoelectric range between pH 7 and 9. Type B has a narrower isoelectric range between pH 4.7 and 5.4. Gelatine in solution containing no non-colloidal ions other than H+ and OH- is known as isoionic gelatine. The pH of this solution is known as the Isoionic Point (pl). These solutions may be prepared by the use of ion exchange resins' [23].

3.2.2 Chemical Derivatives

'Chemical Derivatives (24) – Gelatine may be chemically treated to bring about significant changes in its physical and chemical properties. These changes are the result of structural modifications and/or chemical reactions. Typical reactions include acylation, esterification, deamination, cross-linking and polymerization, as well as simple reactions with acids and bases' [23].

3.2.3 Gel Strength

'Gel Strength (24) – The formation of thermal reversible gels in water is one of gelatine's most important properties. When an aqueous solution of gelatine with a concentration greater than approximately 0.5% is cooled to approximately 35- 40°C it first increases in viscosity,

and then later forms a gel. The rigidity or strength of the gel depends upon gelatine concentration, the intrinsic strength of the gelatine depends on pH, temperature, and the presence of any additives. The intrinsic strength of gelatine is a function of both structure and molecular mass. The first step in gelation is the formation of locally ordered regions caused by the partial random return (renaturation) of gelatine to collagen-like helices (collagen fold). Next, a continuous fibriller three-dimensional network of fringed micelles forms throughout the system probably due to non-specific bond formation between the more ordered segments of the chains. Hydrophobic, hydrogen, and electrostatic bonds may be involved in the cross bonding. Since these bonds are disrupted on heating, the gel is thermos reversible. Formation of the cross bonds is the slowest part of the process, so that under ideal conditions the strength of the gel increases with time as more cross bonds are formed. The total effect is a time-dependent increase in average molecular mass. The gel forming quality of gelatine is a significant physical quality parameter. The measurement of this property is very important from both a control standpoint and as an indication of the amount of gelatine required by a particular application' [23].

3.2.4 Viscosity

'Viscosity (25) - The established method for the determination of viscosity involves efflux time measurement of 100ml of a standard test solution from a calibrated pipette viscometer. In certain case viscosity is determined at concentrations at which the gelatine is to be used. Molecular weight distribution appears to play a more important role in the effect on viscosity than it does on gel strength. Some gelatines of higher gel strength may have lower viscosities than gelatines of lower gel strength. The viscosity of gelatine solutions increases with increasing gelatine concentration and with decreasing temperature; viscosity is at a minimum at the isoionic point [23].

3.2.5 Protective Colloidal Action

'Protective Colloidal Action (25)– Gelatine is a typical hydrophilic colloid capable of stabilizing a variety of hydrophobic materials. The efficiency of gelatine as a protective colloid is demonstrated by its Zsigmondy gold number which is the lowest of any colloid. This property is especially valuable to the photographic and electroplating industries' [23].

3.2.6 Coacervation

‘Coacervation (25)– A phenomenon associated with colloids wherein dispersed particles separate from solution to form a second liquid phase is coacervation. Extensive coacervation studies have been conducted with gelatine. A common application of coacervation is the use of gelatine and gum Arabic to produce oil-containing microcapsules for carbonless paper manufacture. Coacervation is also useful in the photographic industry [23].

3.2.7 Colour

‘Colour (25)– The colour of gelatine depends on the nature of the raw material used and whether the gelatine represents a first, second or further extraction. Pork skin gelatines usually have less colour than those made from bone or hide. Generally speaking, colour does not influence the properties of gelatine or reduce its usefulness’ [23].

3.2.8 Turbidity

‘Turbidity (26) – Turbidity may be due to insoluble or foreign matter in the form of emulsions or dispersions which have become stabilized due to the protective colloidal action of the gelatine, or to an isoelectric haze. This haze is at a maximum at the isoelectric point in approximately 2% solutions. At higher concentrations or different pH the haze will be appreciably less’ [23].

3.2.9 Ash

‘Ash (26)– The ash content of gelatine varies with the type of raw material and the method of processing. Pork skin gelatines contain small amounts of chlorides or sulfates. Ossein and hide gelatines contain primarily calcium salts of those acids which are used in the neutralization after liming. Ion exchange treatment may be used for demineralizing or de-ashing of gelatines’ [23].

3.3 Manufacturing of gelatines

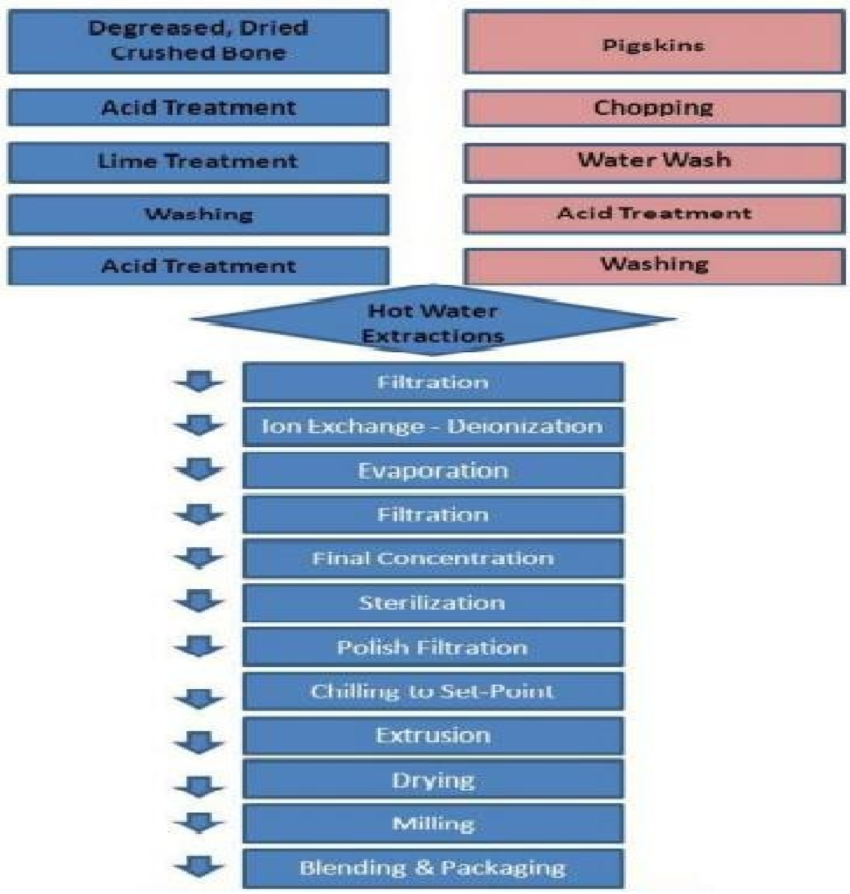


Figure 3: The processing of extraction gelatine from bone and pigskin [23]

3.3.1 Raw materials:

The sources of gelatine are cattle bones, cattle hides, and pork skins. Several alternative sources include poultry and fish. With bone from the slaughter of cattle is cleaned, degreased, dried, sorted, and crushed to a particle size of about 1-2 cm to make it easy for the next step. With pigskin they are chopped in to small pieces [23].

3.3.2 Acid and alkaline treatment:

With bone, cattle and ossein which are produced by type B or using baso treatment: The pieces of bone are treated with dilute hydrochloric acid to remove mineral salts. From this point on in the manufacture of Type B gelatine, both cattle hide and ossein receive similar treatment. For the production of Type B gelatine, both ossein and cattle hide pieces are subjected to lengthy treatment with an alkali (usually lime) and water at ambient temperature. Depending on previous treatment, the nature of the material, the size of the pieces, and the

exact temperature, liming takes 5-20 weeks, usually 8-12. The degree of alkalinity is controlled by using titration with acid, or by making test extractions. Ossein usually requires more liming time than cattle hides. using lime is added to maintain an excess, thereby compensating for any consumed. Addition the evolution of ammonia will be occurs during liming some deamination of the collagen, with. After previous processing, the raw material is thoroughly washed with cold water to remove excess lime; the pH adjusted with acid; and the product extracted with hot water to recover the soluble gelatine [23].

With big skin the short time required for pre-treatment prior to extraction, and the minimization of wastewater generated are important economic factors in the manufacture of gelatine from this raw material and for production of Type A gelatine using cold water to wash and then soaked in cold dilute mineral acid for several hours in order to achieve maximum swelling. There are two mostly acids used is Hydrochloric acid and Sulfuric acid. After that the remaining acid is then drained off and again using cold water to wash the material on several times. The pigskins are then ready for extraction with hot water. The pH, time, temperature, and number of extractions varies from processor to processor depending on product needs, type of equipment employed, timing of operations, and economics. These pre-treated materials are then hydrolysed to gelatine which is soluble in hot water [23].

3.3.3 Extraction:

Type A and Type B are then ready to extraction and they influence both quality and quantity the properties of Gelatine. Extraction is normally carried out in stainless steel vessels equipped which is controlled regulation of heating and temperature. The number of extractions varies, 3-6 is typical. The first extraction generally takes place at 50-60° C, subsequent extractions being made with successive increases in temperature of 5-10° C. The final extraction is carried out close to the boiling point. Extracts are kept separate, analysed, and subsequently blended to meet various customer specifications. The initial extraction usually provides a superior product, compared with subsequent extractions. Earlier extractions have higher molecular weights, higher viscosity, higher gel strength, and lighter colour. The later extractions are made at increasingly higher temperatures; the resulting product has lower molecular weight, lower gel strength, and darker colour. The dilute gelatine solutions from the various hot water extractions are filtered, deionized, and concentrated by crossflow membrane filtration and/or vacuum evaporation. The gelatine solution is then chilled and either cut into ribbons or extruded as noodles, and the gelled material is deposited as a bed onto an

endless, open weave, stainless steel belt. The belt is passed through a drying chamber, which is divided into zones in each of which the temperature and humidity of the drying air is accurately controlled. Typical temperatures range from about 30° C in the initial zone up to about 70° C in the final zone. The air is usually conditioned by filtration, dehumidification and tempering. Drying involves progressive increases in air temperature, often with exhaustion of moist air and replenishment with conditioned air. Drying time is 1-5 hours, depending on the quality and concentration of the material and the exact conditions employed. The rate of drying is carefully controlled to avoid melting and case hardening. The gelatine leaves the dryer with a moisture content of approximately 10%. The dried bed is then broken into pieces that are ground to the required particle size. Dried gelatine is tested for quality and gel strength according to standard methods developed by the Gelatine Manufacturers Institute of America. Although gelatines are sometimes referred to as edible, pharmaceutical, photographic or technical, these terms refer only to their uses, and not to the methods of manufacture [23].

3.4 Application of gelatines

Gelatine is usually used at low concentrations relatively in water or polyhydric alcohols in the manufacture of marshmallows, sweets and a whole range of dessert products. One of the the major reasons why it is so widely used in the food industry are because of its high-quality gels in dilute solution with a clean 'melt in the mouth' texture

• It gives elastic gum-like textures which dissolve in the mouth at higher concentrations

• It is an effective foaming and emulsifying agent

• As a polyelectrolyte it is able to flocculate suspended particles.

It is widely used to bind tablets in the pharmaceutical industry and in the manufacture of hard and soft gel capsules which wrap many drugs and nutritional supplements. It also has other medical uses. It is also used the photographic industry which utilises both its surface activity and its gel-forming ability to suspend particles of silver chloride and light sensitives dyes without agglomeration.

3.4.1 In food uses

The food industry is still a large user of gelatine. The major users today include manufacturers of

• Frozen cream products which is used 0.25% of a 250 Bloom gelatine to inhibit the crystallisation of ice and sugar.

• Ice cream which is used 0.5% of a similar 250 Bloom gelatine to prevent crystallisation.

• Marshmallows which may incorporate with up to 1.5% gelatine (of good Bloom strength) to prevent crystallisation.

• Lozenges, wafers and sweet coatings where up to 1% gelatine may be added to limit dissolution.

• It is a low-calorie sweets and spreads ingredient where its excellent water-holding capacity is utilised. It has an energy value of 14.7kJ/g so a 2% gelatine gel will contain less than 30kJ per 100g, i.e. (less than 8k calories per 100g). The development of low-fat and low-calorie foods has been intensively researched in recent years and the possibility of using mixed gels of, for example, egg white and gelatine in fat-free products so that the water is partitioned between the two gel phases, one of which is dispersed within the other to give the appropriate mouthfeel has been researched

• Meat products such as corned beef and luncheon meats where it is primarily used to hold water. In the manufacture of pasteurised canned hams and other such products gelatine (in the form of granules or portions of sheets) may be introduced into the product just prior to cooking so that the hot water released on heating the meat matrix dissolves the gelatine so that on cooling, a gel forms around the product to fill the space left as the meat shrinks. In pork pie manufacture a similar approach is adopted except that a hot gelatine solution, at over 45ºC to reduce microbial contamination, is injected into the cooled pies after baking. Pies are best 'jellied' at a core temperature of 65–70ºC, when the pastry is sufficiently cool to minimise the absorption of the gelatine solution. Usually a 6% solution of Bloom strength 160–200g is used. Lower core temperatures than 65–70ºC may be used and this will help minimise any softening of the pastry due to migration of water from the gelatine solution/gel. However, this is not usually feasible because of the possibility of microbial contamination.

• Novel dairy products where small amounts of gelatine can produce creamier stir yoghurts, gelled products with good organoleptic properties. If adding directly the gelatine in to the cold fresh milk to enable it to swell and subsequently dissolve during processing (heating).

• Apply in drinks industry as a flocculating agent. Both extremely high Bloom gelatines, and low Bloom which are mostly in the form of the leaf gelatine and powdered gelatines (ca. 80 Bloom grams) respectively are used. Clarification of beer using gelatine is widely used in North and is being increasingly used in Europe, outside Germany. Unlike the clarification

of wine, juices and beer where the gelatine is re flocculated, in lemonades it remains in the drink to stabilise the essential oils. [27]

3.4.2 Pharmaceutical uses

Gelatine is an ingredient of pastes, pastilles, pessaries, and glycerol suppositories and isotopic solutions containing 0.5–0.77 gelatine and a suitable bactericide may be used as artificial tears.

Capsules: One of the major of gelatine is the uses in the health/medical field as the main constituent of hard and soft (flexible) capsule shells. The hard gelatine capsule is a form off unit solid dosage. It consists of two pieces which are a cap and a body, which have the form of fit one over the other and open-ended cylinders. Following its processing a cold metal forming mould is dipped into a hot gelatine solution. Forming of gelatine gels around the mould as it is withdrawn then create a continuous film. This is then dried, removed from the mould, cut into the standard length and the two pieces combine together. They are produced by a certain specialist companies who provide them to the pharmaceutical industry where they are filled with active mixture to produce the final dosage form. They have been using significantly in Europe in the last 20 years. It is estimated that the consumption of them is in excess of 100,000 million. For hard gelatine capsules its walls need to be fairly rigid and strong and so it is necessary to use a high Bloom gelatine and the viscosity is used to control the wall thickness. 30–40% gelatine solution generally was prepared in hot (60ºC–70ºC) demineralised water in stainless-steel pressure vessels and it takes two to three hours. After this time it was moved to vacuum to remove entrapped air bubbles. Gelatine in hot solution hydrolyses and loses its desired physical properties; addition the quantity prepared at any one time is governed closely by its consumption rate to make sure that it is not held at these temperatures for too long. The other main raw material is the colorant. Capsules are made in a vast range of variety colours and to achieve this two sorts of colorants are used; soluble dyes and pigments. All of these are synthetic in origin, they are also dyed by natural way but it is costly. The colorants which can be used are governed by legislation which, unfortunately, varies widely from country to country. This means that sometimes the same colour has to be prepared with different dyes, depending upon the area of the world in which the finished product is to be sold.

We use the capsule manufacturing machine to make it which its parameter usually about 10m long and 2m wide. In 1932, R. P. Scherer developed the first continuous method of

encapsulation, the Rotary Die Method. This was later refined to give a fully automated process for the production of soft gelatine capsules. Today this process may be described as follows. Liquid material is fed from two tanks into the encapsulation machine, one contains molten gel material at 60–65ºC and the other contains the medicinal fill material usually at 25ºC. Molten gel (approximately 40% gelatine plus the plasticiser) flows down two heated pipes into two heated spreader boxes and then onto two large cool-casting drums where flat gelatine ribbons are cast.

Gelatine sponges. Gelatine is also used as the base for the foam cubes which is used by dentists to absorb blood during treatment and aid stop bleeding. The aerated gelatine gels have a high absorption capacity and the good compatibility of gelatine with human tissue rules out allergic reactions to a high degree.

Blood substitutes. To counteract high blood losses blood substitutes, which have optimum dwelling periods in the blood stream and thus allow blood volume to be regulated, are used. Here infusion therapy using suitable gelatine solutions is important, the solutions are so designed to have similar viscosities to blood and gelatine has among other advantages the fact that it is not stored in the body but is completely decomposed. Obviously high purity is essential for such gelatines and DGF Stores are the major suppliers. [27]

3.4.3 Photographic uses

Modern silver bromide photographic materials are mostly composed of emulsions containing gelatine on a backing material (paper or film). There are three functions of gelatine:

- It is a bonding agent for the photosensitive silver bromide.
- It is essential the gelatine swells and forms a solution when heated for the fabrication of the emulsion, which transfer into a gel on cooling and, after the water has been extracted, changes into a durable state.
- The swelling capacity of gelatine make sure that the photographic baths, that are essential for the chemical reactions during the processing of the exposed photographic materials, penetrate into the emulsion and can be removed easily by rinsing. Over the last decades, films became about 1,000 times more sensitive than their predecessors. However, the spectrum of gelatine applications includes much more than just prints, movie. slides, and cine films. In film industry, photographic gelatine to various types of repro-films for the printing trade. To technical and scientific photographic emulsions, such as nuclear trace emulsions for localising radio isotopes in nuclear medicine, to infrared sensitive emulsions for taking pictures in the ‘dark’, in astronomy, and in geology and photogrammetry for pictures taken from great

heights. Nowadays the highest demands are made on photographic gelatine for manufacturing X-ray films. [27]

II. EXPERIMENT PART

4 THE AIM OF EXPERIMENT

Gelatine plays an important role in our life. Recently, most of gelatine which is almost extracted from mammalian sources especially bovine and porcine is used wide range market but it seems that at this moment these sources are unsuitable for some religious and ethnic communities (14). It is not only because of culture but also due to the fear of bovine spongiform encephalopathy. Therefore, seeking alternative sources from other species rather than mammalians could be potential objects to produce collagen and gelatine. In some study recently year scientists have published the extraction gelatine from variety sources such as chicken head or feet, fishes, or even insects which has been concerned by society. By-product from these sources are becoming popular and researched by many countries, the two method of pre-treatment by using acid or alkali to remove non-collagen protein which are usually used in those researches resulting many different outcome, optimum conditions of extraction gelatine is one of the factors to determine which one will be economic efficiency, besides that using enzymes to enhance extracted gelatine is an effective method but it is not common and widely use in production. Therefore, we are doing the method by using enzyme protease to increase extracted process of uncommon chicken paw and expecting gelatine from this source could be an alternative product replacing mammalian's gelatine and fewer studies have been carried out to investigate chicken paws gelatine and it has led to limit information about products. The purpose of this study is to process hen paws which contain rich in collagen into gelatines, From the raw material fats and other non-collagenous proteins need to be removed. To produce gelatine from chicken paws, based on enzymes pre-treatment techniques for the best conditions of extraction gelatine, the experiments will be proposed by the methods "DOE – doing of experiments" – factorial schemes which we expect it will have better properties, yield of prepared gelatines will be studied. Prepared gelatines will be analysed (gel strength, viscosity, ash content, dry matter content, melting point of gels, transmittance etc.) and compare the physical, chemical, thermal and rheological properties.

5 MATERIAL AND METHOD OF WORK FLOW

5.1 Biological materials

5.1.1 Hen paws

Figure 4: Milled hen paws in package

The raw material was supplied by RACIOLA Uhersky Brod, which cut into 10mm small pieces and then were stored in cold storage. Its composition was showed on the table below, where: A in dry matter

Table 7: Composition of raw hen paws

Dry matter (%±SD)	**Proteins A (%±SD)**	**Portion of collagen from total proteins (%±SD)**	**Fat A (%±SD)**	**Mineral substances A (%±SD**
43.3 ±1.0	49.5 ±1.3	77.2 ±1.1	33.4 ±2.2	19.0 ±1.3

5.1.2 Enzymes

1. Lipolase

Lipases are one of the most commonly used classes of enzymes in biocatalysis. Lipases catalyze the hydrolysis of triacylglycerols to diacylglycerol, monoacylglycerol, glycerol and free fatty acids. The reaction reverses under anhydrous conditions and the enzyme is able to synthesize new molecules by esterification, alcoholysis and transesterification. All reactions can be performed with high region and enantios-electivity under mild reaction conditions. Lipases have been used on a variety of substrates and show very broad substrate specificity due to the ubiquity in nature and the heterogeneity of lipases from different sources.

2. Protamex

Producer: Novozymes

Description: Protamex is a Bacillus protease complex developed for the hydrolysis of food proteins.

Product Type: Protamex is a light brown, free-flowing, non-dusting micro-granulate with an average particle size of approximately 250-450 microns. The colour may vary from batch to batch and colour intensity is not an indication of product strength. The product is readily soluble in water.

Application: In contrast to many other endo-proteases, Protamex will produce non-bitter protein hydrolysates even at low degrees of hydrolysis.

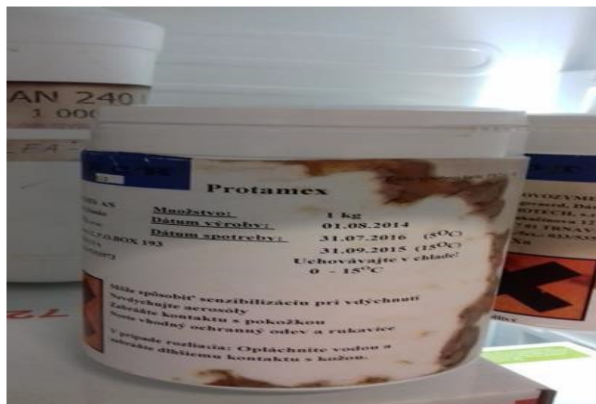


Figure 5: Enzyme protamex in package

5.2 Instruments and chemicals

5.2.1 Instruments

Table 8: Instruments and Equipment used during experiments

No.	Name	Trade mark and manufacturer	Describe
1	Kitchen sieve	Czech republic	
2	PE bottles	Czech republic	
3	Shaking machines SL 2and SL 3, KAVALIER	Czech republic	
4	1000ml measuring cylinder	Czech republic	

5	Volumetric flask	Czech republic	
6	Dispensing bottles	Czech republic	
7	Stirring bars	Czech republic	

8	Beakers	Czech republic	
9	Blalance scale,BCBC 100, KERN 770	Germany	
10	Oven, WTC BINDER	Germany	
11	Erlenmeyer Flasks	Czech republic	

12	Heater, SCHOTT GERATE GMHB	Germany	
13	Texture Analyzer, TA1000, LEONARD FARNELL&CO LTD	England	
14	Furnace, L91 NABER THERM	Germany	
15	Desiccator	Czech republic	

16	Viscometer	Czech republic	
17	Vessel	Czech republic	
18	Soxhlet LTHS 250	Czech republic	
19	pH meter, pH 526, MULTICAL	Germany	

20	Blender, b/013977, VWR	Italia	
21	Thermos spec-tronic, HELIOUS, EPSILON	USA	
22	Firer	Czech republic	

5.2.2 Chemicals

1. NaCl 0,2M
2. NaOH 0,03M
3. Acetone
4. HCl 2%

5.3 Methodology

Basic part of experiments: Factorial schemes with 3 studied factors on two levels (minimum and maximum) + central experiment (2 repetitions). Using STAT- DOE-FACTORIAL-ANANLYZE FACTORIAL DESIGN to analyse the regression and achieve the graph to show which factors will have the positive effect on yield of extraction, gel strength, viscosity and the ash.

Optimisation part of experiments: Factorial schemes with 3 studied factors on two levels (minimum and maximum) + central experiment (2 repetitions). After optimising we will obtain at which conditions the yield of extraction and gel strength will be the best and then it should be repeat again to support exactly the first results.

Working strategy:

5.3.1 Separation of fat and other non-collagenous parts from the hen paws

Hen paws were chopped into small pieces and washed and stored in refrigerator. After thawing it was washed in tap water to remove dirty and unwanted component. And then it will be treatment with NaCl 0.2M and NaOH to remove globulins and glutelin. After that it was removed fat through out to stages, the first was incubated with the addition enzyme LIPOLASE 5% and the second was shake with acetone achieve non-fat tissue. After all the preparation of pure collagen was ready for the next step.

5.3.2 Gelatine extraction

For gelatine extraction stage we did prepare 10 experiments with different enzyme proteaza concentrations, incubation times, extraction times + 1 blank without adding enzyme. The processing was presented at the below table 9

Completed experiments and calculation, analysis of the results of gelatine yields. With 3 factorial schemes are enzyme proteaza concentrations, incubation times, extraction times at 2 level minimum and maximum to optimize the result.

Analysis of prepared gelatines (statistical evaluation) was using the software MINITAB (version 14 and higher) to design optimal conditions for optimisation part of experiments

Table 9: Experiments of Processing conditions extraction of first (main) fraction of gelatine

Experiment No.	**Processing conditions** **Extraction of first (main) fraction of gelatine**		
	Factor A: Amount of added enzyme (%)	***Factor B:*** Time of enzyme treatment (h)	***Factor C:*** Extraction time of main fraction of gelatine (min)
1	0.2	24	30
2	0.2	24	120
3	0.2	72	30
4	0.2	72	120
5	0.8	24	30
6	0.8	24	120
7	0.8	72	30
8	0.8	72	120
9	0.5	48	75
10	0.5	48	75
Blind experiment (WITHIUT ADDITION OF ENZYME):			
11	0	48	75

Table 10: Experiments of Processing conditions extraction of second fraction of gelatine

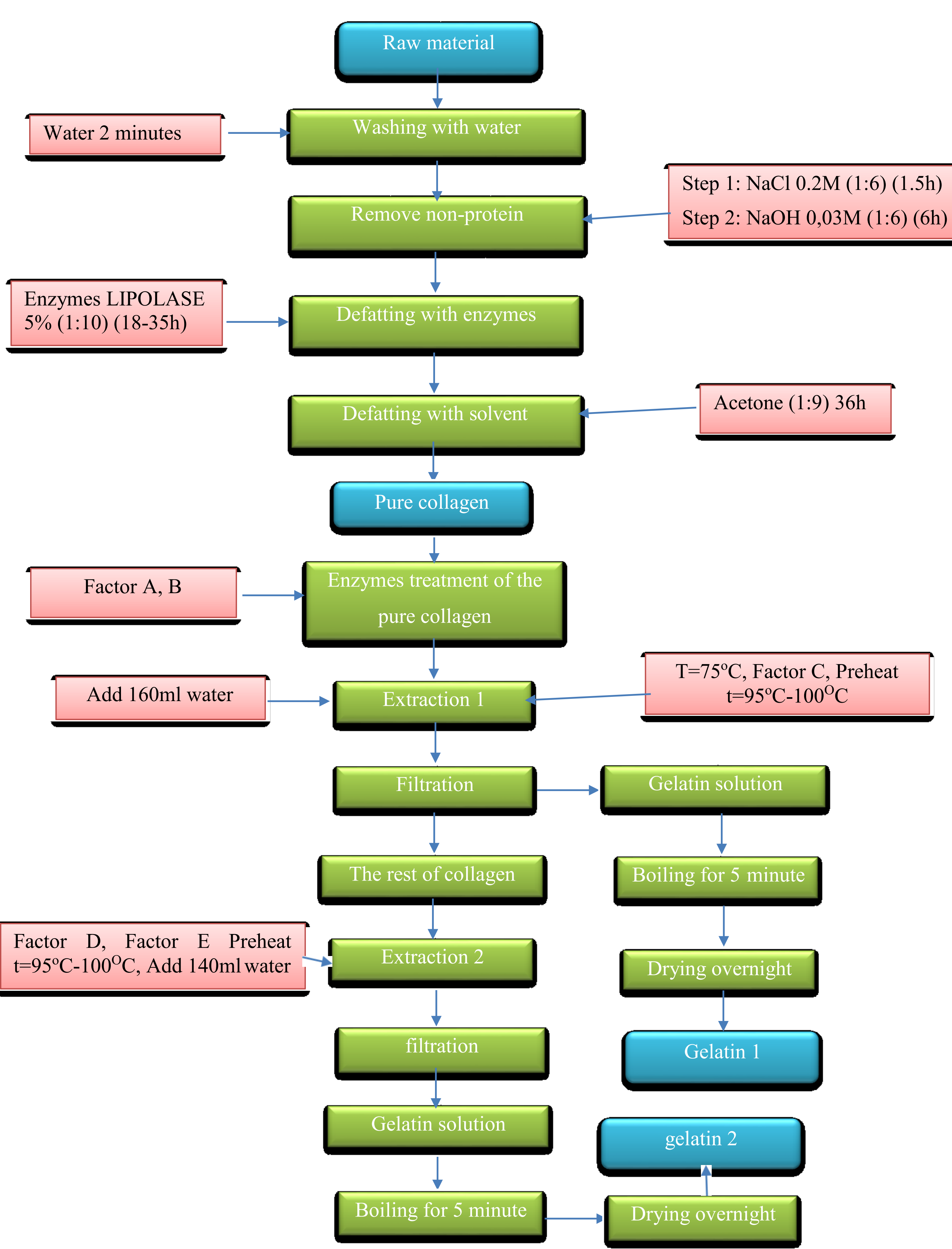
Experiment No.	**Processing conditions: Extraction of second (side) fraction of gelatine**	
	Parameter D Extraction temperature of second (side) fraction of gelatine (°C)	***Parameter E*** Extraction time of second (side) fraction of gelatine (min)
1	95	30
2	95	120
3	100	30
4	100	120
5	80	30
6	80	120
7	85	30
8	85	120
9	90	30
10	90	120
11 (Blind experiment:)	90	120

5.3.3 Optimisation part of experiments

Using Minitab to optimize the result through 3 factors: amount of enzyme treatment, time of enzyme treatment and time of main extraction on 2 levels: maximum and minimum.

After analysis we did obtain 2 experiments which performed good properties and high efficient that based on the results of gelatine yields and their quality (gel strength) the optimal conditions of processing of hen paws into gelatines will be proposed.

5.3.4 Work flow


Raw material
Water 2 minutes
Washing with water
Remove non-protein
Step 1: NaCl 0.2M (1:6) (1.5h)
Step 2: NaOH 0,03M (1:6) (6h)
Enzymes LIPOLASE 5% (1:10) (18-35h)
Defatting with enzymes
Defatting with solvent
Acetone (1:9) 36h
Pure collagen
Factor A, B
Enzymes treatment of the pure collagen
Add 160ml water
Extraction 1
T=75°C, Factor C, Preheat t=95°C-100°C
Filtration
Gelatin solution
The rest of collagen
Boiling for 5 minute
Factor D, Factor E Preheat t=95°C-100°C, Add 140ml water
Extraction 2
Drying overnight
filtration
Gelatin 1
Gelatin solution
gelatin 2
Boiling for 5 minute
Drying overnight

5.3.4.1 Washing in cold running water

Aim: Removing Albumins

Process: On a kitchen sieve wash the raw material properly for approx. 2 minutes. Raw material then transferred into a beaker with cold water and let in there for approx. 5 min. On a kitchen sieve wash the raw material properly for another approx. 2 minutes

Figure 6: Raw material was washed under tap water

5.3.4.2 Removing of non-protein

a. Treatment in 0.2 M NaCl

Aim: Removing Globulins

Process: Mix the raw material with 0.2 M NaCl in a ratio of 1:6. Modestly shake for 1.5 h at room temperature, filter out (on the sieve) the liquid and then wash the raw material properly for approx. 1 minute with running cold water

b. Treatment in 0.03 M NaOH

Aim: Removing Glutelins

Process: Mix the raw material with 0.03 M NaOH in a ratio of 1:6 and modestly shake at room temperature for approx. 6 hours, filter out (on the sieve) the liquid and then wash the raw material properly for approx. 1 minute with running cold water, mix the raw material with 0.03 M NaOH in a ratio of 1:6 and modestly shake for at room temperature overnight. Filter out (on the sieve with 1 layer of polyamide cloth) the liquid and then wash the raw material properly for approx. 2 minutes with running cold water

c. Mechanically squeeze out the raw material
Aim: Removing as much as water as possible
Process: In a small portion of the material determine the dry matter content (it is important to know for the next step).

5.3.4.3 Defatting with the act of enzyme

Mix the tissue with water at the ratio of 1:10 (the tissue has to be soaked thoroughly), then add enzyme LIPOLASE – the amount of 5 % (based on the dry matter of wet tissue from the previous step). Agitate at room temperature for the prescribed period of time in prescribed number of defatting steps; measure the pH of the mixture at the beginning and at the end the agitating cycle (see the table). Filter through a sieve (with 1 layer of polyamide cloth) and after filtration wash with running tap water for approx. 2 minutes. After defatting spread the tissue onto a Petri dish a gently dry at 35-40ºC for 18-35 hours

Table 11: Scheme of shaking cycles

Defatting cycle	**Day**	**Day time**	**pH**		**Remark**
			At the beginning (after 20 min)	At the end	
1.	1.	Morning (8 a.m.) – afternoon (3 p.m.)	11.69	11.79	
2.	1.-2.	Afternoon (3 p.m.) – morning (8 a.m.)	11.20	11.20	
3.	2.	Morning (8 a.m.) – afternoon (3 p.m.)	9.96	9.96	
4.	2.-3.	Afternoon (3 p.m.) – morning (8 a.m)	8.87	7.06	

5.3.4.4 Defatting with the act of solvent

Mix the tissue with acetone solvent (1:1) in a ratio of 1:9 and shake for 6-8 hours at room temperature on a shaking machine; then change the solvent for the one and shake till the morning on the next day (shaking period in may be prolonged). Filter-out the solvent and place defatted tissue (collagen) in thin layer on the sheet plate; let the remnants of the solvent evaporate (for overnight)

After defatting of tissue, we did analysis the amount of fat contains inside the tissue, the result showed that the fat was 11,83%.

Figure 7: Evaporating solvent of collagen tissue

5.3.4.5 Milling of the tissue (collagen)

Mill the (smaller amounts of) tissue using a (vertical) blender for a period of 15 seconds; if necessary blend again (after cooling down the tissue); blending time should not be too long, otherwise the temperature of the tissue can rise (which is unwelcomed). Store the blended tissue in a vessel at room temperature (and avoid sun-shines)

Figure 8: Milled collagen at room temperature

5.3.4.6 Enzyme treatment of the tissue (pure collagen)

a) Mix the tissue with distilled water at a ratio of approx.1:10 (**20 g of tissue + 200 ml water**)

b) Gently shake for approx. 45 minutes at room temperature; then adjust pH to 6.5-7.0. Make a record of the amount of chemicals used for pH adjustment

c) Add proteolytic enzyme PROTAMEX in the amount according to ***Factor A*** (see schedule of experiments). The amount of enzyme is based on dry matter of processed tissue

d) Gently shake at room temperature for the time according to ***Factor B*** (see schedule of experiments). During the first 5 hours (after 1st, 2nd and 5th hours) check the pH of the mixture

and adjust if necessary to 6.5-7.0, make a record of the amount of chemicals used for pH adjustment. After finishing the whole shaking period measure the pH (but do not adjust any more)

e) Filter the mixture through a (kitchen) sieve equipped with 3 layers of polyamide cloth. Filtrate, liquid part (***Hydrolysate***) in a vessel heat to the boiling point (97-100ºC) and boil for 5 minutes (inactivation of the enzyme occurs). The whole volume of hydrolysate cast onto a plate (with anti-adhesive foil) and dry at 60ºC for approx. 36-48 hours. Scrape off the dried hydrolysate and weigh it; then store in a closed sack at room temperature

2. Washing the enzyme treated tissue: The tissue retained on the sieve wash thoroughly with cold tap water for approx. 2 minutes (to firstly remove as much as enzyme as possible). The mix the tissue with enough surplus of 0.03 M NaOH and shake intensively on a shaking machine for approx. 10 minutes, then filter out, shortly wash with cold tap water; repeat the whole procedure once more. The tissue retained on the sieve wash thoroughly with cold tap water for approx. 2 minutes (to remove hydroxide). Finally mix the tissue with enough surplus of cold tap water and shake intensively on a shaking machine for approx. 10 minutes, then filter out, shortly wash with cold tap water; repeat the whole procedure once more

5.3.4.7 Extraction of first (main) fraction of gelatine

a) Transfer washed tissue into the beaker and add distilled water in a ratio of approx. 1: 8. Based on initial amount of the tissue at the beginning of the procedure (20 g; now it is a bit less) add 160 ml distilled water. Mark (with a permanent marker) a line indicating the upper level of water; during the extraction step add the water (it will evaporate)

b) Heat the mixture to the extraction temperature of 75ºC; use a large hot plate (use always the same plate with all the experiments). After the temperature of 75ºC is reached extract the gelatine for the time according to ***Factor C*** (see schedule of experiments). Record the time of reaching the temperature from the ambient temperature to the extraction temperature (i.e. 75ºC). During the extraction gently mix the mixture (using a magnetic stirrer)

c) Filter the mixture through a (kitchen) sieve equipped with 3 layers of polyamide cloth. The filtrate – liquid – (***The main fraction of gelatine***) immediately heat up in the beaker to the boiling temperature (97-100ºC) and boil for 5 minutes (to inactivate the rest of enzyme). Cast the whole volume of gelatine solution onto a plate (with anti-adhesive foil) and dry at

45ºC for approx. 48 hours. Scrape off the dried main fraction of gelatine and weigh it; then store in a closed sack at room temperature; it will bet hen analysed.

Figure 9: Extraction gelatine from pure collagen at 75 ºC

5.3.4.8 Extraction of second (side) fraction of gelatine

a) Transfer the undissolved tissue remained after the extraction of first (main) fraction of gelatine into the beaker and mix with the distilled water in a ratio of approx. 1: 7. Add approx. 140 ml water, mark (with a permanent marker) a line indicating the upper level of water; during the extraction step add the water (it will evaporate)

b) Heat up the mixture to the extraction temperature according to ***Factor D*** – see schedule of experiments (use the same heater plate as before) and as soon as the extraction temperature is reached extract gelatine for the time according to ***Factor E*** – see schedule of experiments. Record the time of reaching the temperature from the ambient temperature to the extraction temperature. During the extraction gently mix the mixture (using a magnetic stirrer)

c) Filter the mixture through a (kitchen) sieve equipped with 3 layers of polyamide cloth. Do not forget to weigh the cloth (the undissolved part of the tissue will be dried on it lately to calculate the amount of remnant undissolved tissue). The filtrate – liquid – (***The side fraction of gelatine***) immediately heat up in the beaker to the boiling temperature (97-100ºC) and boil for 5 minutes (to inactivate the rest of enzyme) Cast the whole volume of gelatine solution onto a plate (with anti-adhesive foil) and dry at 45ºC for approx. 48 hours. Scrape off

the dried side fraction of gelatine and weigh it; then store in a closed sack at room temperature; it will bet hen analysed

5.3.4.9 Undissolved part of the tissue

Remained on the cloth after the extraction of side fraction of gelatine dry on a petri dish at 103°c (overnight) and weigh. Calculate a mass balance efficiency of the extraction.

5.4 Evaluation of efficiency of the process and quality of prepared products [24]

5.4.1 Measuring gelatine gel strength

Principle: According to the standard procedure, the gel strength of gelatine is a measure of the rigidity of a gel formed from a 6.67% solution and prepared according to certain arbitrary prescribed conditions. Bloom is a measure of force (weight) required to depress a prescribed area of the surface of the sample a distance of 4 mm. Take a photo of every prepared gelatine gel; the photographs will be then compared

Procedure

1. Weigh 7.50 ± 0.01 g of gelatine into the bloom bottles using an analytical balance.

Methods according to GMIA: 112 g of gelatine gel of concentration 6.67 % (w/w) is prepared in a standard vessel.6.67 % (w/w) solution= *6.6667 g gelatine + 93.3333 g water (∑=100 g)* = **7.5 g gelatine + 104,5 g water (∑=112 g)**

In case you do not have enough gelatine (6.67 g) use following method (B or C) for measuring gelatine gel strength (make a note which procedure is used – for re-calculating the final result). Method B using 3g of gelatine with 42g water in 1/2 volume vessel and method C using 1.5g of gelatine with 21g water in 1/4 volume vessel.

Recalculation regarding measurement of gelatine gel strength in different vessels:

Method A standardised vessel

Method B vessel: outer diameter of 50 mm, inner diameter 40 mm, height 50 mm **Correction factor (gel strength is less): 1.26**

Method C vessel: outer diameter of 40 mm, inner diameter 35 mm, height 50 mm **Correction factor (gel strength is less): 1.64**

2. Add 105.0 ± 0.2 g of distilled water at 25 ± 2°C from an automatic pipette. Stir with a stirring rod while adding the water, using the pointed end to remove all gelatine in the groove at the bottom of the bottle. Wash any gelatine adhering to the rod into the bottle with the last portion of water.

3. Insert a perforated stopper and allow the sample to hydrate 1-3 hours at room temperature.
4. Place the sample bottle in the 65°C bath. Stir or swirl periodically, and after 8-10 minutes remove the bottle from the bath. Swirl the bottles several times to be sure that all of the gelatine is in solution. Replace the bottle in the bath, insert a thermometer into the sample and stir just enough to effect thorough mixing until the temperature of the sample is 61°C. The total time in the 65°C bath shall not exceed 15 minutes.

5. When the temperature of the solution reaches 61°C and the sample is completely dissolved and thoroughly mixed, remove the thermometer and transfer to a viscometer and determine the viscosity according to the designated procedure (see Viscosity Procedure). Collect all of the effluent from the viscometer in the original bottle and immediately replace the stopper.
6. Temper the sample by placing the bottle in the 45°C bath for 30-45 minutes or letting it stand for 15-20 minutes at room temperature.

Note: If the viscosity is not to be determined or if the viscosity is to be determined after the jelly strength, insert the stopper and temper immediately after the 65°C bath as above.

7. After tempering, gently swirl the solution in the Bloom bottle to remove condensation from the sides of the bottle. Remove the stopper and gently remove all foam from the centre of the gelatine solution surface with a spoon or other convenient means. Replace the stopper and place the sample bottle in the 10.0 ± 0.1°C water bath for 16-18 hours.

8. Set up Stevens LFRA and TAXT2 according to Annex 1.

9. Remove the jelled sample from the 10°C bath and quickly wipe the water from the exterior of the bottle. Remove the stopper and centre the bottle on the texture analyser platform so that the plunger contacts the sample as close its midpoint as possible. (If there is any foam at this area, start the determination over). The determination is to be done immediately after removal of the bottle from the chill bath.

10. Press “Start” (LFRA) or “Run” (TA.XT2) to run the determination. If using the LFRA, check the zero weight often. Repeat as needed until all bloom determinations are complete.

5.4.2 Determination of gelatine gel melting temperature on DSC

Temperature increase: 5ºC / min, into a DSC crucible weigh-in 10-20 mg of the sample after gelatine gel strength measurement; close the crucible hermetically (closed sample in a crucible can be stored for weeks (months) at room temperature. Use the following method (as previously proved to be the best one): DSC crucible cool down to a temperature of approx. 6ºC and hold at this temperature for 10-30 min; then heat by 5ºC / min to temperature of 50ºC; finally cool down to the initial temperature (6ºC) with the same speed (5ºC / min); endotherm peak during the heating equals gel melting temperature; a transition during cooling should be gel gelling temperature. Do not discard the crucible after measurement, keep

5.4.3 Determination of kinematic viscosity of gelatine

Principle: The viscosity of a 6.67% gelatine solution is determined at 60ºC by measuring the flow time of 100 mL of the solution through a standard pipette. Use the sample after determination of gelatine gel strength, use capillary viscometer

Calculation of the Viscosity

The viscosity (to the nearest millipoise) at 60ºC of any sample with efflux time t (in seconds) may be calculated from the following equation:

$$V = (At - B/t) \times d$$

V = Viscosity, in millipoises (mP.S) A, B = A and B pipette constants (Refer to Annex I, Calibration of Viscosity Pipette, to obtain A and B constants, if not available) t = efflux time, in seconds d = solution density

Refer to Section D, Calibration, to obtain A and B constants, if not available.

For a 6.67% gelatine solution at 60ºC d = 1.001

5.4.4 Determination of gelatine clarity

Principle: The clarity of a 6.67% gelatine solution is determined at 45ºC by measuring the percent transmittance through a 1 cm cuvette at 640 nm.

Procedure

1. Weigh 7.50 ± 0.01 g gelatine into bloom jar or 150 mL beaker, add 105.0 ± 0.2 g deionized water, stirring often to suspend all gelatine particles, cover and let stand 1 – 3 hours at room temperature. Dissolve the sample in a 65ºC water bath for 10 – 15 minutes, stirring or swirling as required. Remove the sample to a 45ºC water bath and hold until sample temperature is 45 ± 1ºC. 6. Calibrate the spectrophotometer to 100% transmittance with deionized

water blank at 640 nm according to the manufacturer's instructions. Transfer an aliquot of the sample solution to the cuvette and record the percent transmittance value at 640 nm.

5.4.5 Determination of dry matter content of gelatines

Principle: A weighed sample of gelatine is maintained for 16 to 18 hours at 105 ± 2ºC and is then reweighed. The moisture content is defined as the percentage loss in weight of the sample.

Procedure: Sample Preparation: Wash the evaporating dish very carefully in hot water. Place the dish in the drying oven at 105ºC for at least one hour. Cool dish in the desiccator until room temperature is reached. Weigh approximately 5.0 g of gelatine to the nearest milligram and note the weight of the test sample (m0) and the weight of the sample together with the evaporating dish (m1).

Determination: Place the evaporating dish containing the sample in the drying oven at 105 ± 2ºC for 16 to 18 hours. Cool the dish in the desiccator until room temperature is reached and weigh to the nearest milligram (m2), weigh and calculate the percentage of residue

Result: The moisture content, expressed as a percentage by weight, is equal to:

$$\% \text{ Moisture} = [(m1) - m2) / m0] \text{ X } 100\%$$

where: m0 is the weight in grams of the test sample m1 is the weight in grams of the test sample and the evaporating dish, before drying M2 is the weight in grams of the test sample and the evaporating dish, after drying

5.4.6 Determination of ash content of gelatines

Principle: Gelatine is ashed in a crucible at 650ºC using a muffle furnace. The residue is determined by differential weighing and the result expressed as a weight percentage of the original sample.

Procedure: Sample Preparation: Weigh approximately 5.0 g of gelatine to 0.001 g in a suitable crucible that has previously has been ignited, cooled and weighed. Add 1.5 to 2.0 g of paraffin to avoid loss due to swelling. Heat, gently at first on an electric hot plate or in a muffle furnace, until the substance is thoroughly charred. Finish ashing in a muffle furnace at 550ºC for 15 to 20 hours.

Determination: Cool the sample in a desiccator. Weigh the sample and calculate the percentage of residue

Result: The ash content, expressed as % ash, is equal to: % ash = [weight of ash] / [weight of sample] X 100%

5.4.7 pH of gelatine solution

Principle: The pH of a 1.5 % gelatine solution is determined by potentiometry at a temperature of 35 ± 1°C using a pH meter.

Procedure: Weigh 1.60 ± 0.01 g gelatine into a bloom jar or 150 mL beaker. Add 105.0 ± 0.2 g deionized water, stirring often to suspend all gelatine particles Cover and let stand 1 – 3 hours at room temperature Dissolve the sample in a 65ºC water bath for 10 – 15 minutes, stirring or swirling as required. Transfer the sample to the 35ºC water bath and temper to 35ºC. Perform a two-point calibration on the pH meter, using pH 4 and pH 7 buffers, at 35ºC. Determine the pH of the gelatine solution according the pH-meter instructions. Swirl the solution well using the pH probe to ensure the electrode is sufficiently saturated. Rinse the electrode with warm distilled water once testing is complete.

6 Result and discussion

Table 12: Results of experiments of Processing conditions extraction of first (main) fraction of gelatine

Experiment No.	**Processing conditions** **Extraction of first (main) fraction of gelatine**			**Results of the processing**								
	Factor A Amount of added enzyme (%)	***Factor B*** Time of enzyme treatment (h)	***Factor C*** Extraction time of main fraction of gelatine (min)	Yield of hydrolysate [%]	Yield of gelatine after first extraction step [%]	Gel strength of gelatine after first extraction step [g]	Total yields of the Process [%]	Mass balance mistake (%)	Viscosity (mPa.S)	clarify	pH	Ash (%)
1	0.2	24	30	3.3	8.24	317	13.74	2.2	5.26	1.1	8.9	1.97
2	0.2	24	120	3.8	11.54	270	19.23	5.4	7.34	0.8	9.05	2.5
3	0.2	72	30	4.4	9.34	341	14.84	4.4	5.77	1.8	9.28	2
4	0.2	72	120	4.4	12.91	300	20.05	6.6	5.77	1.1	9.12	2.13
5	0.8	24	30	6.04	14.29	359	17.59	5.5	6.8	0.8	9.36	2.3
6	0.8	24	120	6.59	14.84	320	20.34	3.85	7.34	0.7	9.07	1.4
7	0.8	72	30	7.14	15.93	413	20.88	6.59	5.77	1.4	8.87	1.6
8	0.8	72	120	6.79	21.42	328	28.01	4.4	5.77	0.7	8.7	1.7
9	0.5	48	75	5.49	14.29	373	20.88	6	8.36	1.1	8.96	1.7
10	0.5	48	75	4.4	15.93	350	24.93	5.5	6.3	0.7	8.96	1.8
Blind experiment (WITHOUT ADDITION OF ENZYME):												
11	0	48	75	1.65	11	376	18.69	1.65	7.83	0.8	9	1.9

Table 13: Results of experiments of Processing conditions extraction of second fraction of gelatine

Experiment No.	Use the undissolved tissue remained after extraction of first (main) fraction of gelatine	Processing conditions Extraction of second (side) fraction of gelatine						
		Parameter D Extraction temperature of second (side) fraction of gelatine (°C)	***Parameter E*** Extraction time of second (side) fraction of gelatine (min)	Yield of gelatine after second extraction step [%]	Gel strength of gelatine after second extraction step [g]	The ash (%)	Viscosity (mPa.S)	pH
1		95	30	5.5	196	2.6	3.65	8.34
2		95	120	7.69				
3		100	30	5.5				
4		100	120	7.14				
5		80	30	3.3	224	2.8	4.72	9.44
6		80	120	5.5				
7		85	30	4.95				
8		85	120	6.59				
9		90	30	6.59	286	2.5	5.77	8.58
10		90	120	8.24				
		Blind experiment:						
11		90	120	7.69				

6.1 Optimization of yield gelatine

Pareto Chart of the Standardized Effects
(response is Yield of extraction 1(%), α = 0.05)

Term
12.71
A
C
B
AB
BC
ABC
AC
0 2 4 6 8 10 12 14
Standardized Effect

Factor Name
A Amount of enzyme(%)
B Time of enzyme treatment(h)
C Main extraction time(min)

Figure 10: Pareto chart of standardized effects on yield of gelatine

The analysis for the response of yield of gelatine using analyse factorial design (table 14) was significant at $P<0.05$ with R^2=96% which P = 0,033 for amount of enzyme, it means there is the effect of amount of enzyme on gelatine yield. The coefficient of gelatine content of hen paws was obtained by the regression, the insignificant coefficient was dropped from the model which was presented on the table, and the graph showed how it was effected on yield of gelatine of three factors presented in the figure 5, the equation used in generating the three dimensional response are as follows:

% Yield of extraction 1 =4.15 + 12.52 X_1

Table 14: Parameters estimate for percent yield gelatine of hen paws obtained by regression analysis

Coefficients	T-Value	P-Value	VIF
X_1	5.39	0.033	1.00
X_2	2.36	0.142^{ns}	1.00
X_3	2.83	0.105^{ns}	1.00
X_1* X_2	1.26	0.336^{ns}	1.00
X_1* X_3	-0.18	0.872^{ns}	1.00
X_2* X_3	1.17	0.362^{ns}	1.00
X_1* X_2* X_3	1.04	0.409^{ns}	1.00

ns: Not significant at P < 95%. other coefficients were significant at P < 95%

Where: X_1 Amount of enzyme

X_2 Time of enzyme treatment

X_3 Main extraction time

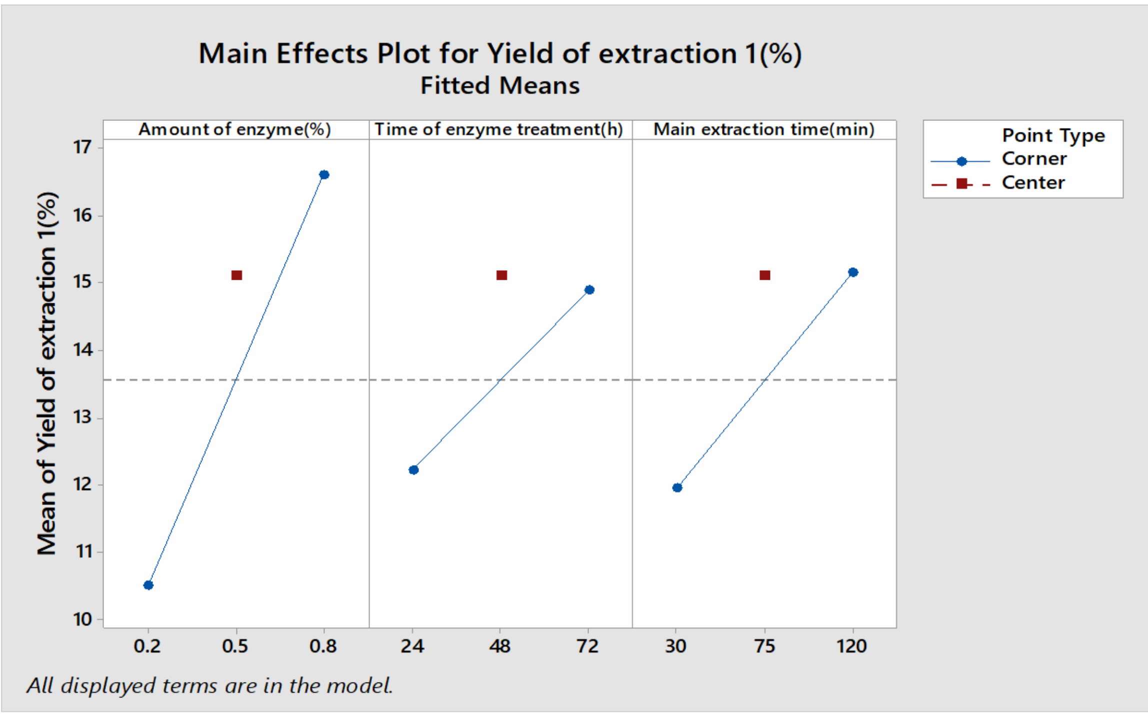


Figure 11: Main effects plot for yield of extraction 1

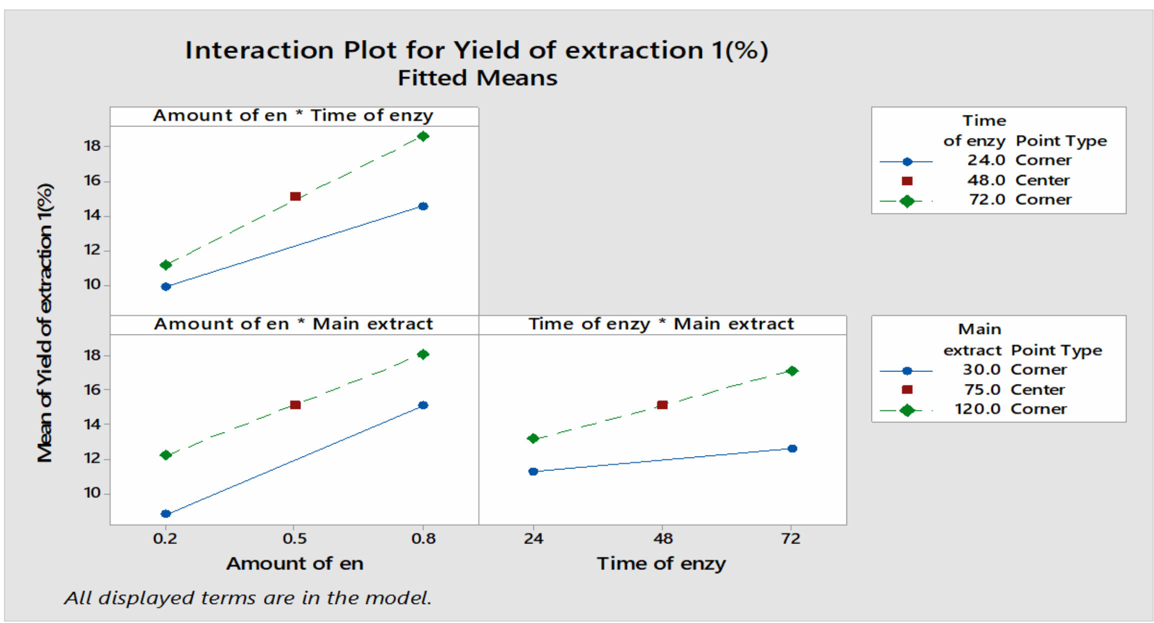


Figure 12: Interaction plot for yield of extraction 1

Figure 6,7 indicated the effect of amount of enzyme, time of enzyme treatment and main extraction time in that the amount of enzyme has significant with the yield of extraction while time of enzyme treatment and main extraction time has no much meaning with gelatine content after extraction. It also supports for regression model result as the amount of enzyme was the only factor effect on gelatine yield.

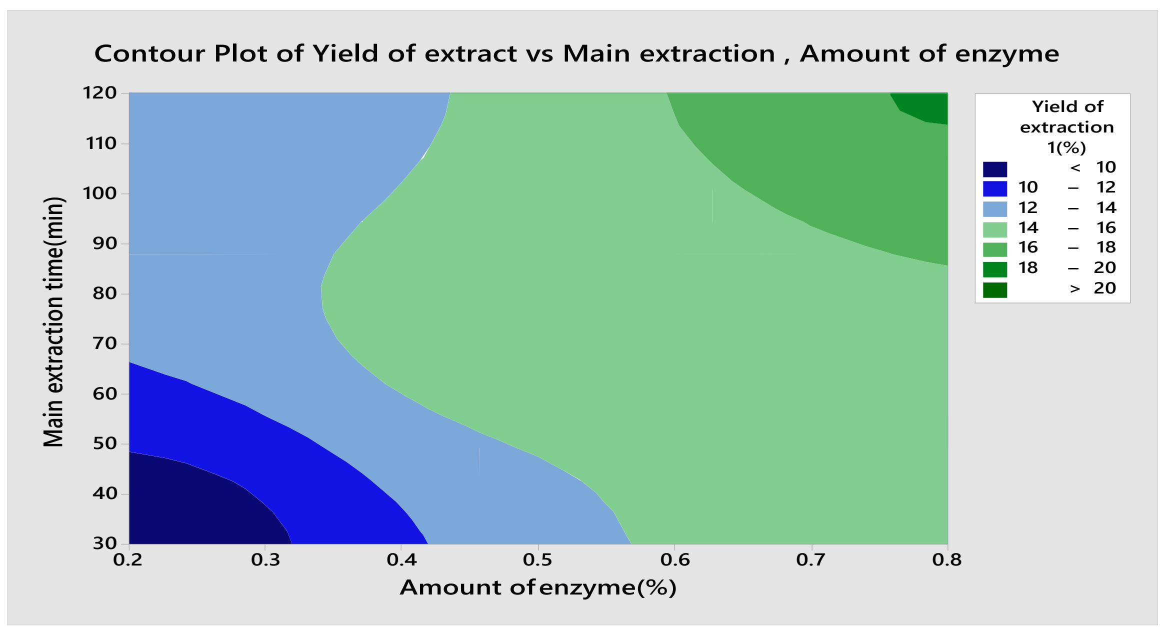


Figure 13: Contour plot of yield gelatine under effect of amount of enzyme and main extraction time

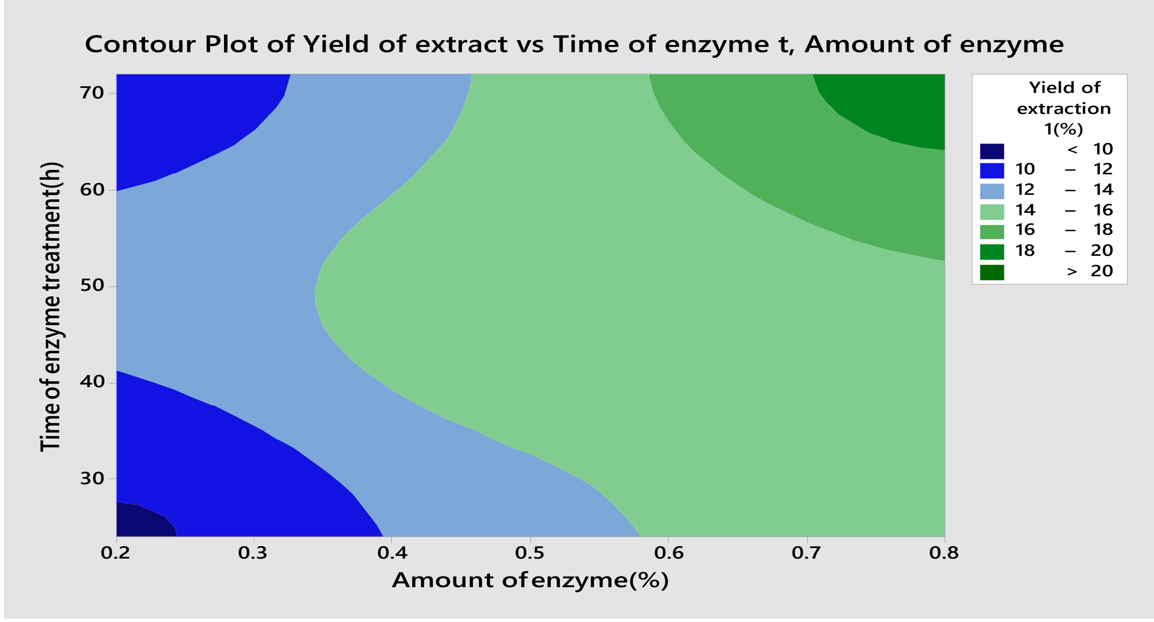


Figure 14: Contour plot of yield gelatine under effect of amount of enzyme and time of enzyme treatment

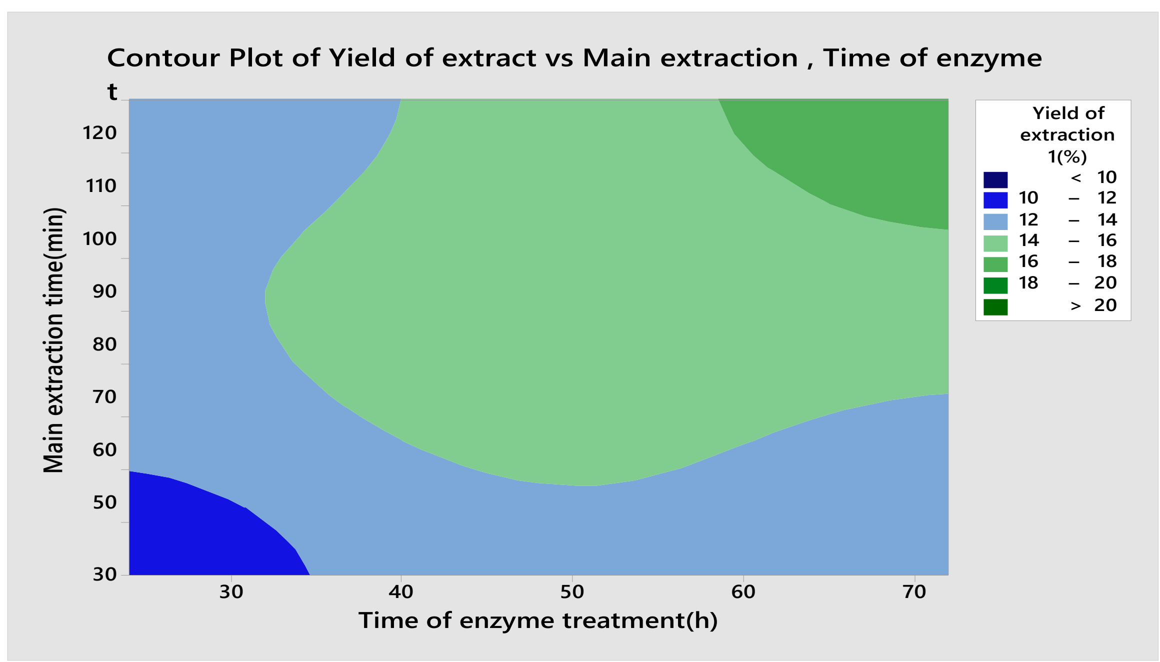


Figure 15: Contour plot of yield gelatine under effect of time of enzyme treatment and main extraction time

The extraction of gelatine was taken place under three factors amount of enzyme, time of enzyme treatment and extraction time of main extraction. Each of them had effect significantly on yield of gelatine. The response graphs showed (figure 13) an increase in gelatine yield on increase both the amount of enzyme and main extraction time, in figure 14,15 gelatine yield also increased on three of the concentration of enzyme and time of enzyme treatment and main extraction time, so the concentration of enzyme around 0,8%, time of enzyme treatment is greater than 70 hours and main extraction time up to 120°C are suitable for obtaining maximum yield of hen paws. Comparing to table 12, non-enzyme treatment has 18.69% in totally gelatine content while whole of samples added 0.8% enzyme have the yield which are greater than non-enzyme sample. Combining with regression evaluate Amount of enzyme play an important role to obtained the highly yield of extraction, it means the increasing or decreasing of time enzyme treatment and main extraction time had no much significant effect on the gelatine contain. This is cost effective in that no extra energy will be required for incubation time of enzyme for long periods time extraction.

After optimizing the yield of extraction, we did this experiment once again and compared the result with the first one to confirm that the optimization was significant.

Table 15: The comparison between the first experiment and repeat experiment.

Properties	**Yield of Extraction 1(%)**	**Yield of extraction 2(%)**	**Yield of gelatine totally(%)**
No.8	21.42	6.59	28.01
No.8 repeat	21.97	6.04	28.01

This table shows that there is no much difference between two experiments so we can conclude that the optimization of yield of extraction or amount of gelatine will be around 21.42%-21.97% at the conditions: 0.8% amount of enzyme treatment, 120h of main extraction and 72h time of enzyme treatment. Beside that the gel strength, viscosity and Ash of the no.8 and No. repeat are 328 and 345, 5.77 and 5.26, 1.7 and 2.1 respectively, we can also see that there is no big difference between them.

Comparison of the yield of gelatine at the optimal conditions (28.01%) and the yield of gelatine at the conditions without enzyme treatment (18.69%) for extraction indicated that using of enzyme had the positive influence on efficiency of extraction. In some case study of scientist, they found the number of amount of gelatine in animals and each of them contributed on the research works. The Comparison of yield of gelatine on this thesis and the result of previous studies showed that according to Norizah they found yield of gelatine in dried chicken skin about 16% was lower (12.01%) compared to extracted hen paws gelatine, the result indicated that the extraction procedure using enzyme treatment may be optimal for gelatine extraction [17]. Comparing to L.Du they also used alkaline-pre-treatment to obtain pure collagen, then extraction took place under conditions of first extraction at 50°C for 18h and then second extraction at 60°C for 6h, the yield of gelatine obtained totally of chicken head and turkey head are 52.29% and 62.76%, these yield of gelatine are pretty higher than hen paws [18], it is maybe due to protein content of hen paws is lower than chicken head and turkey head. It concludes that the yield of gelatine is not only affected by enzyme, time and temperature of extraction but also the source of raw materials.

6.2 Effect of different factors on gel strength of gelatine

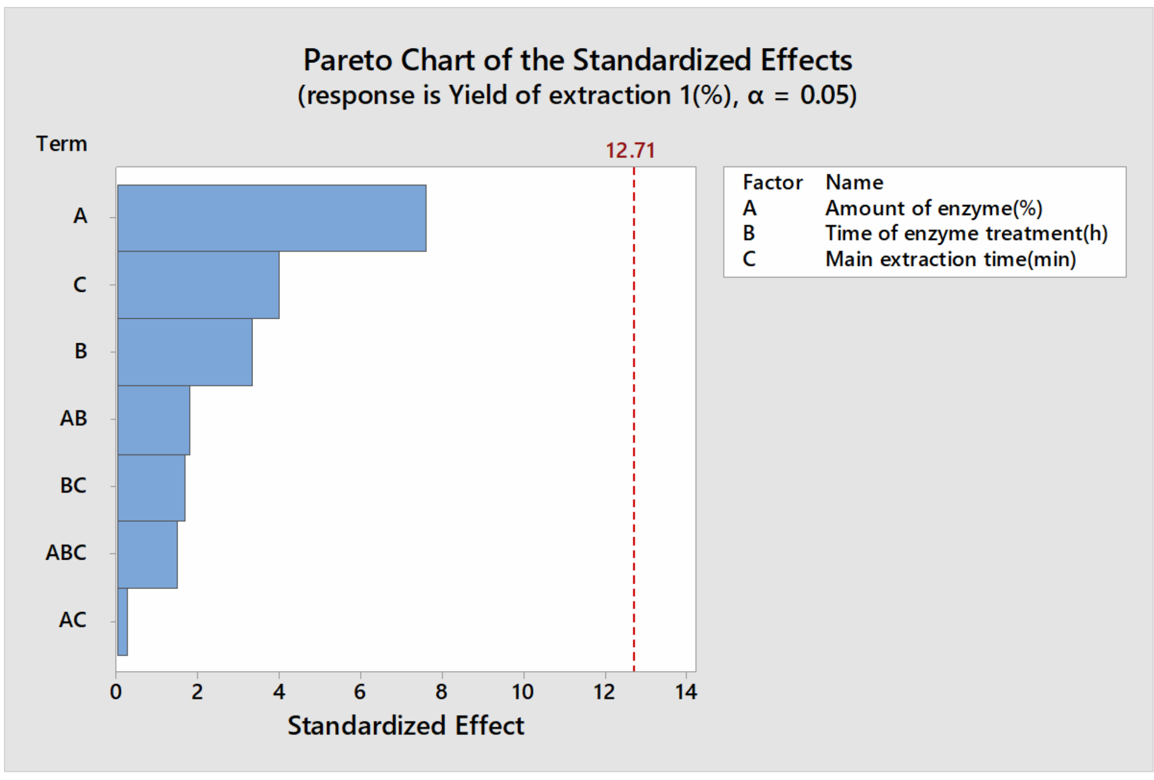


Figure 16: Pareto chart of standardized effects on gel strength

Table 16: Parameter estimates for percent gel strength of hen paws obtained by regression analysis

Coefficients	T-Value	P-Value	VIF
X_1	2.29	0.149^{ns}	1.00
X_2	1.39	0.300^{ns}	1.00
X_3	-2.53	0.127^{ns}	1.00
X_1* X_2	0.10	0.933^{ns}	1.00
X_1* X_3	-0.43	0.709^{ns}	1.00
X_2* X_3	-0.48	0.68^{ns}	1.00
X_1* X_2* X_3	-0.62	0.598^{ns}	1.00

$^{ns:}$ Not significant at P < 95%. other coefficients were significant at P < 95%

Where: X_1 Amount of enzyme

X_2 Time of enzyme treatment

X_3 Main extraction time

The analysis for the response of gel strength using analyse factorial design (table 16) was significant at P<0.1 with R^2=87.8% there is no P less than 0,1, it means there is no effect of any factor on gel strength. The coefficient of gel strength of hen paws was obtained by the

regression, the insignificant coefficient was dropped from the model which was presented on the table, and the graph showed how it affected on gel strength of three factors presented in the figure 16, the equation used in generating the three dimensional response are not significant in this case

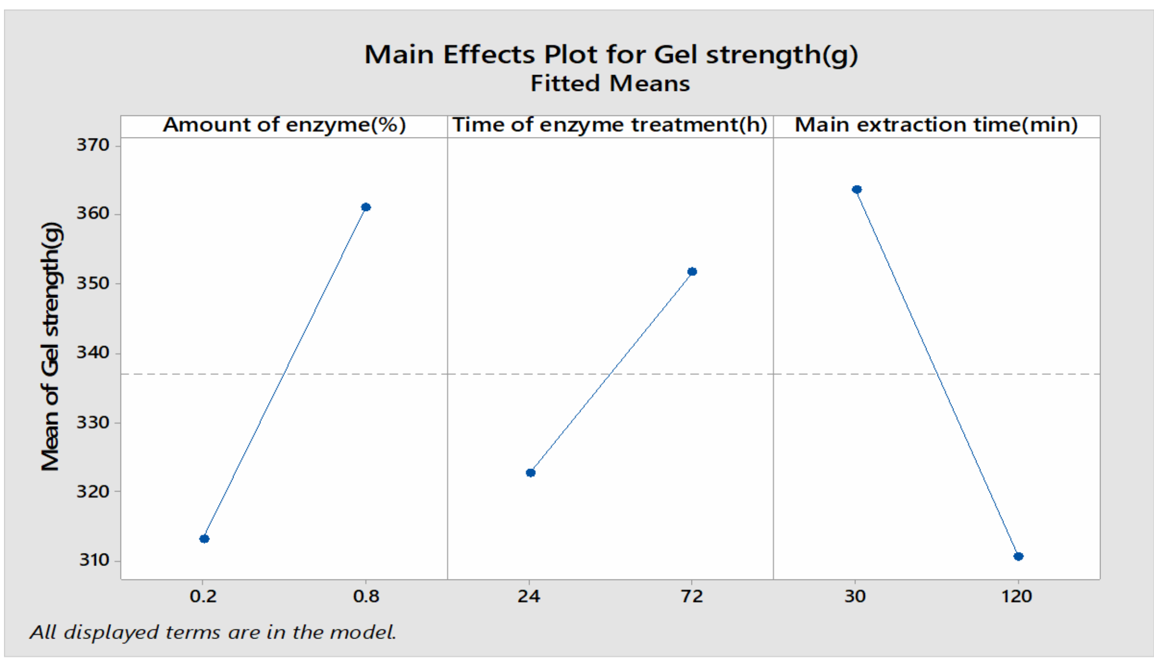


Figure 17: Main effects plot for gel strength

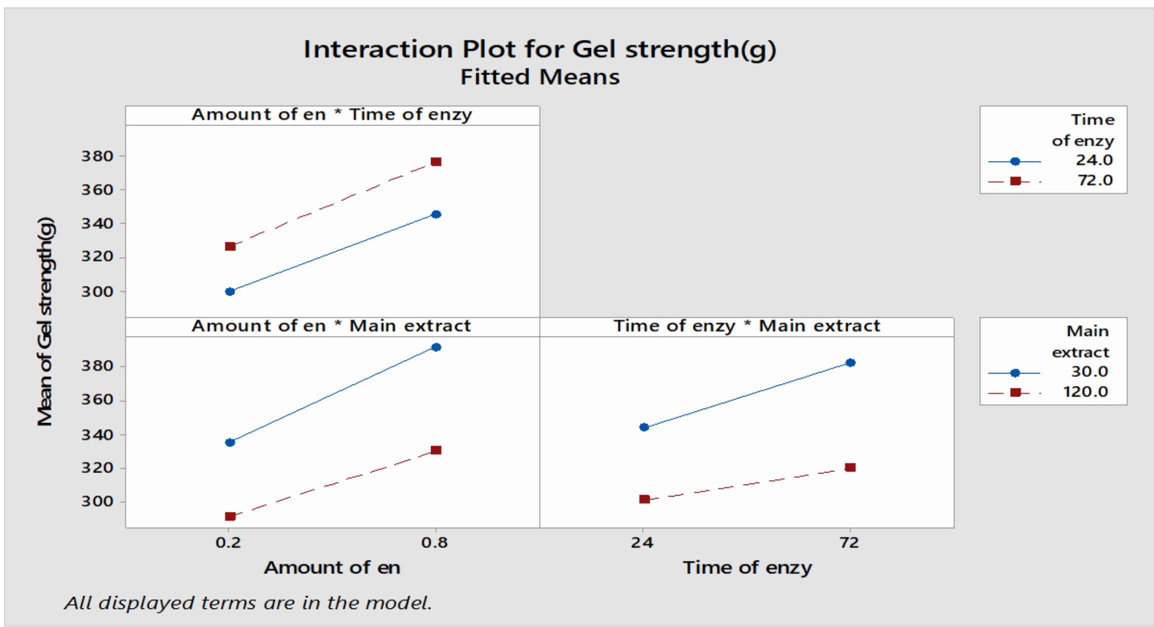


Figure 18: Interaction plot for gel strength

Figure 17,18 pointed out the effect of amount of enzyme, time of enzyme treatment and main extraction time in that the amount of enzyme has significant with the gel strength independently, while the gel strength increased with increased both amount of enzyme and time

of enzyme treatment but decreased when increased the main extraction time. When it was combined together the result found there is no factor which has a positive influence on the gel strength of gelatine, it means without conditions we can obtain the same result of gel strength.

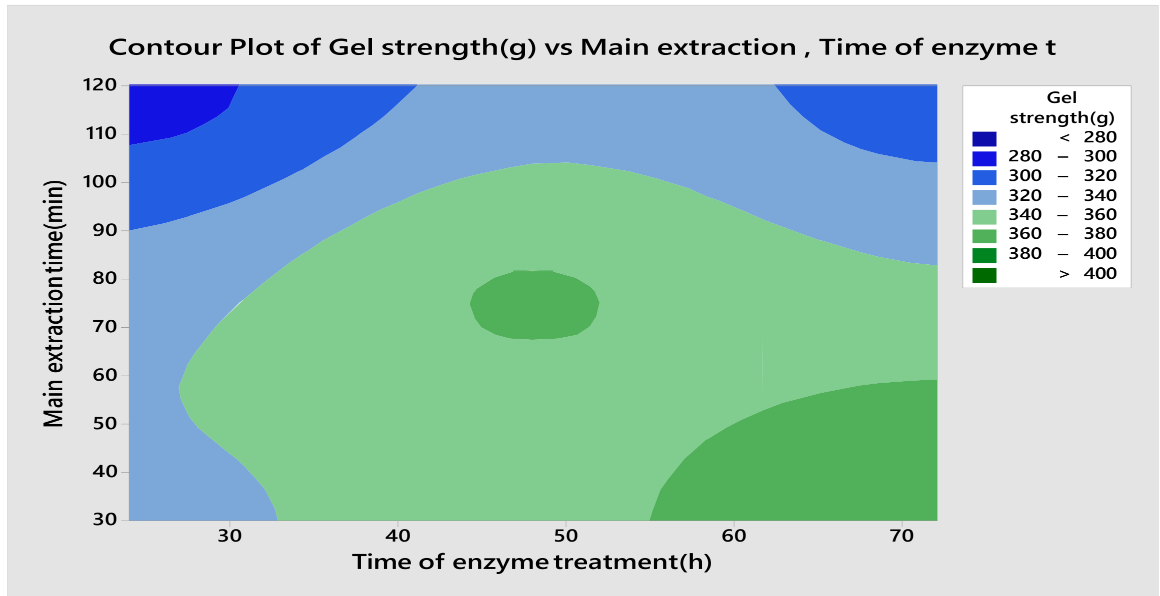


Figure 19: Contour plot of gel strength with main extraction, time of enzyme treatment

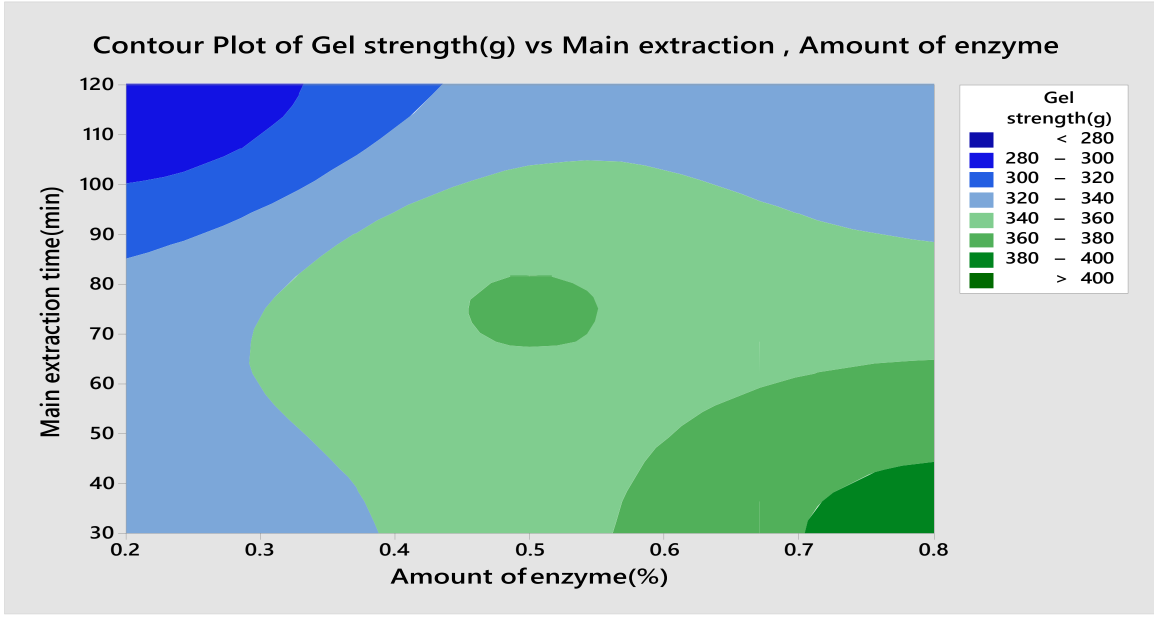


Figure 20: Contour Plot of gel strength of gel strength with Main extraction, amount of enzyme treatment

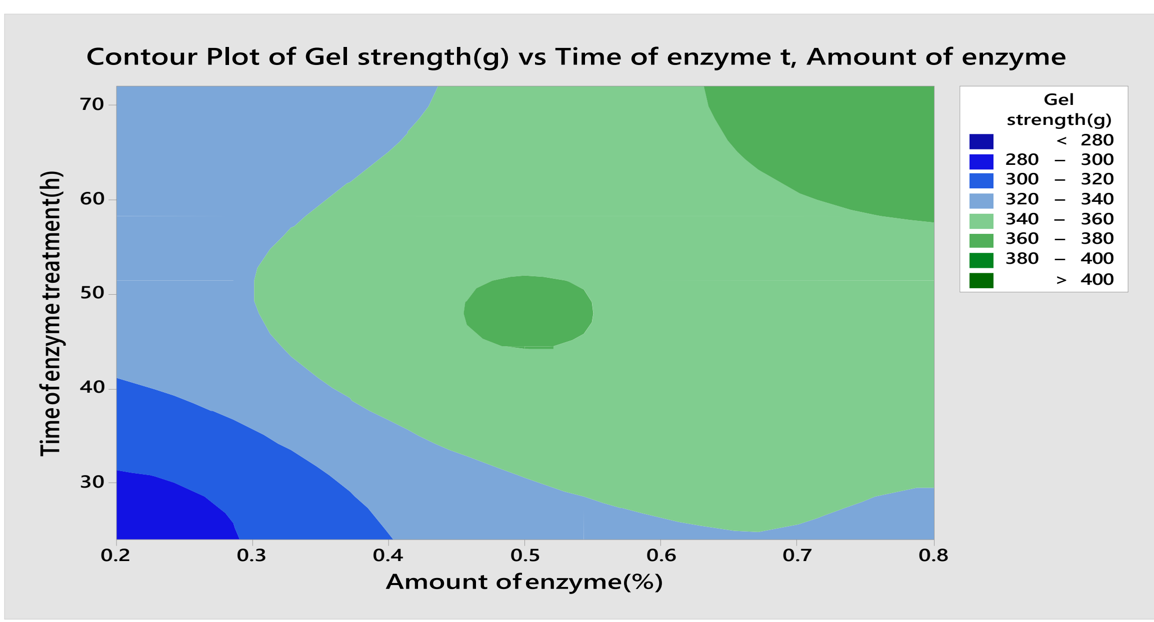


Figure 21: Contour plot of gel strength with time of enzyme treatment, amount of enzyme

Under three factors amount of enzyme, time of enzyme treatment and extraction time of main extraction we did extract gelatine from hen paws. Each of them had effect significantly on yield of gelatine. The response graphs showed (figure 19) the increase of the amount of enzyme and decrease of main extraction time lead to the highest gel strength, in figure 20 shows that the gel strength also increased on the increase of concentration of enzyme and time of enzyme treatment, so the concentration of enzyme around 0,8%, time of enzyme treatment is greater than 70 hours and main extraction time around 30°C are suitable for obtaining maximum gel strength of hen paws. Comparing to table, non-enzyme treatment has 340 g it is quite similar with samples which extracted under conditions. Combining with regression evaluate there is no factor which has positive effect on gel strength, it means the increasing or decreasing of time enzyme treatment and main extraction time and amount of enzyme had no much significant influence on the gel strength. This is cost effective in that no extra energy will be required for using enzyme, incubation time of enzyme for long periods time extraction.

After optimizing the gel strength, we did this experiment once again and compared the result with the first one to confirm also that the optimization was significant.

Table 17: The comparison between the first experiment and repeat experiment.

Properties	Yield of Extraction 1(%)	Gel strength(g)	Viscosity (mPa.S)	Ash(%)
No.7	15.93	413	5.26	1.6
No.7 repeat	15.38	402	5.26	1.5

In This table we can see there is no difference between two experiments so it led to the conclusion that the optimization of gel strength or amount of gelatine will be around 402-413 g at the conditions: 0.8% amount of enzyme treatment, 30 h of main extraction and 72h time of enzyme treatment.

6.3 Effect of different factors on ash

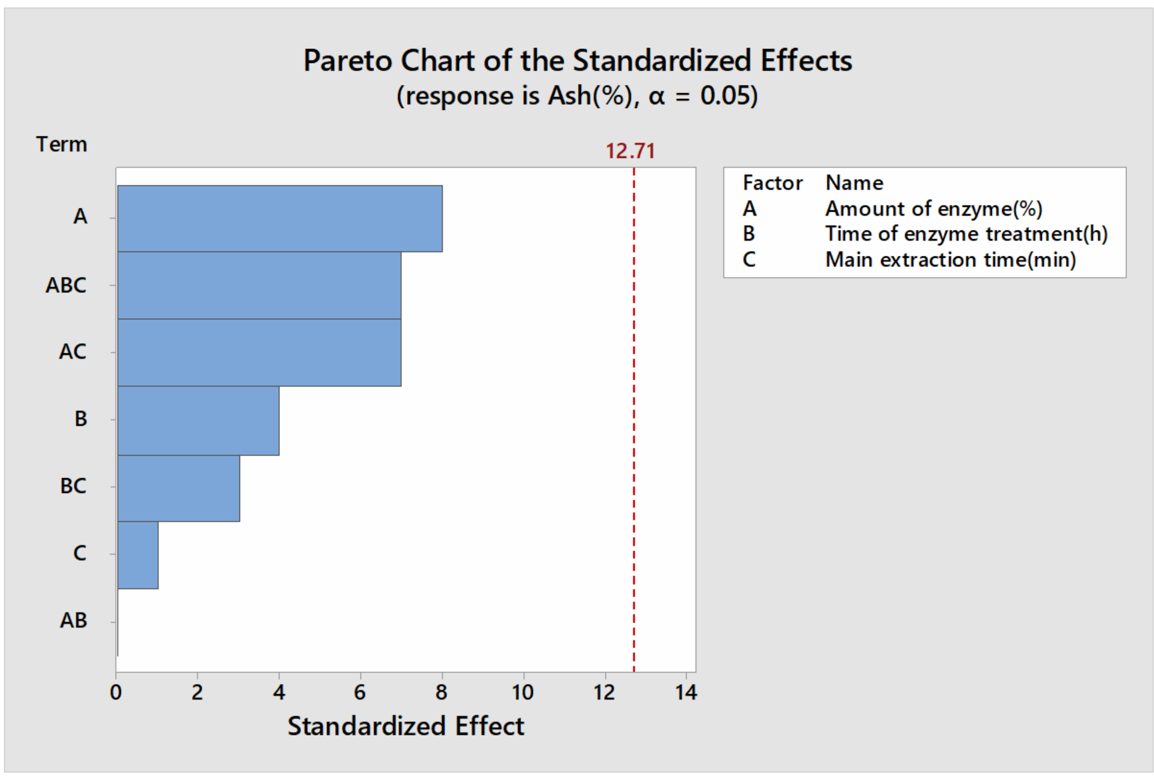


Figure 22: Pareto chart of standardized effects on the ash

Table 18: Parameter estimates for percent the ash of hen paws obtained by regression analysis

Coefficients	T-Value	P-Value	VIF
X_1	-3.05	0.093	1.00
X_2	-1.52	0.267^{ns}	1.00
X_3	-0.38	0.740^{ns}	1.00
$X_1 * X_2$	0.00	1.000^{ns}	1.00
$X_1 * X_3$	-2.66	0.117^{ns}	1.00
$X_2 * X_3$	1.14	0.372^{ns}	1.00
$X_1 * X_2 * X_3$	2.66	0.117^{ns}	1.00

$^{ns:}$ Not significant at P < 95%. other coefficients were significant at P < 95%

where: X_1 Amount of enzyme

X_2 Time of enzyme treatment

X_3 Main extraction time

The analysis for the response of yield of gelatine using analyse factorial design (table18) was significant at P<0.1 with R^2=93.16% which P = 0,093 for amount of enzyme, it means there is the influence of amount of enzyme on ash. The coefficient of ash content of hen paws was obtained by the regression, the insignificant coefficient was dropped from the model which was presented on the table, and the graph showed how it is effected on ash of three factors presented in the figure 22, the equation used in generating the three dimensional response are as follows:

$$Ash = 1.317 + 2.250 X_1$$

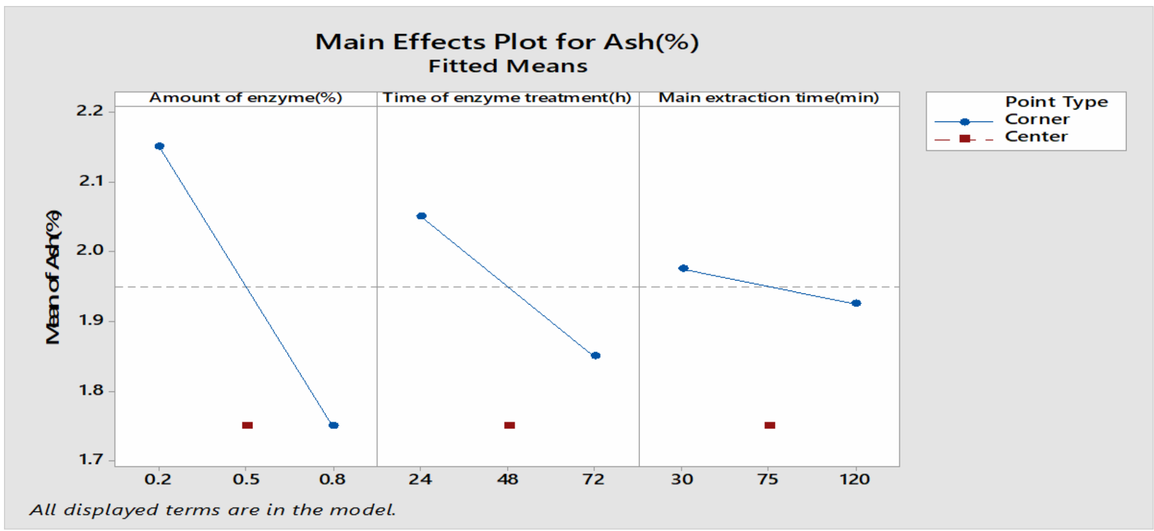


Figure 23: Main effects plot for the ash

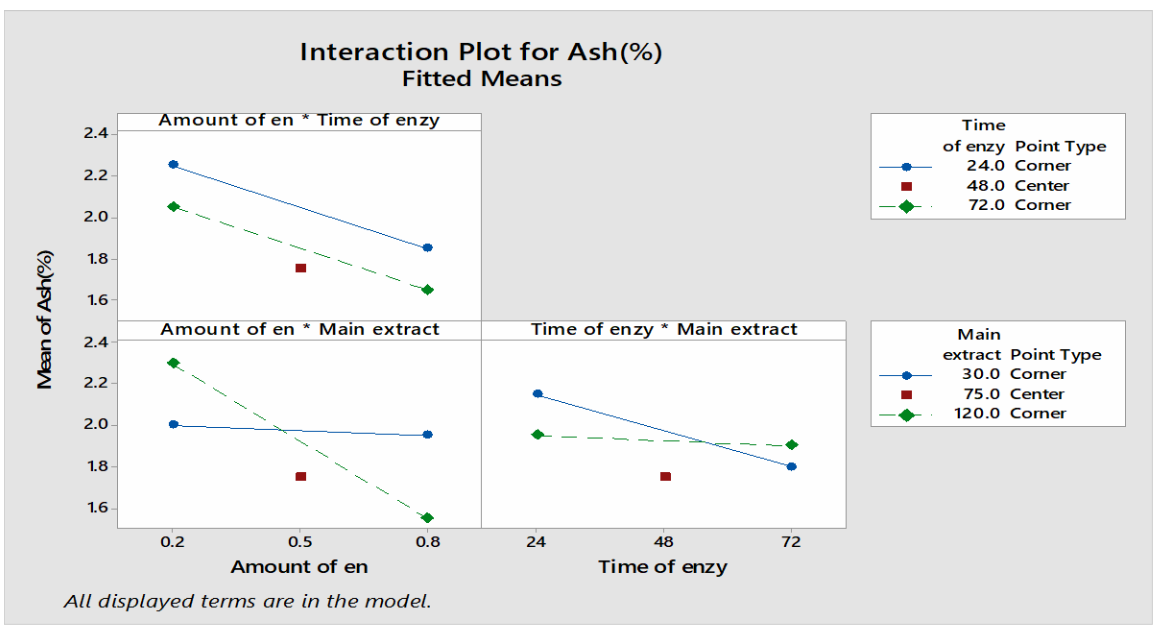


Figure 24: Interaction plot for the Ash

Figure 23, 24 show that the main factor effect on the ash is amount of enzyme, it tends to decrease when increasing the amount of enzyme while an influence of the time of enzyme treatment and main extraction time are not that much on the ash.

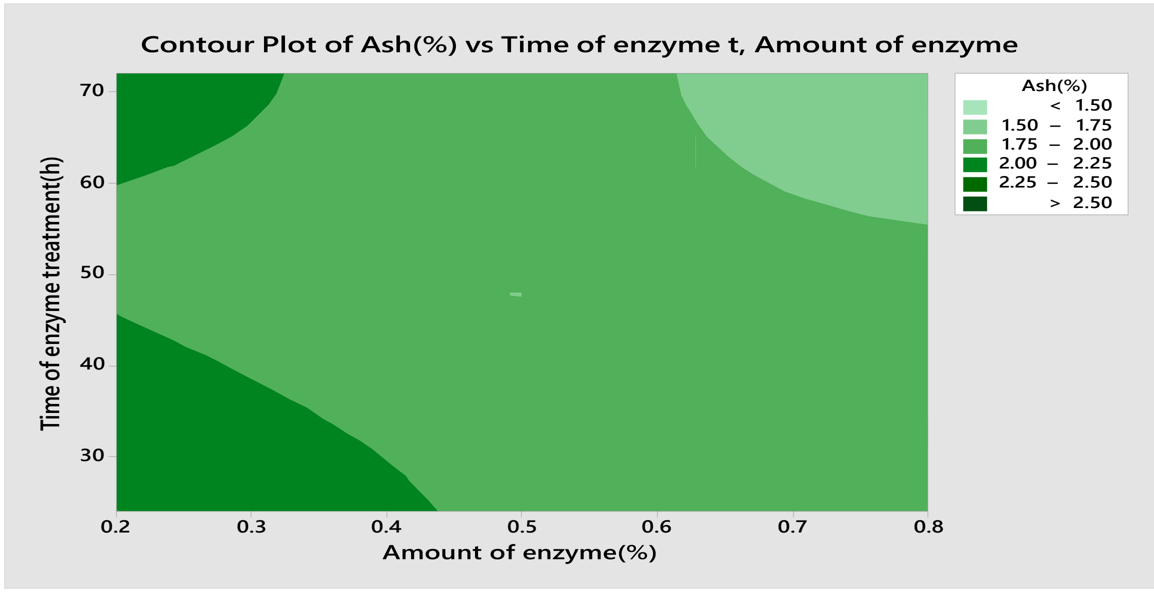


Figure 25: Contour plot of ash (%) with time of enzyme treatment, amount of enzyme

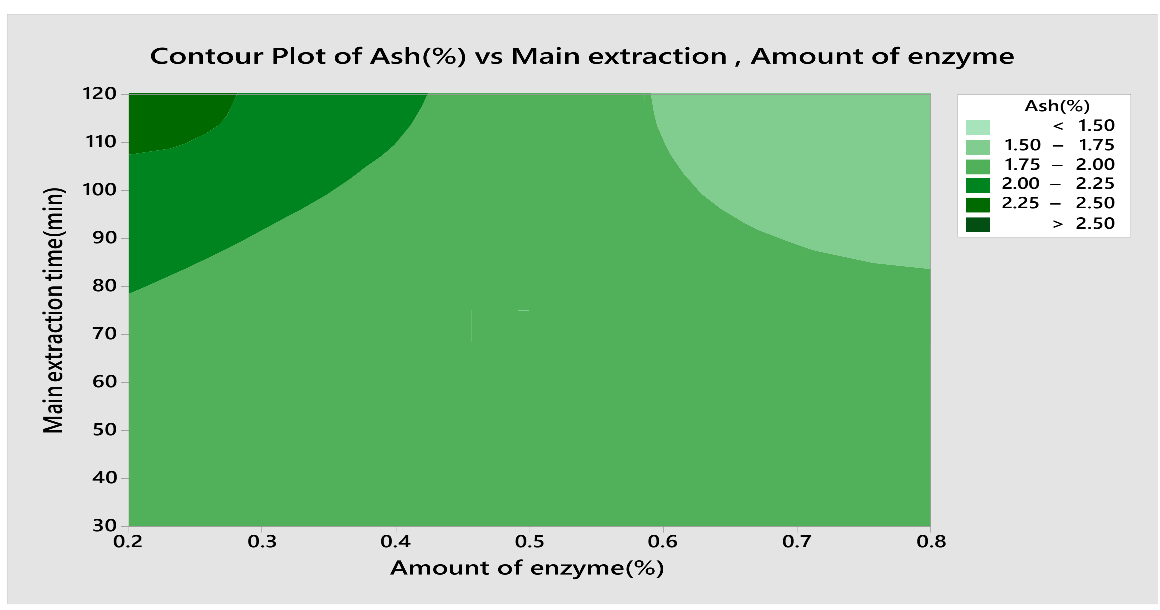


Figure 26: Contour plot of ash (%) with main extraction, amount of enzyme

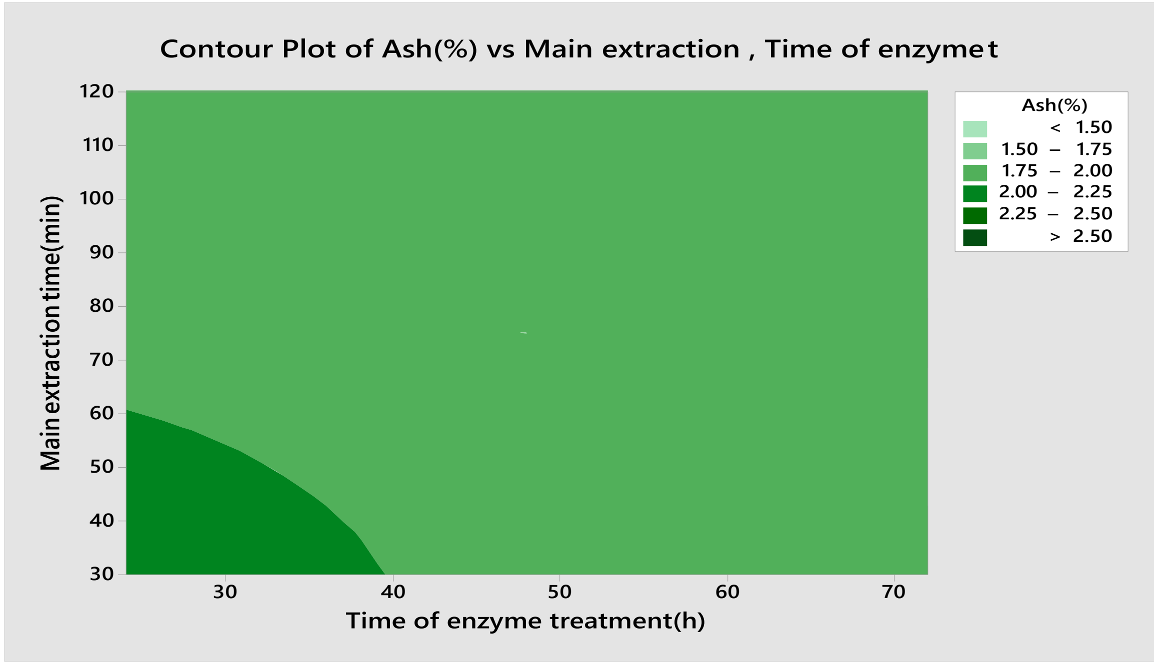


Figure 27: Contour plot of ash (%) with main extraction, time of enzyme treatment

In the industry of producing gelatine from animal the ash is one of the factor that directly effect on the quality of gelatine, the lower the ash on gelatine the greater gelatine has, figure 25,26,27 show the ash under conditions, it was showed that at the higher of all conditions the ash will be lowest, at amount of enzyme 0.8%, extraction time 120h and time of enzyme treatment 72h the ash will be lower 1.5% and according to gelatin handbook the gelatine should be lower 2% will be better for application in food, cosmetic or photography industry [23].

6.4 Effect of different factors on viscosity

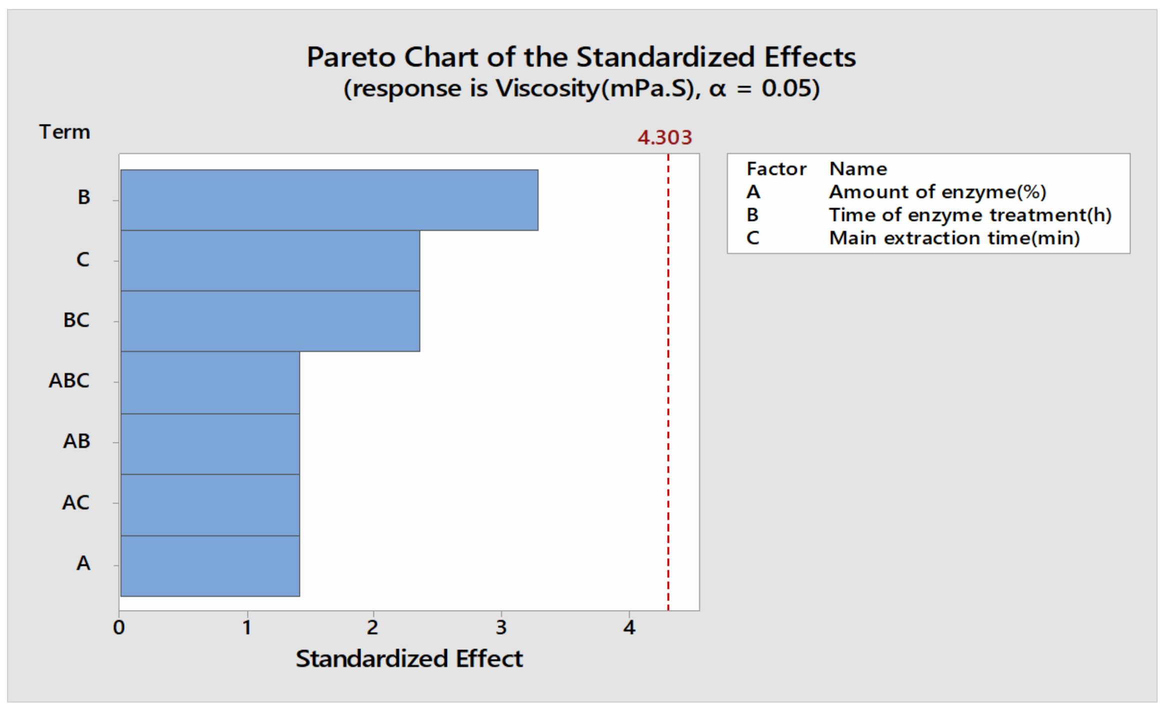


Figure 28: Pareto chart of standardized effects on viscosity

Table 19: Parameter estimates for percent viscosity of hen paws obtained by regression analysis

Coefficients	T-Value	P-Value	VIF
X_1	1.41	0.293^{ns}	1.00
X_2	-3.30	0.081	1.00
X_3	2.36	0.143^{ns}	1.00
X_1* X_2	-1.41	0.293^{ns}	1.00
X_1* X_3	-1.41	0.293^{ns}	1.00
X_2* X_3	-2.36	0.143^{ns}	1.00
X_1* X_2* X_3	1.41	0.293^{ns}	1.00

$^{ns:}$ Not significant at P < 95%. other coefficients were significant at P < 95%

Where: X_1 Amount of enzyme

X_2 Time of enzyme treatment

X_3 Main extraction time

The finding for the response of viscosity using analyse factorial design (table 28) was significant at P<0.1 with R^2=93.16% which P = 0,081 for amount of enzyme, it means there is the positive relative of time of enzyme treatment on viscosity. The coefficient of viscosity of hen paws was obtained by the regression, the insignificant coefficient was dropped from

the model which was presented on the table, and the graph showed how it is effected on viscosity of three factors presented in the figure 28, the equation used in generating the three dimensional response are as follows:

$$\text{Viscosity} = 3.11 + 0.0382\ X_2$$

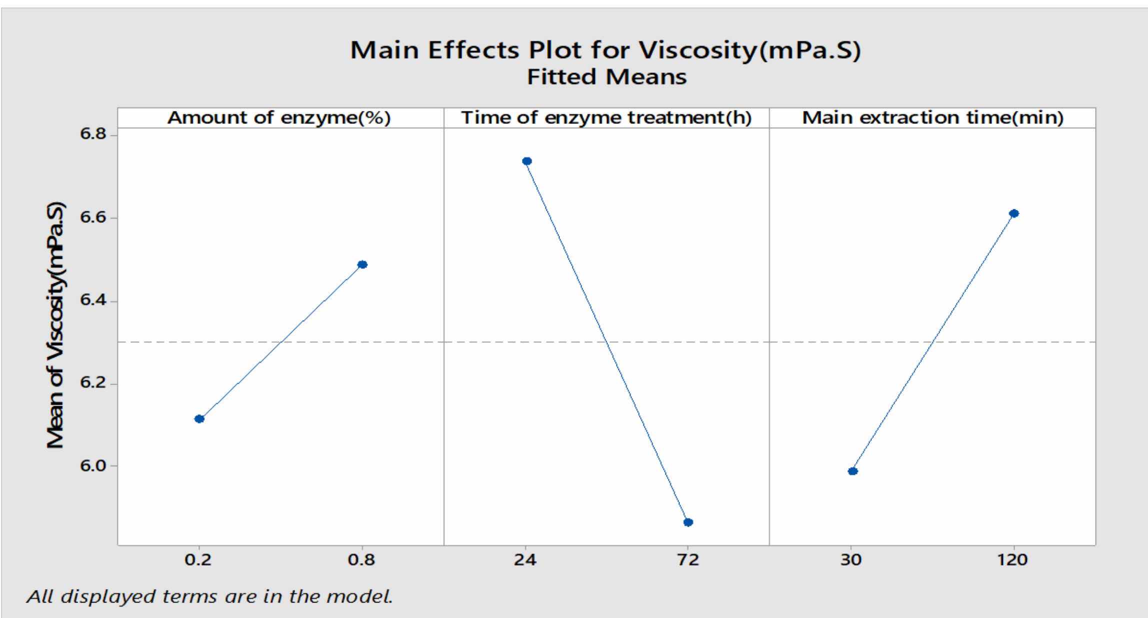


Figure 29: Main effects plot for viscosity (mPa.S)

Figure 29,30 indicated the main factor effect on the viscosity is time of enzyme treatment, the amount of enzyme and the main extraction also influence on the viscosity but the impact all of them independently, while the increasing of the amount of enzyme and main extraction time led to the increasing of the viscosity, the viscosity tends to decrease when increase the time of enzyme treatment.

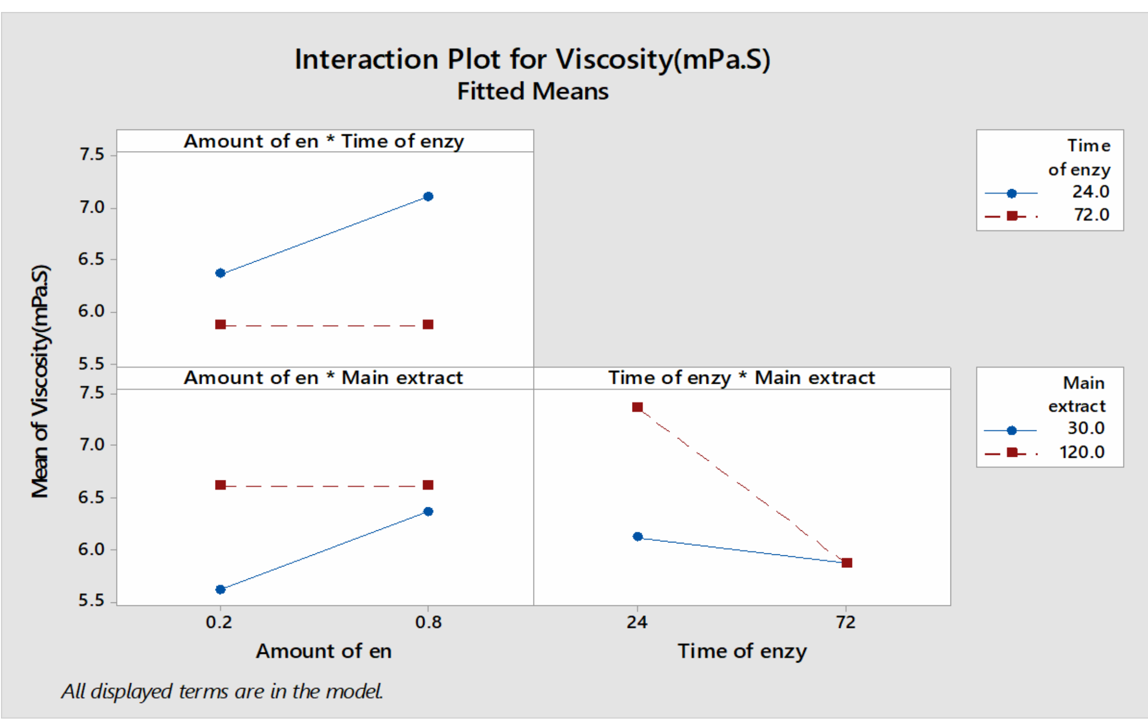


Figure 30: Interaction plot for Viscosity (mPa.S)

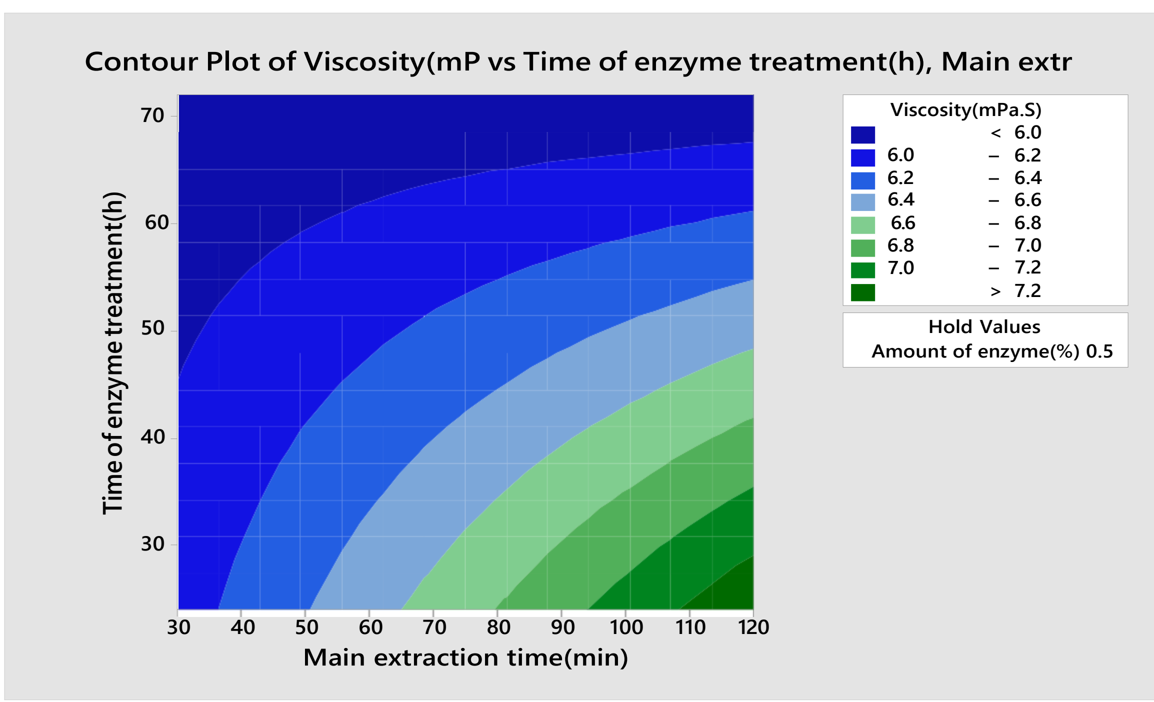


Figure 31: Contour plot of Viscosity (mPa.S) with time of enzyme treatment, main extraction

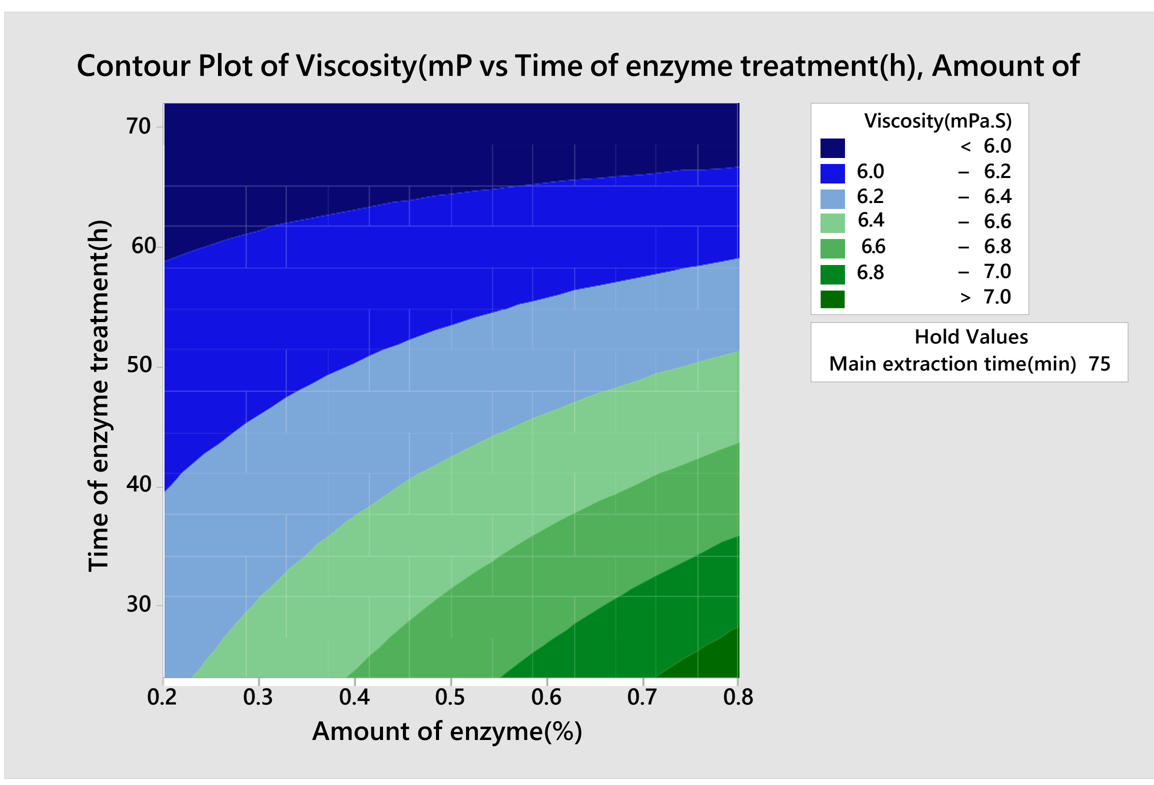


Figure 32: Contour plot of viscosity (mPa.S) with time of enzyme treatment(h), amount of enzyme

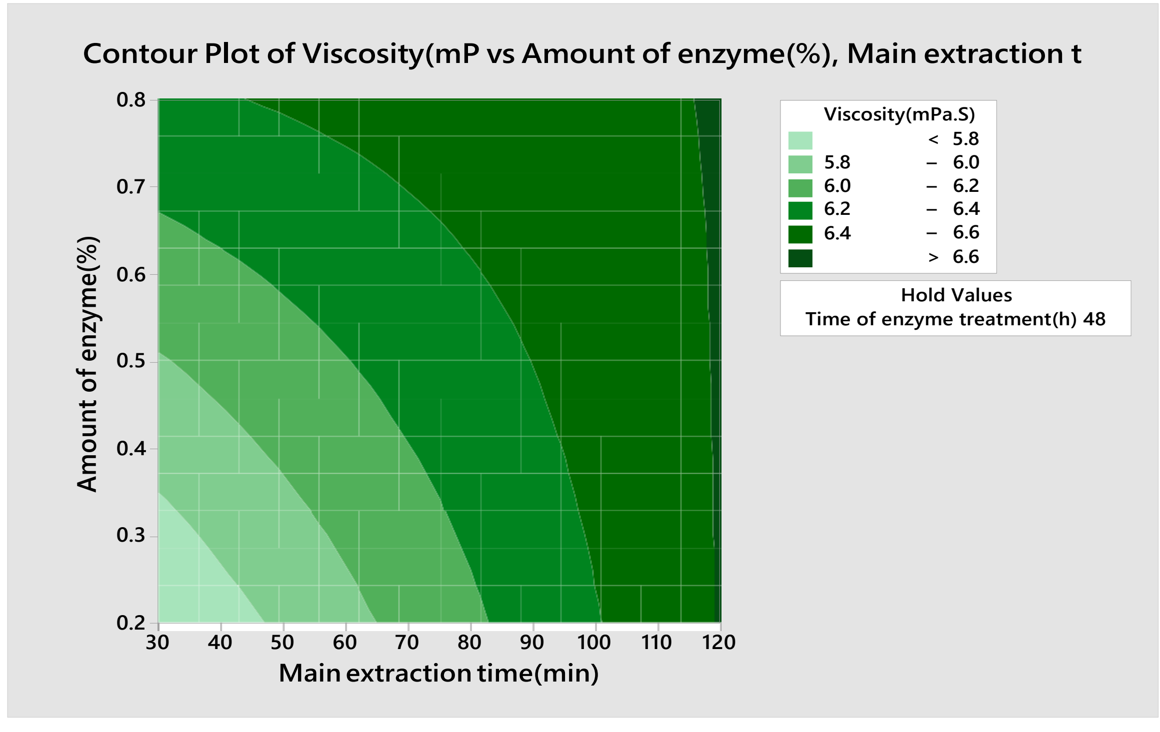


Figure 33: Contour plot of viscosity (mPa.S) with amount of enzyme (%), main extraction treatment

The finding showed that at the time of enzyme treatment is from 60h to 72h and at the amount of enzyme 0.2-0.4%, main extraction time from 30 to 60h the viscosity of gelatine will be less than 6 mPa.S, at the conditions of amount of enzyme 0.6-0.8%, main extraction time around 120h and time of enzyme treatment less than 30h the viscosity of gelatine will be greater than 7.0 mPa.S, And all others area of the conditions of the amount of enzyme, main extraction time and time of enzyme treatment respond the viscosity around 6.0 to 7.0 mPa.S.

6.5 Properties of hen paws gelatine

6.5.1 Properties of gelatine of extraction 1

Table 20: Properties of gelatine after first extraction of samples

No.	Gel strength (g)	Viscosity (mPa.S)	Clarity	Dry matter (%)	Ash(%)	pH
1	317	5.26	1.1	92	1.97	8.9
2	270	7.34	0.8	93.7	2.5	9.05
3	341	5.77	1.8	93.6	2	9.28
4	300	5.77	1.1	93.5	2.13	9.12
5	359	6.8	0.8	92.4	2.3	9.36
6	320	7.34	0.7	94.7	1.4	9.07
7	413	5.77	1.4	94.6	1.6	8.87
8	328	5.77	0.7	94.4	1.7	8.7
9	373	8.36	1.1	94.1	1.7	8.96
10	350	6.3	0.7	94.2	1.8	8.96
11	376	7.83	0.8	94.2	1.9	9
Average	340.6	6.6	1.0	93.8	1.9	9.0

The result found that after first extraction we did obtain gelatine with expected properties, according to GMIA [23], the ash should be lower than 2%, comparing to the average result the ash was 1.9% and it was a good finding. The gel strength of commercial gelatine should be around 50-300g, the average of gelatine after first extraction is a bit higher (340,6g) the result of gel strength depends on concentration, pH, temperature, or the presence of any additive. The differences among these gelatines in gel strength could be due to the intrinsic characteristics of protein like the molecular mass weight, amino acid content, type of extraction treatment or also the stability of triple- helix structure of collagen through hydrogen bonding regarding to Norziah [22]. Gel strength of chicken gelatine (6.67% (w/v) has value

355g compared with bovine gelatine (229g). According to Norizah compared to other alternative sources like fish gelatine, chicken gelatine showed higher gel strength than for tilapia (181 g and 263 g), horse mackerel (280g), sin croaker and shortfin scad (125 and 177 g, respectively) [17]. Comparing with the result of L. Du [18] the gel strength of gelatine after first extraction of chicken head and turkey head are 247.9g and 368.4 respectively. The ash of hen paws gelatine is 1.9% lower than 2% it means this value has significant in industry. And at this topic we also found the average of viscosity, clarify, pH was around 6.6 mPa.S, 1.0, 9.0 respectively. The dry matter of collagen should be higher 90% and the result responded as the expectation.

6.5.2 Properties of gelatine of extraction 2

Due to the amount of yield of gelatines on extraction 2 were very low so the combination of experiments was used at the similar extraction temperature 2 to evaluate the properties of gelatines, the results showed in the table below:

Table 21: Properties of gelatine after second extraction of samples

No.	Gel strength (g)	Viscosity (mPa.S)	Ash(%)	pH
1	196	3.65	2.6	8.34
2				
3				
4				
5	224	4.72	2.8	9.44
6				
7				
8				
9				
10	286	5.77	2.5	8.58
11				
Average	235	4.71	2.63	8.8

Table 21 points out at the similar extraction temperature it has different properties of gel strength, viscosity, the ash and pH, at the temperature 90°C we found the highest gel strength of gelatine comparing to gel strength at 95-100°C and 80-85°C are around 196 and 224 respectively, it could may be the temperature of extraction effected on the properties of hen paws gelatines. The average gel strength was 235g, it is lower than gel strength of gelatine extraction 1, the viscosity, the ash and pH are 4.71, 2.63 and 8.8 respectively.

6.5.3 DSC analysis of 3 levels of experiments

The Differential scanning calorimetry was used to observe the melting behaviour of hen paws gel gelatines samples

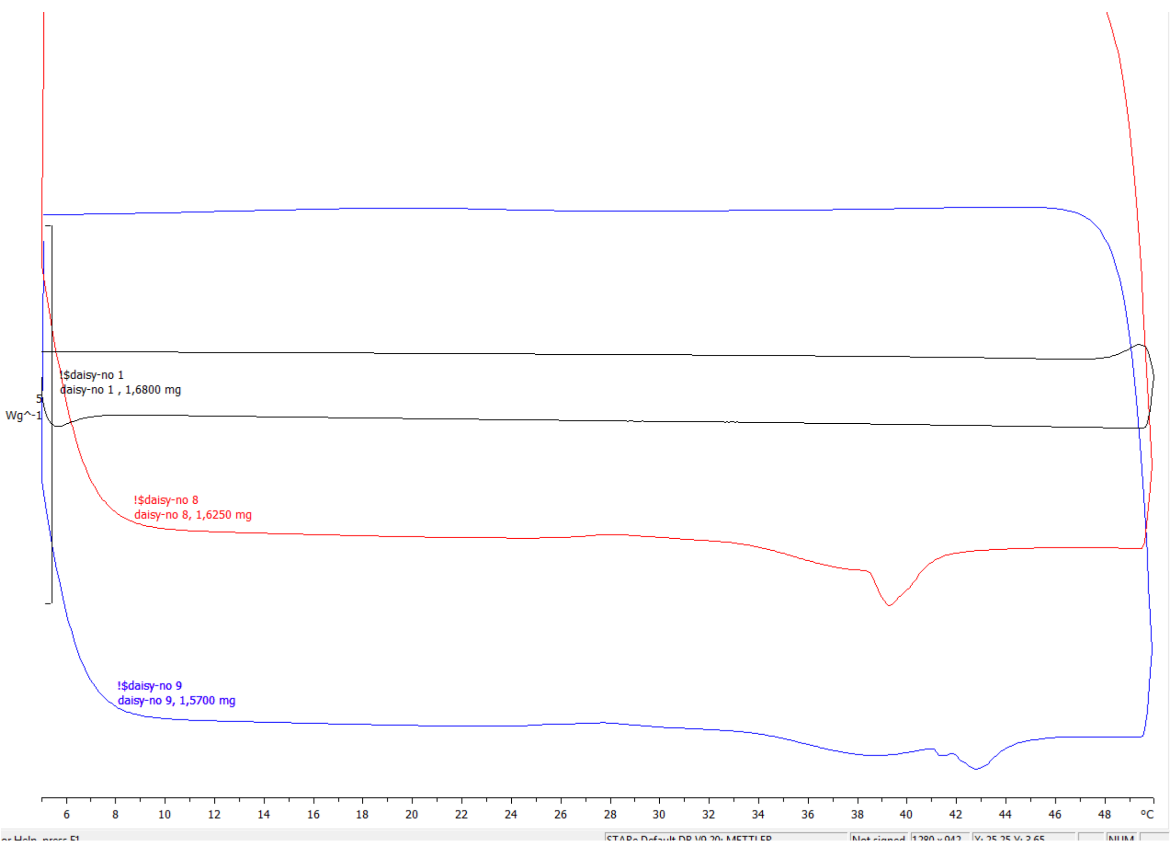


Figure 34: Melting temperature of three differently levels of experiment by using DSC analyser

The finding points out that the melting temperature of 3 samples has the difference, the experiment at the conditions 0.2% amount of enzyme treatment, 24h time of enzyme treatment and 30 minutes of main extraction we did see any melting temperature peak but at the optimal conditions we mentioned above the melt temperature peak reached at 39ºC and at the central conditions 0.5% amount of enzyme treatment, 48h time of enzyme treatment and 75ºC the melting temperature were a bit higher (43ºC). It means the structural stability of the centre sample is greater than two other experiments.

It seems that the amount of enzyme, time of enzyme treatment and main extraction time affected on melting peak of temperature and it could be because of technical errors so we didn't obtained any peak of melting temperature of sample at conditions number 1.

CONCLUSION

This master thesis accomplished the extraction of gelatine from hen paws under conditions of amount of enzyme treatment, time of enzyme treatment and main extraction time, then optimising the yield of gelatine and gel strength by using Minitab software, the plots were responding at the condition of 0.8% amount of enzyme treatment, 72h time of enzyme treatment and 120 minutes of main time extraction we did obtain the highest yield of gelatine (28.01% in total after first and second extraction), at this optimal conditions, its properties were also analysed with 328 g gel strength, 5.77 mPa.s of viscosity, 0,7 clarify value, pH 8.7 and the ash was about 1.7%, these properties were quite suitable for gelatine standard and in industry. This optimum object was done once again with the yield of gelatine was about 28.01% in total and gel strength (402 g) to make sure that it was virtual valuable.

Similarly, at the conditions 0.8% amount of enzyme treatment, 72h time of enzyme treatment and 30 minutes of main time extraction, the gel strength of gelatine was maximum (413g), and after repeating once again this experiment we obtained the gel strength of gelatine was about 402 g and it is not big different with the result of the first experiment. Thus, its properties were also analysis following to standard and made the comparison with the commercial gelatines.

Firstly, we did extract collagen from hen paws by using enzyme lipolase by several steps of pre-treatment which used NaOH 0.03M, after removing un-wanted substance, non-proteins and fat, we obtained pure collagen.

Secondly, we used enzyme protemax to gain efficiency of extraction gelatine from hen paws, the concentration of enzyme 0.2%, 0.5%, and 0.8% at three level 24h, 48h and 72h of time of enzyme treatment and at factor main time extraction were at 30min, 75min and 120min which were composite rotatable designed into eleven experiments. After extraction, we did obtain the yield of gelatines after extraction 1 and extraction 2, and then gelatines were stored at the shelf of laboratory for next steps of analysing its properties.

Finally, gelatines were used to analysis of some properties include viscosity, ash, clarify, pH, dry matter, and gel strength. After analysing all its properties, we used Minitab software to optimise the yield of gelatine and gel strength which were mentioned above and made the comparison with others publication to make the conclusion and have more experience for next study.

REFERENCES

[1] NORIZAH MHD SARBON 1, FARAH BADII, NAZLIN K. HOWELL.: Preparation and characterisation of chicken skin gelatine as an alternative to mammalian gelatine, Food Hydrocolloids, UK, 2012, doi:10.1016/ j.foodhyd.2012.05.009.

[2] PAVEL MOKREJS, ROBERT GAL , DAGMAR JANACOVA , MÁRIA PLSKOVA 2 and MICHAELA ZACHAROVA.: Chicken Paws By-products as an Alternative Source of Proteins, Oriental Journal Of Chemistry, 2017, Vol. 33, No.(5), ISSN: 0970-020 X.

[3] DANISH MEAT RESEARCH INSTITUTE.: Rentek Database – Kødbranchen. Slag-teriernes for skinings institute (Danish Meat Research Institute). Denmark, 1995.

[4] FINNISH ENVIRONMENT INSTITUTE.: Finnish expert report on best available techniques in slaughterhouses and installations for the disposal or recycling of animal car-casses and animal waste. Report 539. Finnish Environment Institute. Helsinki, Finland, 2002.

[5] IRSHAD A.: Abattoir by-Product Utilization for Sustainable Meat Industry: A Review, J Anim Pro Adv 2015, 5(6): 681-696, DOI: 10.5455/japa.20150626043918, ISSN: 2251-7677.

[6] OLE PONTOPPIDAN AND MIRKO MISELJIC.: Slaughterhouses and producers of Animal By-products in the Nordic Countries, Tema Nord 2016:551, ISSN 0908-6692

[7] K. JAYATHILAKAN, KHUDSIA SULTANA, K. RADHAKRISHNA, AND A. S. BAWA.: Utilization of by-products and waste materials from meat, poultry and fish pro-cessing industries: a review, 2011 Febuary 20th, Doi: 10.1007/s13197-011-0290-7

[8] GELATIN MANUFACTURERS INSTITUTE OF AMERICA, STANDARD TESTING METHODS FOR EDIBLE GELATIN, July 2013.

[9] NEWS AGRICULTURE FORESTRY SEAFOOD OF MINISTRY OF INDUSTRY AND TRADE, Viet Nam, 2018.

[10] L. DU, Z. KHIARI , Z. PIETRASIK ,AND M. BETTI .: Physicochemical and func-tional properties of gelatines extracted from turkey and chicken heads, Poultry Science 92 :2463–2474, 2013, doi.org/ 10.3382/ps.2013-03161

[11] Noi tang nhap khau re bang mot phan ba hang viet [vid.21 -1-2019]. https://kinhdoanh.vnexpress.net/tin-tuc/hang-hoa/noi-tang-nhap-khau-re-bang-mot-phan-ba-hang-viet-3815936.html.

[12] WIKIPEDIA. Chicken feet. [vid.21-1-2019].: https://en.wikipedia.org/wiki/Chicken_feet.

[13] NEWS AGRICULTURE FORESTRY SEAFOOD OF MINISTRY OF INDUSTRY AND TRADE, Viet Nam, 2018.

[14] P. HASHIM, M. S. MOHD RIDZWAN, J. BAKAR.: Isolation and Characterization of Collagen from Chicken Feet, World Academy of Science, Engineering and Technology International Journal of Bioengineering and Life Sciences, 2014, Vol:8, No:3.

[15] D.C.LIU, Y.K.LIN, AND M.T. CHEN.: optimum condition of extracting collagen from chicken feet and its characteristics, Taiwan, 2001

[16] POLIANA FERNANDES ALMEIDA1,2 AND SUZANA CAETANO DA SILVA LANNES1.: Extraction and Physicochemical Characterization Of Gelatine From Chicken By-Product, Journal of Food Process Engineering, 2013, doi:10.1111/jfpe.12051, ISSN 1745-4530.

[17] NORIZAH MHD SARBON 1, FARAH BADII, NAZLIN K. HOWELL.: Preparation and characterisation of chicken skin gelatine as an alternative to mammalian gelatine, Food Hydrocolloids, UK, 2012, doi:10.1016/ j.foodhyd.2012.05.009.

[18] L. DU, Z. KHIARI, Z. PIETRASIK, AND M. BETTI.: Physicochemical and functional properties of gelatines extracted from turkey and chicken heads, Poultry Science 92 :2463–2474, 2013, doi.org/ 10.3382/ps.2013-03161.

[19] N. HUDA, E.K. SEOW, M.N. NORMAWATI AND N.M. NIK AISYAH.: Preliminary Study on Physicochemical Properties of Duck Feet Collagen Malaysia, International Journal of Poultry Science 12 (10): 615-621, 2013, ISSN 1682-8356.

[20] ROSEMARY ANIMA OFORC.: Preparation Of Gelatine From Fish Skin By An Enzyme Added Process, Thesis, Department of Food Science and Agriculture Chemistry Macdonald Campus of McGill University Montreal, Canada,1999.

[21] ABDALBASIT ADAM MARIOD, HADIA FADOL ADAM.: Review: Gelatine, Source, Extraction and Industrial Applications, 2013, Acta Sci. Pol., Technol. Aliment. 12(2), 135-147.

[22] M.H. NORZIAH A,*, A. AL-HASSAN A , A.B. KHAIRULNIZAM A , M.N. MORDI B , M. NORITA C.: Characterization of fish gelatine from surimi processing wastes: Thermal analysis and effect of transglutaminase on gel properties, 2008.

[23] GELATIN MANUFACTURERS INSTITUTE OF AMERICA, GELATINHANDBOOK, January 2012.

[24] GELATIN MANUFACTURERS INSTITUTE OF AMERICA, STANDARD TESTING METHODS FOR EDIBLE GELATIN, July 2013.

[25] BAILEY, A. J.: 'The biological diversity of collagen: A family of molecules' in Advances in Meat Research Vol. 4, (eds) A. G. Ward and A. Courts, 1985.

[26] VEIS, A.: Macromolecular Chemistry of Gelatine, Academic Press, London, 1964.

[27] GELATIN: Handbook of hydrocolloids, D. A. Ledward, University of Reading

LIST OF ABBREVIATIONS

DSC	Differential scanning calorimetry
GMIA	Gelatine Manufacturers Institute of America
FTIR	Fourier Transform Infrared spectroscopy
Ns	Non- significant
EFG	Extracted fish gelatine
CFG	Commercial fish gelatine
HBG	Halal bovine gelatine
DC	Duck feet collagen
FC	Fish Collagen
CC	Cow Collagen
PAGE	Polyacrylamide gel electrophoresis
IEP	Isoelectric Point

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