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# **Development of hamburger patties based on rest raw materials from carp**

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Nordic Master in Aquatic Food Production – Safety and Quality



# **Development of hamburger patties based on rest raw materials from carp**

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## Preface

This master thesis project is a part of the international M.Sc. programme *Aquatic Food Production - Safety & Quality* (AQFood). It was carried out at the members of the NVKP\_16-1-2016-0023 government project, respectively at The Fishmarket Ltd. in Hungary in collaboration with Wessling Hungary Ltd. and Szent István University, Faculty of Food Science, Department of Refrigeration and Livestock's Products Technology.

Sample preparation was carried out in the manufacturing plant of The Fishmarket Ltd. The microbiological and chemical tests as well as the sensory evaluation were performed in laboratories of Wessling Hungary Ltd. Instrumental tests such as texture analysis and measurement of pH, color and water holding capacity was performed at the Department of Refrigeration and Livestock's Products Technology at Szent István University. I would like to express my deepest appreciation to those who made this thesis possible.

I would like to thank my supervisors Odd-Ivar Lekang at NMBU and Charlotte Jacobsen at DTU for their useful comments, advice and support through the whole process of the master thesis and also my supervisor at The Fishmarket Ltd., Dávid Török for allowing me to participate in this project.

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## Summary

The average Hungarian fish consumption of 4.6 kg/person/ year is one of the lowest while the number of deaths caused by cardiovascular diseases is among the highest in Europe. The identification of these trends made it necessary to seek strategies for the increase of fish consumption. The majority of active fish consumers were introduced to fish at a very young age, therefore a possible way to promote fish consumption would be the development of fish products for catering in schools and kindergartens. Due to the low budget of these institutions, the product has to be based on inexpensive raw materials, which makes the rest raw materials utilized from carp processing ideal for this purpose. As the characteristic odor and taste of fish is largely unappetizing to children, a milder fish taste has to be achieved in the product. The aim of this master thesis is to develop hamburger patties for catering utilizing carp rest raw materials, mixed with minced poultry or pork meat, in order to reduce the cost and to achieve a milder fish taste than 100% fish products, and possibly make them more appealing to children.

Three sets of experiments were performed during the project. Experiment one was evaluating the microbiological (total microbial count, *Enterobacteriaceae* count and the presence of relevant pathogenic microorganisms) and nutritional quality (fat, protein, ash and calcium content and fatty acid composition) of mechanically separated carp meat (MSCM) from back bones and cut offs. Experiment two included the instrumental and sensory evaluations of the effects of different mixing ratios of MSCM with turkey and pork meat. Experiment three was inspecting the effects of different mixing ratios on microbiological quality.

Results of Experiment one showed that mechanically separated carp meat utilized entirely from rest raw materials has low microbiological properties, as the total microbial count of 5-6 log CFU/g and the presence of *Listeria monocytogenes* was detected at time of testing. The raw material was characterized by low protein content about 11.6-12.32% and high fat content about 22.04 to 22.84%. Comparing two batches of MSCM has revealed the effects of the composition of rest raw materials on the nutritional quality of the product.

In Experiment two, the instrumental analysis of hamburger patties shown major differences between pH, water holding capacity, hardness and color of the different

recipes, but no significant difference was found between the sensory scores of the recipes. All recipes received the average sensory scores of 3-4 on a scale of five in all categories.

The results of Experiment three revealed, that all recipes were microbiologically stable during the chilled storage. However, the material's damaged packaging resulted in rapid spoilage of the samples, reaching high microbial counts (respectively  $3.8-9.8 \cdot 10^6$ ) after three days of storage.

The main objective of the project was partly fulfilled, as a product acceptable for consumers was developed from cheap rest raw materials. However, the nutritional quality and packaging of the hamburger patties require further research in order to make a healthier and safer product.

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## 1.0 Introduction

Hungarian fish consumption lags significantly behind the European average. The survey of the European Market Observatory from Fisheries and Aquaculture Products from 2017 shows that compared to the 25.8 kg /person/year mean European consumption, this number is only 4.6 kg/person/ year in Hungary, which is the lowest among all the European countries (EUMOFA, 2017).

Similar trends can be observed regarding the numbers of deaths caused by cardiovascular diseases. Studies conducted in 2007 shows, that from all EU countries, Hungary has the most cases of death caused by cardiovascular diseases. They also highlighted, that the number of these cases have been significantly exceeding the EU average in the last two decades (MOTESZ, 2007).

Beside the significant amount of vitamins (vitamin D), minerals (selenium) and functional proteins, fish has considerable n-3 polyunsaturated fatty acid (PUFA) content, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which can potentially decrease the risk of cardiovascular diseases. By the review of this health claim, EFSA established the recommendation of 250mg per day in order to support this claim (EFSA, 2010).

The correlation found between fish consumption and the risk of cardiovascular diseases indicate the need for increasing the country's fish consumption. About 85% of the Hungarian aquaculture production is carp (AKI, 2017). Being one of the most well-known species among the population, carp could be an excellent raw material for the promotion of fish consumption. The government project NVKP\_16-1-2016-0023 is aiming to improve both the farming and processing technology of carp and to develop nutritional products for gastronomy, retail and catering in order to increase the Hungarian fish consumption, which requires the coordinated work of the government, aquaculture and fish processing industry members.

A major studie (Temesi, 2016, Töröcsik, 2014) examining Hungarian fish consumption identified, that the active fish consumers were introduced to fish at a very young age, therefore a possible way to promote fish consumption would be the development of fish products for catering in schools and kindergartens. They also found that the major unappealing factors of fish consumption among children are the characteristic smell and taste.



In the survey of the National Food and Nutritional Institution, the analysis of the average budget for one person/meal in school and kindergarten catering showed, that depending on the region, about 1-1.5 euro can be spent on one person's meal. Furthermore, the survey indicated, that only 3% of the schools serves fish every week and only 40% serves it every two weeks, generally using canned or frozen fish, most likely because of the high price of fish meat (Bakacs et al., 2014). This indicates, that products developed for this market have to be inexpensive.

As part of the above mentioned government project, the aim of this master thesis is to develop hamburger patties for catering utilizing carp rest raw materials, mixed with minced poultry or pork meat, in order to reduce the cost and to achieve a milder fish taste, which would possibly make it more acceptable for children.

The main challenges of the project were the low availability of the raw materials and its microbiological quality. Due to the detection of high microbiological load and pathogenic microorganisms, both the subsequent theoretical section and the experimental work had a focus on microbiology.

### 1.1 Common carp (*Cyprinus carpio*)

Common carp (*Cyprinus carpio*) is one of the most important fish species of temperate zone freshwater aquacultures (FAO, 2018) due to its fast growth rate, easy cultivation and high feed efficiency ratio (Tokur & Ozku, 2006). In 2016, 15 tons of carp was produced in Hungarian aquacultures, making it the country's main aquaculture species (AKI, 2017).



Figure 1. Common Carp (*Cyprinus carpio*)

Common carp (Figure 1.) is an omnivorous fresh water fish species. According to their appearance, we distinguish between the native wild carp (*Cyprinus carpio morpha hungaricus*), whose body is narrow, elongated, covered with small scales and the noble

carp (*Cyprinus carpio morpha acuminatus*). The body of the latter is rounder, the mouth is larger, and the scales can be full, incomplete or completely missing. Their common feature is the shorter and longer pairs of mustache on the upper lip. Most of their bodies are olive-green or brown, the center of their body is greenish yellow, and their stomach is yellowish white. They generally occur in standing water and slow-moving rivers warming up in the summer. For commercial use, most of the carp comes from pond farms. The optimal temperature for its growth is 23 °C. From the fish processing's point of view, three year old fish with the weight of 1-2 kg is the most valuable (Darázs & Aczél 1987).

## 1.2 Microflora of carp

Fresh fish is highly perishable due to their poikilothermic nature, aquatic environment, high post-mortem pH and their biological composition including the high water content of their meat and the presence of large amounts of non-protein-nitrogen (Huss & Gram, 1996). The bacterial flora on newly harvested fish depends more on the environment in which it is caught than the fish species. The organisms from the environment around the fish become closely associated with the external surfaces of the fish, as fish are continuously exposed to the microorganisms present in water. There may also be accumulation of the organisms at sites of damage, such as missing scales or abrasions (Austin, 2006).

Allen, Austin and Colwell (1983) reported that the predominant component of the aerobic, heterotrophic bacterial flora in streams and rivers comprises Gram-negative asporogenous rods, namely representatives of *Aeromonas*, *Pseudomonas*, *Enterobacteriaceae*, and Gram-positive spore-bearing rods of the genus *Bacillus*. Many of the taxa isolated in this study have been associated with the normal microflora of fish. This is supported by the data collected by Al-harbi and Uddin (2004), who investigated the seasonal variation of tilapia intestines cultivated in ponds. Besides isolating the above mentioned microorganism groups in the intestines, they found seasonal variations in the colony counts. They found a generally high bacterial load in the intestines except during winter. One of the reasons possibly being that the high ambient temperature in the water body was close to optimum for many mesophilic bacteria in natural systems. Fish caught in very cold, clean waters generally carry a lower number of bacteria whereas fish caught in warm waters have slightly higher counts. Very high numbers, i.e.,  $10^7$  cfu/cm<sup>2</sup> are found on fish from polluted warm waters. Microorganisms are found on all the outer

surfaces (skin and gills) and in the intestines of live fish. The total number of organisms vary tremendously, normally ranging between  $10^2$ - $10^7$  cfu/cm<sup>2</sup> on the skin surface and between  $10^3$  and  $10^9$  cfu/g on the gills and in the intestines.

The flesh of healthy live fish is sterile as its immune system prevents the bacteria from growing in the flesh. When the fish dies, the immune system collapses and bacteria are allowed to proliferate freely. On the skin surface the bacteria mainly colonize the scale pockets. During storage, they invade the flesh by moving between the muscle fibers. Since only a limited number of organisms actually invade the flesh and microbial growth mainly takes place at the surface, spoilage is to a large extent a consequence of bacterial enzymes diffusing into the flesh and nutrients diffusing to the outside. It has also been reported that the bacterial flora on temperate water fish species tend to continue growth immediately after the fish have been caught and a lag phase is rare even when the fish are already iced, because the microflora is already adapted to the chill temperatures (Huss, 1995).

Due to the climate, the water temperatures and the microflora of these waters the germ count on the carps' integument, gills and in their digestive system is higher compared to that of marine or cold-water species. Mahmoud *et al.* (2004) reported that the colony count measured on the integument, the gills and in the digestive system of carp samples was  $1.2 \times 10^4$  cfu/cm<sup>2</sup>,  $1.6 \times 10^5$  cfu/g and  $7.9 \times 10^6$  cfu/g, respectively. Several authors reported the short shelf life of carp meat (Gelman, Pasteur, & Rave, 1990; Mahmoud *et al.*, 2004) reaching  $10^6$ - $10^7$  cfu/g after 3-4 days of storage.

Beside the microbiological spoilage of fish, the presence and growth of microorganisms pathogenic to humans can also be a limiting factor of the shelf life of fishery products. Surendraraj *et al.*, (2009) detected several members of enteric bacteria on the samples of carp cultivated in aquaculture ponds, such as *Salmonella*, *Shigella*, *Yersinia* and pathogenic *E.coli*. Other pathogenic bacteria associated with fresh water fish include *C. botulinum*, *C. perfringens* and *Staphylococcus spp.* (Surendraraj *et al.*, 2009). *Clostridium botulinum* is a spore-forming anaerobic bacterium that produces neuroparalytic toxins lethal to man and animals. Only the non-proteolytic types B, E, and F have the property of growing and producing toxin at temperatures as low as 3.3°C. Of these, type E is the most prevalent in marine and freshwater environments (Eklund *et al.*, 1984). *C. botulinum* type E is the most prevalent in lake sediments and in fish intestine.

It does not grow or produce toxin in living fish but is carried passively. *L. monocytogenes* is a psychrotrophic pathogen with the ability to grow at refrigerated temperatures, widely distributed in the general environment including fresh water, coastal water and live fish from these areas. Contamination or recontamination of food could also take place during processing (Novotny et al., 2004).

### 1.3 Yield and composition of carp meat

Carp is recognized as a low/medium price specie that has been sold predominantly live or dead as a whole fish. Carp processing is a seasonal operation, since most of the carp are produced in fish ponds where the production is seasonal (Váradi, 1995). Furthermore, one third of the yearly carp consumption of Hungary happens traditionally at Christmas time (Temesi, 2016). As carp was mainly distributed as whole fish or alive, carp processing technology is underdeveloped in the country. In 2014 (MK, 2014), the sales of live fish was banned in Hungary, increasing the importance of processed fish products. Although several small-scale fish-processing plants have been established in Hungary, the process is highly labor intensive, generally made by hand. The first step of the process is stunning. The fish is generally paralyzed by electricity or concussion. After stunning, the carp is descaled and gutted. It is followed by beheading, filleting and boning. As the boniness is a major throwback of the consumer acceptance of carp, it is a crucial step of the processing. The bones are eliminated by cutting into the fillets in 0.5-1 cm distances usually by hand or a set of revolving blades. The availability of well-designed equipment for producing fillets is quite limited, especially because the preferred morphology is for the carp produced to be as round as possible with the maximum amount of flesh on the back. This makes the use of filleting machines difficult (Vallod, 1995).

The yield from filleting is usually low and it represents only 35-40% of live weight (Bauer & Schlott, 2009) whereas for trout, this figure is about 50%. The flesh has a good consistency, but often described with a characteristic flavor of stale water (Vallod, 1995). There are two major characteristics adversely affecting consumer acceptance, namely the bones and the off-flavor. Carp it is characterized by certain types of unpleasant odours and taste from semi-volatile compounds that confer a discernible muddy and musty odour from geosmin (GSM) and 2- methylisoborneol (2-MIB) produced by planktonic and benthic algae (particularly cyanobacteria), fungi, bacteria, and actinomycetes, present in the environment which carp is grown in (T. Zhang et al., 2016).

Although bones are a major attribute of almost all fish species, intramuscular bones represent a specific problem in carps. There are 43 intramuscular bones on each side of the fish, 26 above the lateral line and 17 below the lateral line. These bones can be found in the flesh approximately 1/3 of the depth below the body surface (Váradi, 1995).

The flesh of the common carp contains about 69-80% water, 16-20% protein, 3-12% fat, and 1.1-1.3% ash depending on the culturing conditions and season (Bauer & Schlott, 2009; Tokur & Ozku, 2006). It contains most of the amino acids common in fish and essential in human nutrition. The proportion of unsaturated fatty acids is 80%, with high levels of linoleic, linolenic and arachidonic acids. Furthermore, carp is also a source of eicosapentaenoic acid and docosahexaenoic acid (X. Zhang et al., 2019). The low filleting yield makes the utilization of the rest raw materials particularly important in order to improve the economy of processing and to minimize the negative environmental impact.

#### 1.4 Health effects of fish consumption

Fish is a source of energy and protein with high biological value, and contributes to the intake of essential nutrients, such as iodine, selenium, calcium and vitamin A and D, with well-established health benefits. Fish also provides n-3 long-chain polyunsaturated fatty acids (LCPUFA), and is a component of dietary patterns associated with good health. Most European Food-Based Dietary Guidelines recommend a minimum of two servings of fish per week for older children, adolescents, and adults to ensure the provision of key nutrients, especially n-3 LCPUFA, but also vitamin D, iodine and selenium (EFSA, 2014). The essential benefits of omega-3 polyunsaturated fatty acids, especially eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), are well recognized. n-3 PUFAs are important components of cell membranes for maintaining the membrane fluidity (Kidd, 2007), are essential for neural development of fetus (Janssen & Kiliaan, 2014) and have beneficial effects on maintenance of normal blood pressure, normal blood concentrations of triglycerides and normal cardiac function (EFSA, 2010). Unfortunately, human do not have the ability to synthesize n-3 PUFAs de novo due to the genetic absence of  $\Delta 12$  desaturase which converts 18:1n-9 to 18:2n-6 and  $\Delta 15$  desaturase which converts n-6 PUFAs to n-3 PUFAs. Therefore, human must obtain enough n-3 PUFAs from daily diet (X. Zhang et al., 2019). The intake of about 250 mg

EPA and DHA per day is required to obtain the claimed effect. In the fish species most commonly consumed in Europe, mean n-3 LCPUFA content varies from 200 mg/100 g (cod and whiting) to 2 500 mg/100 g (herring and tuna). Also Atlantic salmon provides n-3 LCPUFA in high amounts (1 800 mg/100 g). The freshwater fish species, such as carp, have an n-3 LCPUFA content of around 300 and 600 mg/100 g (EFSA, 2010). Freshwater fish usually contain lower level of EPA and DHA than marine fish species due to the lack of n-3 PUFAs in their natural food chain (X. Zhang et al., 2019).

### 1.5 Rest raw materials

Considering the quick growth of the world population, the global demand for food is predicted to double by the year 2050, making it crucial to increase the utilization of existing protein and lipid sources and to develop new sustainable food products (Lopes et al., 2015). Fish processing activities are resulting in a considerable amount of waste, such as trimmings, belly flaps, heads, frames, fins, skin and viscera often thrown away or used for the production of low-value goods (such as silage and fish meal), resulting in a low profit for the fish industry. However, these by-products can contain a substantial amount of utilizable nutrients which could be suitable for more valuable products using the right utilization methods (Alonso *et al.*, 2010; Rustad, Storrø & Slizyte, 2011).

### 1.6 Quality changes of rest raw materials during handling and storage

Even though the muscle tissue of freshly harvested fish is considered to be sterile, the intestines and gills contain a significant amount of bacteria, which can produce degradation enzymes resulting in spoilage. Such enzymes like proteases, peptidases and lipases are also present in different fish organs. In live fish these enzymes are regulated by different biochemical systems, which are inactivated right after death, resulting in the development of off-flavor and the release of nutrients supporting microbial growth. Proteolysis degrades protein into smaller peptides and amino acids and by doing that, decreases molecular weight, which is important for proteins to be able to act as functional ingredients in food or for other applications. This makes it crucial to inactivate the spoilage bacteria, to minimize or control the proteolysis or to separate the above-mentioned organs from the more valuable parts. To ensure high quality, it is important to process or freeze by-products immediately after production (Alonso et al., 2010).

Oxidative activities are not completely inhibited by frozen storage conditions, particularly in the presence of catalysts such as, light, transition metals, heme groups, etc. Therefore, a variety of oxidation products are formed, which are normally small molecules that interact with the other components and reduce the nutritional and sensory properties of the product (Aubourg & Medina, 1999; Thorkelsson et al., 2009). Freeze denaturation of proteins is also a limiting factor for the shelf life of frozen fish meat (Kim & Park, 2006).

#### 1.6.1 Effects of oxidation in rest raw materials

Both proteins and lipids are targeted for oxidative reactions in fish rest raw materials during processing and storage, which is generally initiated by reactive oxygen species (ROS). ROS is a collective term to include oxygen radicals and non-radical derivatives of oxygen. The oxygen radicals are superoxide anion ( $O_2^{\cdot-}$ ), hydroxy ( $HO^{\cdot}$ ), peroxy ( $ROO^{\cdot}$ ), alkoxy ( $RO^{\cdot}$ ), and hydroperoxy ( $HOO^{\cdot}$ ) radicals. ROS react with lipids, proteins, sugars, and vitamins; produce undesirable volatile compounds; change the functionalities of proteins, lipids, and carbohydrates by forming oxidized dimers and trimers. By destroying essential fatty acids, amino acids, and vitamins; and by producing carcinogens, ROS cause food products to be less acceptable for consumers. They react easily with polyunsaturated fatty acids and aromatic amino acids, which have electron-rich double bonds in the molecules. The higher the unsaturation of fatty acids, the greater the reactions with oxygen radicals (Choe & Min, 2005).

Fresh fish are especially susceptible to oxidation, due to their high content of polyunsaturated fatty acids. The basic mechanisms of lipid oxidative reactions can be characterized by three distinctive steps: initiation, propagation and termination reactions. The first step in lipid oxidation is the removal of a hydrogen from a methylene carbon in a fatty acid (RH). This becomes easier as the number of double bonds in the fatty acid increases, which is why polyunsaturated fatty acids are particularly susceptible to oxidation. The initiation step can be catalysed by  $HO^{\cdot}$  or by certain iron-oxygen complexes. The fatty acyl radical ( $R^{\cdot}$ ) reacts rapidly with  $O_2$  to form a peroxy radical ( $ROO^{\cdot}$ ). Because  $ROO^{\cdot}$  is more highly oxidized than the fatty acyl radical or the fatty acid itself, it will preferentially oxidize other unsaturated fatty acids and propagate a chain reaction. Lipid hydroperoxides ( $ROOH$ ) formed in the propagation reaction are both products of oxidation and substrates for further reaction with  $Fe^{2+}$  and  $Cu^+$  to yield  $ROO^{\cdot}$  and alkoxy radicals ( $RO^{\cdot}$ ).  $Fe^{2+}$  reductively cleaves  $ROOH$ . Both  $ROO^{\cdot}$  and



RO• can initiate further reactions. The RO• can also undergo  $\beta$ -scission and degrade to alkyl radicals ( $R'CH\cdot_2$ ) and a range of aldehydes ( $R''CHO$ ) depending on the particular hydroperoxide present. ( $R'CH\cdot_2$ ) can initiate further chain reactions resulting in the formation of ethane and pentane, while the aldehydes, including hexanal, malondialdehyde and 4-hydroxynonenal, can react readily with  $\epsilon$ -amino groups of proteins to yield Maillard-type complexes (Buckleyh, 1998).

This phenomenon can be influenced by both intrinsic and extrinsic factors, such as the fatty acid composition, the concentration of pro-oxidants, endogenous ferrous iron, myoglobin, enzymes, pH, temperature, ionic strength and oxygen concentration. Hydrolysis, induced by lipases and phospholipases, is also promoting the oxidation of lipids, by producing free fatty acids that undergo further oxidation to produce low molecular weight compounds that are responsible for the rancid off-flavor and taste of fish and fish products. Metmyoglobin formation is also positively correlated with lipid oxidation. Myoglobin and other heme compounds function as prooxidants in muscle tissues. Enzymatic and non-enzymatic reducing systems, converting iron from the inactive ferric form to the active ferrous state, can accelerate oxidation of meat (Chaijan et al., 2006).

Myoglobin is a globular heme protein localized in muscle fibers, being known to be a major contributor to the color of the muscles, depending on its derivatives and concentration. Hemoglobin is lost rather easily during handling and storage, while myoglobin is retained by the muscles' intracellular structure, therefore the color of meat heavily depends on the stability of myoglobin. The color-changes in meat are mainly due to the reaction of myoglobin with other muscle components, especially myofibrillar proteins. During the handling and storage of fish, a number of biochemical, chemical and microbiological changes occur, leading to discoloration. Myoglobin is the heme protein responsible for meat color. The oxidation of the central iron atom within the heme group is responsible for discoloration, a change from red oxymyoglobin to brownish metmyoglobin. When ferrous heme iron oxidizes to its ferric form, oxygen is released and replaced by a water molecule. Metmyoglobin formation is positively correlated with lipid oxidation. The mechanisms by which lipid oxidation could enhance myoglobin oxidation have been explained primarily on the reactivity of primary and secondary products derived from unsaturated fatty acids (Faustman, Sun, Mancini, & Suman, 2010). Furthermore, metmyoglobin forms cross-linkages with myosin in the presence of



hydrogen peroxide (Chaijan et al., 2005). Huang *et al.* (2006) associated the darkening of the meat and the loss of redness with the oxidation of myoglobin to metmyoglobin in tuna. This can also occur during frozen storage.

#### 1.6.2 Effects of autolysis in rest raw materials

Fish flesh is composed of myofibrillar (70–80%), sarcoplasmic (25–20%), connective tissue stroma proteins (0.3–3%), polypeptides, nucleotides and non-protein nitrogen compounds. The initial steps in deterioration of raw fish during its storage on ice consist of hydrolytic reactions catalyzed by endogenous enzymes, which produce nutrients that allow bacteria proliferation (Hernández et al., 2003). Hydrolysis of lipids and phospholipids can lead to the formation of degradation products, such as fatty acids, which reduce the sensory quality and the oxidative stability of fat; while, proteolytic degradation results in reduced molecular weight of proteins which affects their functional properties (Falch, Sandbakk, & Aursand, 2006). The concentration of autolytic enzymes is the highest in the internal organs, which makes the rest raw material containing viscera more vulnerable towards autolysis. There are also proteolytic enzymes in fish muscles and other tissues that are activated by the pH drop due to post mortem glycolysis that leads to the softening of the muscle and the formation of peptides and free amino acids (Thorkelsson et al., 2009).

Texture is one of the most important quality factors of fish as a food. Although it is desirable to accelerate meat flesh softening in most terrestrial animals, the opposite is the case in fish meat. The initial softening in texture that occurs in many species is considered to be associated with the disappearance of Z-disks, destruction of connectin and general denaturation of collagenous tissue (Hernández et al., 2003). There are several proteolytic systems present in fish muscle tissue, which may be involved in post-mortem muscle degradation. These include the proteasome, a heat-stable alkaline proteinase, matrix metalloproteinases (MMPs), the calpains and the lysosomal cathepsins (Anders et al., 2011). Chéret *et al.* (2007) suggested that cathepsins B, L and/or D could have major roles in degradations of proteins in post-mortem fish muscle. Cathepsins are acid proteases located in the lysosomes. They may be liberated into both the cytoplasm and the intracellular spaces as a consequence of lysosomal disruption occurring after cell death due to a pH fall. The pH of fish drops to about 6.5 during rigor mortis, then it sets back to nearly neutral values. Therefore, the enzymes cathepsin B, L and calpain could have a bigger role in the protein degradation process, since the optimum pH of these

enzymes ranges about 6.5-7.0, while the optimum pH of cathepsin D is under 5.0 (Anders et al., 2011).

### 1.7 Production of mechanically separated meat

The minced fish technology offers a great possibility for the processing of bony fishes like carp. The basic machine of this technology is the meat-bone separator (see Figure 2) that can recover all the edible flesh from the carcass. In this type of equipment, the headed and gutted fish or pieces of fish are fed between a rotating stainless-steel perforated drum and a moving continuous rubber belt under tension. The rubber belt pressure squeezes the relatively soft muscle through the perforations of the drum, while the skin and bone mat on the outside of the drum are scraped off into a waste chute. The drum is open at one end, allowing the separated fish muscle to exit continuously (Váradi, 1995). The mince is usually frozen after the deboning. After frozen storage, fish mince can be used in a variety of products from fish burgers, balls and sticks to chowders, soups and even ready meals (Hall, 2010). While in case of fish, this technology is most commonly used for the utilizations of by-catch and undersized fish, mechanically separated meat is often acquired from meaty bones and by products of poultry and pork meat production. This separated meat has typically higher content of connective tissues, bone, cartilage and calcium (Pospiech et al., 2019).

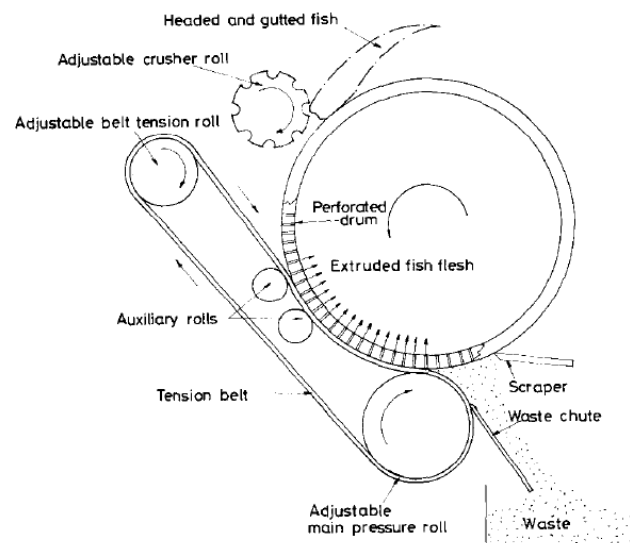


Figure 2. Scheme of the bone-meat separator (Váradi, 1995).

Rest raw materials (see Figure 3) generated in fish fillet processing contain a significant amount of flesh. Compared to commercial fish mince made of whole fish, mechanically deboned frame mince contains bone fragments and reddish colored kidney

juice, which contains high concentrations of the enzyme trimethylamine oxide (TMAO) demethylase. Therefore, the color and flavor quality of mechanically deboned frame mince containing kidney is low compared to commercial mince. Mechanically deboned frame mince is also low in salt extractable protein and high in bone and bacterial counts, impurities, and off-flavor. Products prepared from commercial mince have shown to have firmer texture than products prepared by frame mince. Blended minces (10/90 and 20/80) were reported to be as firm as gels prepared from commercial mince. Two approaches can be proposed to utilize frame meat: one is to develop processing techniques to reduce the high defect levels and the other is to blend minced flesh with minced fillets and/or trimmings to produce an acceptable quality product (Kim & Park, 2006).



Figure 3: Rest raw materials of carp

### 1.8 Utilization of fish mince in hamburger patties

There are several studies focusing on the use of fish species with low or no commercial value, such as Bonefish (*Albula vulpes*), Silver catfish (*Rhamdia quelen*) and Pangasius (*Pangasius sutchi*) (Bochi et al., 2008; Ejaz, Shikha, & Hossain, 2009; Pires et al., 2017) or the utilization of fish waste from major aquaculture species such as European sea bass (*Dicentrarchus labrax*), Gilthead sea bream (*Sparus aurata*) and Rainbow trout (*Oncorhynchus mykiss*) (Husein et al., 2018) in the form of a hamburger product. In general, the soft texture of fish mince was reported to be unfavorable for consumers, therefore numerous additives have been tested to meet consumer expectations of eating quality characteristics (Kasapis et al., 2007).

Hydrocolloids are polysaccharides, widely used to modify the texture, functional properties, and stability (thermal, mechanical, storage, etc.) of many processed foods. In the case of fish products, interactions of the fish protein with added hydrocolloids may lead to phase separation or synergistic phenomena that would determine the quality and acceptability of these products. Addition of starch was found to give strong gel-like properties to the products. The cooking of starch is accompanied by an increase in viscosity due to the swelling of granules and the continuous leaching, entanglement, and association of amylose molecules. Thermodynamic incompatibility between the polymer and fish proteins further enhances the rigidity of the continuous starch matrix thus producing firm burgers. Traditional fish burgers are made mostly with added starch. However, there are many promising functional ingredients that could improve organoleptic properties including milk protein, citrus pectin, and bovine gelatin (Kasapis et al., 2007). The addition of potato flakes (Husein et al., 2018) or mashed potatoes (Ali et al., 2018) as a source of starch was reported to improve sensory scores of fish burger products. In both cases, the increase in potato-fish ratio increased the consumer satisfaction. Consumers less familiar with fish, such as children, did prefer the products with a higher potato content, with its softer texture and more delicate flavor. On the other hand, consumers who like fish did prefer the ones with a higher fish content and characterized by fresh/raw fish olfactory notes (Husein et al., 2018).

Bochi *et al.*, (2008) were testing different mixtures of filleting waste pulp and mince made of fillets. Their results showed that the ratio of fish pulp from filleting waste has no effect on the fat and moisture retention of the burger patties during cooking. Furthermore, they found no significant differences among. Furthermore, they found no significant differences among the sensory attributes of samples containing different levels of pulp. These results indicate that mechanically separated fish meat from by-products is a viable raw material for such products.

### 1.9 Interaction of fish mince with other meats

Gelation is an important functional property of fish protein affecting the rheological and textural properties of fish products. Gel formation involves partial denaturation of proteins followed by irreversible aggregation which results in a three dimensional network. Myosin is abundant in muscle protein and plays a key role in gel development in fish and meat products (Liu et al., 2010).

Proteins are linear molecules whose cold-set gelation involves the formation of a triple helix as a junction zone. The ordered junction zones constitute the structural knots of the network and are linked together by soluble, disordered regions (Kasapis et al., 2007). Fish myosin possesses  $\alpha$ -helix,  $\beta$ -sheet,  $\beta$ -turn and random coil structures.  $\alpha$ -helix was reported to be the predominant structure of fish myosin at pH 7.0. High  $\beta$ -sheet and  $\beta$ -turn fractions prior to heating were found to decrease the WHC of the thermal gel, although they could improve gel strength. Heating causes the partial transformation of  $\alpha$ -helices into  $\beta$ -sheets and  $\beta$ -turns. The transformation plays an important role in the gelation process (Liu et al., 2010). pH is a determining factor in the mince for higher gel forming ability. A good quality product can be prepared from the mince with around neutral pH. (Ejaz et al., 2009). Liu *et al.*, (2010) reported improved water holding capacity and formation of compact and uniform gels at the nearly neutral pH characteristic for fish meat. This indicates an excellent gel forming ability of fish proteins.

The review of the relevant literature indicate that many researchers have conducted studies on textural and rheological properties of fish and meat products separately; but there is only a few that report exploring the textural, rheological properties and microstructure of the mixtures of fish and other meats. In China, pork is often used to improve the gel strength of surimi-based products, such as fish balls and fish cakes. Fish is also added to comminuted pork-based products such as meat balls to improve the sensory quality.

Heating can induce meat protein to denature. Subsequently, irreversible aggregation and gel networks are formed by intermolecular bonds. Protein-protein interactions between different components are commonplace during the processing of food. The cross-linking of different raw materials is useful due to the complementary interaction between the proteins of different meats. These interactions can affect both the structure and the gel properties of proteins (Liu *et al.*, 2016).

Li *et al.*, (2018) reported that pork and fish mince mixtures can combine the characteristics of pork with those of fish. Pork has good hardness but poor water holding capacity, while fish was found to have good WHC but poor hardness. The texture profile analysis of fish-pork mince mixtures showed that hardness, cohesiveness, gumminess and chewiness of the samples decreased while elasticity increased with the addition of fish.

Furthermore, with the addition of fish, gel strength and breaking force of the samples decreased, while deformation showed fluctuating increase.

Liu *et al.* (2016) reported, that the expressible moisture content of 100% pork gel was significantly higher than that of 100% fish gel, suggesting that 100% fish gel has better water holding capacity. The addition of pork mince to the mixture significantly increased the expressible moisture content compared to 100% fish gels. The low water retention of pork was explained by weaker associations between their proteins and water. Additionally, the water retention was also found to be closely related to the gel microstructure. Higher homogeneity of the network and smaller pore size can entrap more water. Largescale rearrangements during and after the gel formation could induce the release of physically entrapped water in a gel matrix. The 100% fish gel was found to have the most compact and uniform microstructure. The fine gel network of fish gel seemed to be related to its high water retention. Addition of pork decreased the 3-dimensional order and uniformity of the gels.

Gui *et al.*, (2018) were experimenting with different mixtures of fish and chicken mince. They reported, that chicken gels had better gel properties and a higher content of immobilized water, than the mixture or fish gels. Additionally, the gel properties of mixture samples were significantly higher, than pure fish gels. Hashemi and Jafarpour (2016) reported that the addition of up to 50 % fish mince to beef sausage formula, positively interacted in the gel formation process, without diminishing its rheological properties. Sausages containing 35 % and 50 % fish mince showed greater texture parameters such as hardness, cohesiveness and subsequent gumminess.

In summary, a well-balanced mixture of fish mince with other meats has beneficial effects on the products, by making use of the unique functional properties of the particular ingredients, such as the better water holding capacity of fish mince, or the harder texture of pork meat.

## 2.0 Aim of the work

The main objective of the project was to develop a cheap carp product for catering in kindergartens and schools, which utilizes rest raw materials and has acceptable sensory properties for the targeted consumers.

The aim of experiment one was to investigate the possibility of utilizing mechanically separated meat from the filleting waste of carp. The parameters investigated in the preliminary experiment, such as total microbial count and the presence of microorganisms pathogenic to human, were aiming to determine, whether MSCM is suitable for the processing of chilled products. It was also important to investigate the nutritional quality of the MSCM, in order to determine if a product based on this raw material could comply with the requirements for the health claims associated with fish. Therefore, the protein, fat and ash content as well as the fatty acid composition were analyzed.

The main experiments were investigating the consumer acceptance of different mixtures of mechanically separated carp meat (MSCM) with pork and turkey meat. As the proposed products are intended for children more sensitive to fish taste, the main objective was to achieve a hamburger patty with milder, less intensive fish taste than 100% fish products. The intensity of fish taste was evaluated by a consumer test. Beside the taste, the untrained panelists evaluated the smell, color, texture and general appearance of the recipes.

The reviewed literature suggested, that the mixing of fish mince with other meats could be beneficial for the texture of the product. The influence of the mixing ratios of fish and other meats was investigated based on texture analysis and measurement of water holding capacity. Furthermore, the pH and color of the samples was also measured. These instrumental measurements were intended to reveal the differences observed in the sensory scores of the products.

In order to investigate the microbiological stability of the product during chilled storage, an 8 day storage test was conducted, aiming to reveal the shelf life of the patties and the effectiveness of the applied heat treatment.



Overall, the project is aiming to achieve a cheap, nutritious, microbiologically stable, chilled carp product acceptable for children, utilizing rest raw materials of carp production.

### 3.0 Materials and methods

The mechanically separated carp meat was purchased from a local carp aquaculture and fish processing plant (Győr Előre HTSZ.). Meaty backbones, cutoffs and belly flaps were placed into a meat bone separator. The company reported, that the separation of 10 kg fish waste yielded 3 kg separated fish meat. The MSCM was delivered to the processing plant of The Fishmarket Ltd. the next day. Overall, about 30 kg separated meat was produced from almost 100 kg of carp waste in two batches. The amount of mechanically separated meat was limited by the amount of carp processed by the company. After collecting samples for the preliminary experiments, the MSCM was frozen at -18 °C in vacuum bags until later tests. The project was separated into two sets of experiments.

#### 3.0.1 Experiment one – Composition and Microbiology of MSCM

Overall, two batches of mechanically separated carp meat were received during the one month experimental period. Chemical and microbiological analysis was performed on both batches, allowing the comparison of the quality of the raw materials. In order to determine, whether the MSCM is suitable for the production of chill stored hamburger patties, five 100g samples were used for microbiological analysis, and five 300g samples were taken for chemical analysis of the samples. In the microbiological analysis, the total bacterial count, *Enterobacteriaceae* count, Sulphite-reducing *Clostridia* count and *E.coli* count was investigated. Furthermore, the samples were tested for the presence of *Listeria monocytogenes* and *Salmonella ssp.* During the chemical tests, the fat, protein, ash and calcium content and fatty acid composition were analyzed. The calcium content was used as an indirect indicator for bones in the fish paste.





**A**



**B**

Figure 4. A: Mechanically separated carp meat; B: sample preparation for chemical and microbiological analysis.

### 3.0.2 Experiment two – Effects of mixing MSCM with meat in different ratios

In a second set of experiments, hamburger patties were prepared from the mixture of mechanically separated carp meat, minced pork and turkey meat. Due to the high fat content of the MSCM revealed in the first experiment, inexpensive meats were chosen which have low fat content. For the production of hamburger patties, minced pork shoulder meat with 10% fat content and minced turkey upper thigh with the fat content of 7% were used. The minced meat was purchased from a local supermarket. The meats were mixed in a 50/50 and a 30/70 ratio. For control, a 100% fish sample was used. The sample codes and recipes are shown in Table 1.

Table 1. Recipes of the burger patty samples; P30/70: 30-70% mixture of pork and carp meat; P50/50: 50-50% mixture of pork and carp meat; T30/70: 30-70% mixture of turkey and carp meat; T50/50: 50-50% mixture of turkey and carp meat; Control: 100% carp meat.

Sample Code	Recipe for 100g				
	Carp	Pork	Turkey	Starch	Salt
<b>Control</b>	100g	-	-	5g	1.5g
<b>P50/50</b>	50g	50g	-	5g	1.5g
<b>P30/70</b>	30g	70g	-	5g	1.5g
<b>T50/50</b>	50g	-	50g	5g	1.5g
<b>T30/70</b>	30g	-	70g	5g	1.5g

The mechanically separated carp meat, minced meat, starch and salt were mixed together in a bowl, then the patties were shaped in a metal ring with the diameter of 10 cm. As it is highlighted in section 1.7, hydrocolloids are crucial parts of the recipe in hamburger patties, in order to get a product acceptable for consumers. The corn starch, purchased in a local supermarket was added to the samples to improve the water holding capacity and the texture of the patties. The same amount was added to every mixture to allow the comparison of their textural properties. The shaped patties were placed in a -18°C freezer for 30 minutes, in order to be able to put them in a vacuum bag without any deformation. The samples were thawed at room temperature, then heat treated at 90°C for 10 minutes. The heat treatment was performed with an 80016 Roner R Sous-Vide machine (International Cooking Concepts S.A., Barcelona, Spain), an equipment which was responsible for both the heating and the circulation of the water to have a better heat transfer in the samples. After the heat treatment, the hamburger patties were placed in icy water and left there for about 10 minutes, then stored between 0-4°C until later testing. For the pH and water holding capacity, one raw shaped, vacuum packaged hamburger sample was separated per each mixture. On the heat treated samples, color measurement, Warner-Brazler texture test and sensory evaluation was performed.



A



B





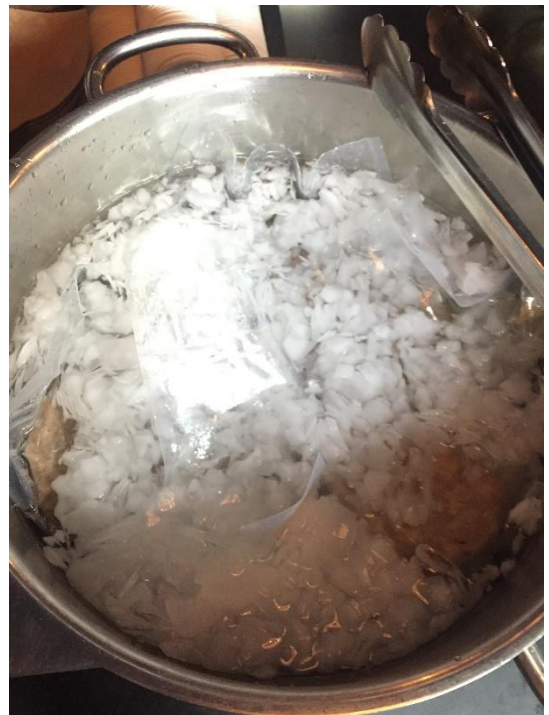
C



D



E



F



G

Figure 5. A: The mixture of pork and turkey meat with mechanically separated carp meat; B: Shaping of the hamburger patties; C: Placing the hamburger patties on a rack for freezing; D: Vacuum packaging of the hamburger patties; E: Heat treatment of the samples with a sous-vide machine; F: Chilling of the samples after the heat treatment; G: The heat treated, vacuum packaged hamburger patties.

### 3.0.3 Third experiment – Effects of different mixing ratios on shelf-life

In order to determine the shelf-life of the product, and to evaluate, whether the applied heat treatment sufficiently eliminated the high microbial count and pathogenic microorganisms detected in the mechanically separated carp meat, an eight day storage test was performed on the heat treated, vacuum packaged hamburger patties. Microbiological analysis was performed immediately and after 3, 6 and 8 days of chilled storage between 2 and 4 °C, where the total bacterial count, *Enterobacteriaceae*, Sulphite reducing *Clostridia*, *E. coli* and *Staphylococcus aureus* counts were investigated, as well as the presence of *Listeria monocytogenes* and *Salmonella ssp.* Three parallel samples were tested per mixture on all four storage days, so in total, 60 · 100g hamburger patties were prepared for the storage test.

## 3.1 Microbiological analysis

### 3.1.1 Total bacterial count

The total bacterial count of the samples were determined according to the MSZ EN ISO 4833-1:2014 method. The total bacterial count of five parallel samples from each batch of mechanically separated meat, and three parallel samples from each mixture of heat treated hamburger patty from each recipe, on the 0<sup>th</sup>, 3<sup>rd</sup>, 6<sup>th</sup> and 8<sup>th</sup> days of storage were analyzed. 10g sample was weighed in sterile conditions and homogenized in 90 ml

salty peptone solution, then a serial dilution was prepared. 1 ml suspension per dilution step was dispersed on a sterile Petri dish with a sterile pipette, then approximately 15 ml, 44-47 °C Plate Count Agar was poured into them. The plates were incubated at 30±1°C for 72 hours. The colonies were counted on the plates, where the colony count was between 30 and 300.

### 3.1.2 Enterobacteriaceae count

*Enterobacteriaceae* count was determined according to the ISO 21528-2:2017 standard. The *Enterobacteriaceae* count of five parallel samples from each batch of mechanically separated meat and three parallel samples of heat treated hamburger patties from each recipe, on the 0<sup>th</sup>, 3<sup>rd</sup>, 6<sup>th</sup> and 8<sup>th</sup> days of storage were analyzed. 10g sample was weighed in sterile conditions and homogenized in 90 ml salty peptone solution, then a serial dilution was prepared. 1ml suspension per dilution step was dispersed on a sterile Petri dish with a sterile pipette, then about 15 ml, 47-50 °C violet red bile glucose (VRBG) agar was poured into them. The inoculum was mixed with the medium by horizontal movements. After the solidification of the medium, another 10 ml layer of the VRBG agar was added, then left on a cold surface until its complete solidification. The plates were incubated at 37±1°C for 24 hours. The characteristic red, pink or purple colonies (with or without precipitation holes) were counted. Five such colonies were randomly chosen from each dish to be subcultured for biochemical confirmation tests. The selected colonies were streaked on nutrient agar and incubated at 37°C for 24 hours. On the well-isolated colonies, an oxidize test was performed, and they were stabbed into tubes containing Glucose OF medium. Colonies that were oxidase-negative and developed yellow color in the Glucose OF medium were confirmed as *Enterobacteriaceae*.

### 3.1.3 Presence of *Salmonella* spp.

The presence of *Salmonella* spp. was determined according to the MSZ EN ISO 6579-1:2017 standard. The presence of *Salmonella* spp. in five parallel samples from each batch of mechanically separated meat and three parallel samples of heat treated hamburger patties from each recipe, on the 0<sup>th</sup>, 3<sup>rd</sup>, 6<sup>th</sup> and 8<sup>th</sup> days of storage were analyzed. 225g ml buffered peptone water was inoculated with 25g homogenized sample, then incubated on 37 °C for 18 hours. 0.1 ml of the culture obtained after the pre-enrichment step was inoculated in 9 ml Rappaport-Vassilidis medium with soy (RVS) broth and 1 ml was inoculated in 9 ml Muller-Kauffmann tetrathionate-novobiocin

(MKTTN) broth. The RVS broth was incubated at 41°C and the MKTTN broth at 37°C for 24 hours. Xylose Lysine Deoxycholate (XLD), Diassalm and Harlequin *Salmonella* agars were inoculated with the cultures obtained in the enrichment step. They were incubated at 37 °C for 24 hours. On the Harlequin *Salmonella* agar, the colonies have green color. On the Diassalm agar, *Salmonella* ssp. has dark, purple mobility zones. On the XLD agar, *Salmonella* colonies have a black center and a lightly transparent zone of reddish color. Biochemical confirmation media was inoculated with the colonies. The hydrogen sulfide- negative strains have a pink color with darker center, the lactose-positive strains have yellow color.

#### 3.1.4 *Staphylococcus aureus* count

The *Staphylococcus aureus* count of the samples was determined according to the MSZ EN ISO 6888-1:2008 standard. The *Staphylococcus aureus* count of five parallel samples from each batch of mechanically separated meat and three parallel samples of heat treated hamburger patties from each recipe, on the 0<sup>th</sup>, 3<sup>rd</sup>, 6<sup>th</sup> and 8<sup>th</sup> days of storage were analyzed. 10g homogenized sample was weighed in sterile conditions and homogenized in 90 ml salty peptone solution, then a serial dilution was prepared. 1 ml suspension was spread evenly on the surface of pre-prepared sterile Baird Parker agar media supplemented with sterile egg-yolk tellurite emulsion. After the agar surface dried, the Petri dishes were incubated at 37 °C for 48 hours. *Staphylococcus aureus* is forming black or grey colonies, surrounded by an opaque zone. The colonies were counted on the plates, where the colony count was between 20 and 200.

#### 3.1.5 *Escherichia coli* count

The *Escherichia coli* count of the samples was determined according to the MSZ EN ISO 4833-1:2014 standard. *Escherichia coli* count of five parallel samples from each batch of mechanically separated meat and three parallel samples of heat treated hamburger patties from each recipe, on the 0<sup>th</sup>, 3<sup>rd</sup>, 6<sup>th</sup> and 8<sup>th</sup> days of storage were analyzed. 10g sample was weighed in sterile conditions and homogenized in 90 ml salty peptone solution, then a serial dilution was prepared. 1ml suspension per dilution step was dispersed on a sterile Petri dish with a sterile pipette, then about 15 ml, 44-47 °C tryptone-bile-X-glucuronide (TBX) agar was poured into them and shaken thoroughly to mix well. After the agar solidified, the Petri dishes were incubated at 44 °C for 24 hours. The colonies were counted on the plates, where the colony count was less than 300.



### 3.1.6 Detection and enumeration of *Listeria monocytogenes*

The presence of *Listeria monocytogenes* was determined according to the MSZ EN ISO 11290-1:2017 standard. The presence and quantity of *Listeria monocytogenes* in five parallel samples from each batch of mechanically separated meat, and three parallel samples of heat treated hamburger patty from each recipe, on the 0<sup>th</sup>, 3<sup>rd</sup>, 6<sup>th</sup> and 8<sup>th</sup> days of storage were analyzed. 225g ml half-Fraser broth was inoculated with 25g of the homogenized sample, then incubated at 30 °C for 25 hours. 0.1 ml of the obtained culture was transferred to a tube, containing 10 ml Fraser broth, then incubated at 37°C for 24 hours. This enrichment medium was inoculated on ALOA and Palcam agar, then incubated on 37°C for 48 hours. On the ALOA agar, *Listeria ssp.* is forming blue-green colonies, surrounded by an opaque halo. On the Palcam agar, *Listeria monocytogenes* is forming grey-green colonies, surrounded by a black zone. For confirmation, 1-1 presumptive *Listeria monocytogenes* colony were selected. The selected colonies were streaked onto the surface of tryptone soy yeast extract agar (TSYEA) and incubated at 37°C for 24 hours. For the confirmation, haemolysis test was performed.

Enumeration of *Listeria monocytogenes* count was performed according to the MSZ EN ISO 11290-2:2017 standard. 10g sample was weighed in sterile conditions and homogenized in 90 ml half-Fraser broth, then a serial dilution was prepared. 0.1ml suspension per dilution step was inoculated on the surface of a Petri dish of ALOA agar. The plates were incubated at 37°C for 48 hours. The presumed colonies of *Listeria monocytogenes* with blue-green color surrounded by an opaque halo were counted on each Petri dish containing less than 150 characteristic colonies. For the confirmation, haemolysis test was performed.

### 3.1.7 Sulphite-reducing Clostridia

The Sulphite-reducing *Clostridia* count of the samples was determined according to the MSZ ISO 15213:2006 standard. Sulphite-reducing *Clostridia* count of five parallel samples from each batch of mechanically separated meat, and three parallel samples of heat treated hamburger patty from each recipe, on the 0<sup>th</sup>, 3<sup>rd</sup>, 6<sup>th</sup> and 8<sup>th</sup> days of storage were analyzed. 10g sample was weighed in sterile conditions and homogenized in 90 ml salty peptone solution, then a serial dilution was prepared. 1ml suspension per dilution step was dispersed on a sterile Petri dish with a sterile pipette, then about 15 ml, 45-50 °C iron sulfite agar was poured into them and carefully mixed by horizontal movements.

After the medium was solidified, another 10 ml of iron sulfite agar was poured into the dish. After the second layer solidified, the petri dishes were incubated in anaerobic jars at 37°C for 24 hours. Black colonies with the diameter of about 1-2 mm were counted in each dish containing less than 150 typical colonies and less than 300 total colonies.

## 3.2 Chemical analysis

### 3.2.1 Fat content and fatty acid composition

The fat content was measured according to the standard MSZ ISO 1444:2000, using a Soxtec 8000 (Foss A/S, Hilleroed, Denmark) extraction unit. For the analysis of fat content, five parallel samples from each batch of mechanically separated carp meat were taken. The drying of the samples was followed by extraction with petroleum ether. The extract was dried then weighed.

The fatty acid composition was determined according to the standard MSZ ISO 5508:1992.  $3\pm 0,1$ g sample was extracted with 20 ml petroleum ether in a Foss Soxtec 8000 extraction unit. The phases were separated by centrifugation, then the solvent was distilled. The methylation of the lipids was performed with a quick trans-esterification method with potassium hydroxide. About  $100\pm 10$  mg sample was weighed into a test tube, then 4ml isooctane and 200  $\mu$ l methanolic potassium hydroxide was added. The mixture was shaken vigorously for 30 seconds. About 1 g sodium hydrogen sulfate monohydrate was added in order to neutralize the potassium hydroxide. After the salt had settled, 0.1 ml from the upper layer containing the methyl esters was diluted with isooctane in a 2ml GC vial. From this solution the determination of fatty acids composition was performed by gas chromatography using HP 6890 GC apparatus (Agilent Technologies Inc., Santa Clara, USA) with a flame ionization detector (FID) and capillary column Supelco SP-2560 (100 m x 0,25 mm x 0,2  $\mu$ m, Sigma Aldrich Ltd., Budapest, Hungary). The injector temperature was 250 °C, the FID temperature was 260 °C. Hydrogen was used as carrier gas with an oven thermal gradient from 50°C for 2 min to 160°C at a heating rate of 10°C/min, from 160 °C to 210 °C at a rate of 3°C/min then to a final temperature of 260°C at a rate of 20 °C/ min. The total running time was 39 min. One microliter of sample was injected. Fatty acids were expressed as percentages of the total fatty acid methyl esters.



### 3.2.2 Protein content

The protein content was measured by the standard Kjeldahl-method according to the MSZ 5874-8:1979 standard, using a K370 protein analyzer (Büchi Labortechnik AG., Flawil, Switzerland). For the analysis of protein content, 250g samples from each batch of mechanically separated carp meat were taken. From the homogenized samples, about 2g was heated with sulphuric acid in the presence of catalyst, turning the nitrogen content of the sample into ammonium ions. The solution was then distilled with a small quantity of sodium hydroxide, which converts the ammonium salt to ammonia. The amount of ammonia present, and thus the amount of nitrogen present in the sample was determined by back titration. The end of the condenser was dipped into a solution of boric acid. The protein content of the sample was calculated by multiplying the amount used for back titration by 6.25.

### 3.2.3 Ash content

The ash content was determined according to the standard MSZ ISO 936:2000 ignition method. For the analysis of ash content, 250g samples from each batch of mechanically separated carp meat were homogenized. 2g of the homogenized sample was dried at 103°C for an hour. The drying of the sample was followed by charring and cremation at 550±25°C in a KD 48P (HAGA Automatika Ltd., Budapest, Hungary) oven for 4 hours. The remains were placed in a desiccator at room temperature to cool down. The result is determined by weighting the remains of the samples.

### 3.2.4 Calcium content

Calcium content was determined by the EPA Method 6010C ICP-OES. An about 200 mg meat sample was placed in a digestion vessel. About 3 ml of nitric acid and approximately 1 ml hydrogen peroxide was added into the vessel. After mixing the sample, it was covered with acid, then the vessel was closed. The microwave assisted digestion of the sample was performed at 180 °C. The digestion was started by applying low microwave energy then the energy supply was slowly raised to the maximum power in order to achieve the digestion temperature. It started with 100W, raised to 600W within 5 min, held for 5 min, then the maximum power was held for 20 minutes. The sample was cooled down to under 40 °C. After the digestion of the sample, the calcium content was measured by an Optima8300 ICP-OES 01 (Perkin Elmer Inc., Waltham, USA) optical emission spectrometer. The wavelength of 317.933 was analyzed.

### 3.3 Water holding capacity

Water holding capacity of the raw hamburger patties was determined by using a filter paper press method (see Figure 5.). About 2 g meat sample was placed on a 5x5 cm dry filter paper, followed by pressing with a 500g weight for 5 minutes. Five 2g samples were taken from each mixture for the test. The filter papers were dried, then the meat juice strain was cut out of the filter paper. The filter paper was weighed on an analytical scale before the test and after cutting out the meat strain and drying. The water holding capacity is described by the ratio of the meat juice strain and the weight of the sample, expressed as mm<sup>2</sup>/g meat. The higher values are indicating poorer water holding capacity, as a bigger meat juice area is a results of a higher amount of moisture released by the sample. The area of the strain was calculated by the following formula:

$$A_2 = \frac{(m_1 - m_2) * A_1}{m_1}$$

$m_1$ : Weight of the filter paper before the test.

$m_2$ : Weight of the filter paper, after cutting out the meat strain.

$A_1$ : Area of the filter paper.

$A_2$ : Area of the meat strain.



Figure 6. Filter paper press method.

### 3.4 Measurement of the pH

The pH of the raw hamburger samples were measured with a Testo 209 one-hand pH/temperature measuring instrument. Five parallel measurements were done on five different points of all mixtures.

### 3.5 Color measurement

The color was measured instrumentally with a MINOLTA CR 400 Chroma meter tristimulus photoelectric colorimeter (Konica Minolta Inc., Tokyo, Japan). CIELab color coordinates were read on the surface of the heat treated burgers, where L\* represents the color lightness on a 0-100 point scale from black to white, a\* describes the position between red (+) and green (-) and b\* is the position between yellow (+) and blue (-). Five measurements were performed on five different points of the surface of all heat treated mixtures. Beside the 10 minute heat treatment on 90 °C, the samples were placed in the oven at 200 °C for 20 minutes, achieving the same discoloration on the surface experienced at the sensory tests, allowing the comparison of the sensory and instrumental data. The  $\Delta E^*_{ab}$  differences were calculated with the following formula:

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} .$$

The  $\Delta E^*_{ab}$  color difference can categorize the numerical data of color coordinates based on the human eye's ability to detect color (see Table 2.).

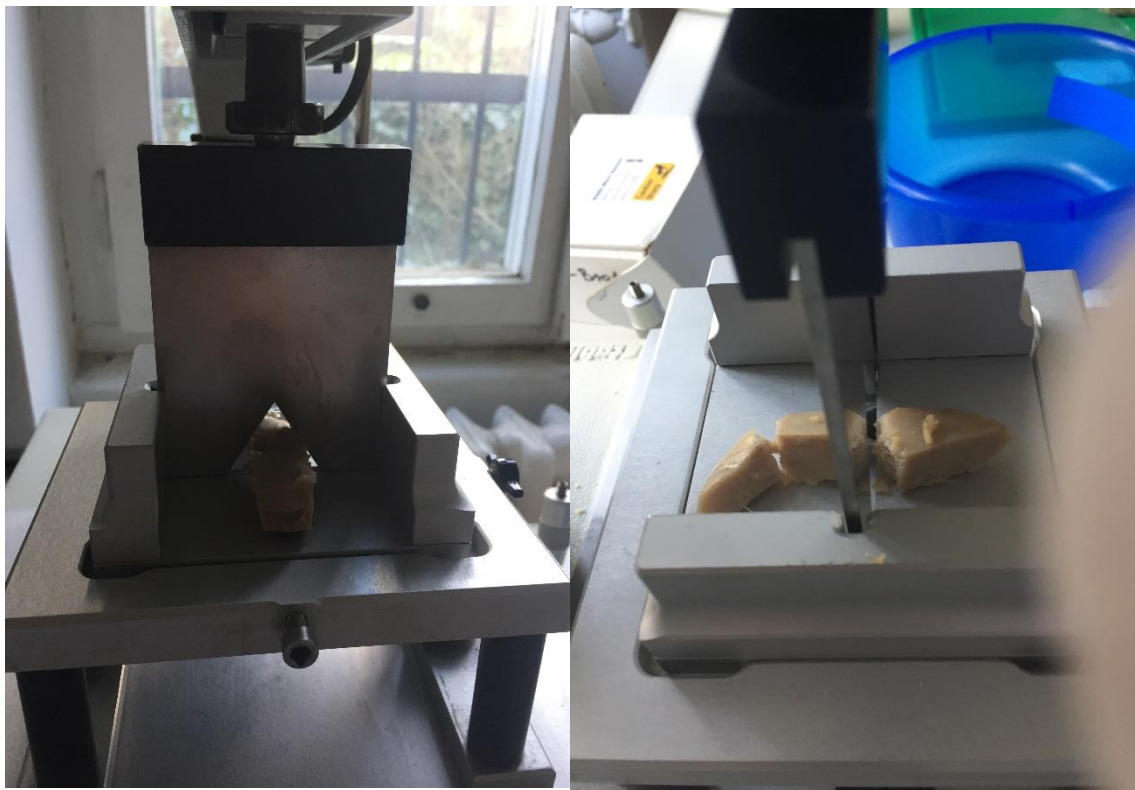
Table 2: The relationship between the sense of color difference and  $\Delta E^*_{ab}$  color coordinates.

$\Delta E^*_{ab}$	Sense of difference
<b>0.....0.5</b>	Not noticeable
<b>0.5....1.5</b>	Barely noticeable
<b>1.5....3.0</b>	Noticeable
<b>3.0....6.0</b>	Clearly visible
<b>6.0...12.0</b>	Big

### 3.6 Texture analysis

The tenderness of the heat treated hamburger patties was investigated with the Warner-Bratzler test, recording the maximum force required to cut through the samples. The hamburger patties were cut to about 1.5 cm wide and about 1.3-1.6 high pieces. Five

parallel measurements were performed on every mixture. For the tests, a TXTA texture analyzer (Stable Micro System Inc. Surrey, UK), equipped with a Warner-Bratzler fixture (see Figure 6.) was used, which consists of a steel frame supporting a triangular shear blade. The samples were placed under the blade, then the blade cut through the samples with a 2mm/s speed. The maximum force and the work required to perform the cut were recorded. Beside the 10 minute heat treatment on 90 °C, the samples were placed in the oven on 200 °C for 20 minutes in order to reconstruct the conditions at the sensory testing, allowing the comparison of instrumental and sensory data. However, as it was difficult to maintain the same hot temperature as during the sensory panel, the samples were all rested after baking, until they reached room temperature, so all samples had the same temperature during the texture analysis.



*Figure 6. Warner-Bratzler test*

### 3.7 Sensory evaluation

Sensory evaluation (see Figure 7.) was carried out by a panel of 54 members recruited from the employees of Wessling Hungary Ltd., where the sensory evaluation was performed. Randomized samples were served for the panelists, evaluating the overall appearance, color of surface, texture, taste and odor, using 5 point scales (1-unacceptable,

2-bad, 3-satisfactory, 4-good, 5-very good). Overall, 60x100g of samples from each recipe were prepared for the test. The 6 additional samples per mixture were used as emergency samples, in case some of the samples get damaged and to perform a test cooking before the start of the panel. The heat treated, vacuum packaged hamburger patties were stored between 0-4°C until the sensory evaluation. The samples were removed from the refrigerator about 30 minutes before cooking. The samples were removed from their vacuum bag and placed in the oven and cooked for 200°C for 20 minutes, until the meat turned golden brown. All five mixtures of hamburger patties were served hot for the panelists. Two panelists were performing evaluation at the same time.



*Figure 7. Sensory evaluation of the hamburger patties.*

### 3.8 Statistical analysis

The statistical analysis of the results was performed with SPSS Statistics 22.0 (IBM Corp., Armonk, NY). Data were expressed as mean  $\pm$  SD. Significant differences were evaluated at  $p < 0.05$  by analysis of variance (ANOVA) and Duncan's multiple range test.



## 4.0 Results and Discussion

### 4.1 Experiment one - Composition and Microbiology of MSCM

In order to determine, whether the mechanically separated carp meat is suitable for the production of a chilled product, the chemical and microbiological properties of the fish pulp was tested. During the experimental phase of the master thesis, two batches of MSCM were received from the supplier. Five parallel samples from both batches were immediately analyzed after receiving the raw materials. The two batches have arrived about one month apart. In the comparison, we were investigating, if there is any difference in the microbiological and chemical quality of the raw materials. For the production of a good quality product, the composition of the raw materials has to be stationary.

#### 4.1.1 Chemical composition of the mechanically separated carp meat

The composition of the mechanically separated carp meat utilized from backbones and cutoffs is shown in Table 3. Overall, both batches were characterized by low protein and high fat content, compared to the properties of carp fillets. Furthermore, the MSCM contains high concentration of calcium, originated from the bone fractures grinded in the mince during the separation process. In order to compensate for this high fat and low protein content, it was decided to use minced meat from relatively low-fat parts of pork and turkey in the recipes of the hamburger patties.

A significant difference ( $p < 0.05$ ) was found between the protein, fat, and calcium content of the two batches. As the two batches were received only a month apart, this change could be caused by a difference in the types of rest raw materials used, rather than the result of seasonal deviations in the meat quality of the fish.

Table 3: Chemical composition of the two received batches of mechanically separated carp meat utilized from backbones and cutoffs.

Parameter	Unit	First batch	Second batch
<b>Protein</b>	w/w%	12.60±0.76*	11.32±0.26
<b>Fat</b>	w/w%	20.04±2.22	22.84±0.87*
<b>Ash</b>	w/w%	0.84±0.04*	0.74±0.15
<b>Calcium</b>	mg/kg	42640±2480	58340±4681*

Mean value ± standard deviation of five individual measurements.

\* Data differ significantly ( $p < 0.05$ ).

Due to the mechanically separated meat (MSM) production process, bones are crushed and an elevated amount of bone particles is to be expected in such meats, which contain high levels of calcium. In MSM, the bone content and consequently the calcium

content are generally higher as compared to fresh meat. Therefore, the calcium content is frequently used as one of the criteria to identify MSM. High bone content means that the pressure used in the deboning process was too high or that the meat to bone ratio was too low (EFSA, 2013). The significant difference in the calcium content of the two batches supports the explanation given for the difference in the other parameters. The higher calcium content indicates that in the second batch, the separated rest raw materials contained more backbones and less meaty cutoffs.

In general, protein content is lower in MSM because raw materials used for mechanical deboning are richer in lipids and it contains more collagen and less myofibrillar proteins, than minced meat. Furthermore, mechanical separation affects the lipid composition of the resulting meat, normally having higher lipid content than fillets. These extra lipids may originate from subcutaneous fat as well as from the skin or from abdominal fat, but it can also originate from the phospholipids from the fraction of bone and from accompanying spinal marrow (Trindade, Felício, & Castillo, 2004). This is in agreement with the data recorded by Tokur and Ozku (2006), comparing the chemical composition of filleted and separated silver carp meat. They observed, that the separation resulted in much higher fat content, than the filleting.

The fatty acid composition of the MSCM is shown at Table 4. As it was expected for fat that is originating in fish, it is mainly composed of unsaturated fatty acids. The predominant fatty acids were oleic acid: 18:1n-9, contributing to 43.77% in the first batch and 47.44 % of the total fatty acid content of the second batch, and palmitic acid: C16:0 as the main saturated fatty acid with 20.04 % in the first batch and 22.84 % in the second batch. The dominant polyunsaturated fatty acids in the first batch were found to be linoleic acid: C18:2, n-6,  $\alpha$ -linolenic acid: C18:3n3 eicosapentaenoic acid: C 20:5 at 5.70%, 1.94% and 1.16% respectively. In the second batch, the eicosapentaenoic acid content was significantly lower (0.46 %) and the linolenic acid was higher (6.51 %). The results are in accordance with the data observed by many authors (Buchtová, 2011; Mai & Kinsella, 1981) while measuring the fatty acid profile of carp.

One of the most important components of fish meat are the polyunsaturated fatty acids. Fish and fishery products are sources of omega-3 polyunsaturated long-chain fatty acids such as eicosapentaenoic acid: 20:5n-3 and docosahexaenoic acid: 22:6n-3. These fatty acids have been demonstrated to be important to human health, and substantial

benefits are reported in relation to diseases of the cardiovascular system by the reduction of blood pressure and of the concentrations of triglycerides in the blood (EFSA, 2009). Marine fish generally contain a higher percentage of n-3 LC PUFA than freshwater fish (Branciari et al., 2017).

The fat content of fish was found to be dependent on the size, age, behavioral and metabolic differences (Fauconneau et al., 1995), sex cycle, nutritional and health status, and certain other conditions in the external environment of the fish (Bauer & Schlott, 2009). However, the main factor controlling the fat content and fatty acid composition was found to be the diet of the fish (Branciari et al., 2017; Fauconneau et al., 1995).

Common carp farmed in aquaculture systems preferably consume cereals (wheat), therefore their fat contains high amounts of oleic acid (C18:1n-9), which can directly come from the vegetable oils in the feed or is produced by the desaturation of saturated fatty acids synthesized in the fish organism from energy-rich supplemented feed. The low proportion of  $\Sigma\text{PUFA}_{n-3}$  suggests that the natural potential of the common carp's organism to produce more polyunsaturated n-3 fatty acids is relatively low when raised in aquaculture systems (Buchtová, 2011). The supply of polyunsaturated n-3 fatty acids in the feed would be required for the enrichment of these lipids in the carcass (Fauconneau et al., 1995).

Although the mechanically separated carp meat had high fat content, it was found to be an unreliable source of EPA and DHA, considering their low amount and the differences in their concentration between the two batches.



Table 4: Fatty acid composition of the two received batches of mechanically separated carp meat utilized from backbones and cutoffs. SFA: Saturated Fatty Acid; MUFA: Monounsaturated Fatty Acid; PUFA: Poly Unsaturated Fatty Acid; UFA: Unsaturated Fatty Acid

% of total fatty acids		First batch	Second batch
Myristic acid	C14:0	1.40±0.02*	1.06±0.03
Palmitic acid	C16:0	20.04±2.22	22.84±0.87*
Margaric acid	C17:0	0.26±0.01*	0.14±0.04
Stearic acid	C18:0	4.97±0.07	5.23±0.06*
Arachidic acid	C20:0	0.07±0.00	0.09±0.01*
Henicosanoic acid	C21:0	0.15±0.08	0.18±0.01
Cerotic acid	C26:0	0.20±0.01*	0.09±0.02
<b>ΣSFA</b>		<b>27.88±0.22*</b>	<b>26.56±0.22</b>
Myristoleic acid	C14:1n9c	0.10±0.01*	0.08±0.01
Palmitoleic acid	C16:1n9c	10.28±0.06*	9.22±0.11
Heptadecenoic acid	C17:1	<0.05	0.17±0.02*
Oleic acid	C18:1n9c	43.77±0.29	47.44±0.39*
Eicosenoic acid	C20:1n9	1.86±0.03	2.11±0.03*
Erucic acid	C22:1n9	0.17±0.21*	0.06±0.03
<b>ΣMUFA</b>		<b>59.62±0.25</b>	<b>62.33±0.49*</b>
Linoleic acid	C18:2n6c	5.70±0.05	6.51±0.16*
α-Linolenic acid	C18:3n3	1.94±0.10*	0.92±0.04
γ-Linolenic acid	C18:3n6	0.13±0.00	0.17±0.02*
Eicosadienoic acid	C20:2	0.24±0.05	0.26±0.01
Eicosatrienoic acid	C20:3n3	0.11±0.01*	0.06±0.03
cis-8,11,14	C20:3n6	0.19±0.01	0.24±0.01*
Eicosatrienoic acid			
Arachidonic acid	C20:4n6	0.50±0.02	0.47±0.04
Eicosapentaenoic acid	C20:5n3	1.16±0.04*	0.46±0.05
Docosahexaenoic acid	C22:6n3	0.38±0.02*	0.28±0.03
<b>ΣPUFA</b>		<b>10.35±0.20*</b>	<b>9.34±0.30</b>
<b>ΣPUFA<sub>n3</sub></b>		<b>3.60±0.14*</b>	<b>1.68±0.11</b>
<b>ΣPUFA<sub>n6</sub></b>		<b>6.52±0.06</b>	<b>7.40±0.19*</b>
<b>ΣUFA</b>		<b>69.97±0.19</b>	<b>71.67±0.64*</b>

Mean value ± standard deviation of five individual measurements.

\* Data differ significantly (p<0.05).

#### 4.1.2 Microbiological properties of the mechanically separated carp meat

The results of the microbiological analysis performed on the MSCM is shown in Table 5.

Table 5: Results of microbiological analysis of the two received batches of mechanically separated carp meat utilized from backbones and cutoffs; TBC: Total bacterial count; CFU: Column forming unit.

Parameter	First batch	Second batch
<b>TBC (log<sub>10</sub> CFU/g)</b>	5.19±0.33	5.74±0.33*
<b><i>Enterobacteriaceae</i> (log<sub>10</sub> CFU/g)</b>	4.14±0.06*	2.67±0.21
<b><i>L.monocytogenes</i> (presence/25g)</b>	positive	positive
<b><i>L. monocytogenes</i> (count/g)</b>	<10	<10
<b>Sulphite-reducing <i>Clostridia</i> (count/g)</b>	<10	<10
<b><i>E.coli</i> (count/g)</b>	<10	<10
<b><i>Staphylococcus aureus</i> (count/g)</b>	<10	<10
<b><i>Salmonella ssp.</i> (presence/25g)</b>	negative	negative

Mean value ± standard deviation of five individual measurements.

\* Data differ significantly (p<0.05).

The total bacterial count and *Enterobacteriaceae* count of both batches reflects the poor microbiological properties of mechanically separated meat utilized from rest raw materials, as the samples reached 5-6 log CFU/g until the time of microbiological analysis, which happened about 2-3 days after the processing of rest raw materials. As the mechanically separated meat was already very close to the  $N=10^7$  CFU/g after 2-3 days of chilled storage- when signs of deterioration start to appear (Broekaert *et al.*, 2011) and considered as the threshold of microbial spoilage-it is recommended to freeze the MSCM until later processing. Significant difference has been found between the two batches in both total bacterial count and *Enterobacteriaceae* count. The higher proportion of *Enterobacteriaceae* in the first batch could be explained by the residues of the intestinal tract on the backbones used for separation, or with the poor hygiene of the processing equipment.

The presence of *Listeria monocytogenes* was detected in all samples. This could be the sign of bad hygienic conditions during the processing of the fish and its raw materials. As *L. monocytogenes* is considered to be a dangerous human pathogenic microorganism, its presence further increases the necessity of frozen storage until the production of fish burger patties.

The results of microbiological analysis was in accordance with the results of Raccach and Baker (1978), who experienced the spoilage of mechanically deboned meat

from cod, pollock and whiting after 3 and 4 days of storage with the corresponding total bacterial count of  $1.0\text{-}2.5 \times 10^8\text{CFU/g}$ . Payandan, Sayyed-alangi and Shamloofar (2017) were also reporting  $6.75 \log \text{CFU/g}$  total bacterial count in carp mince after three days of chilled storage.

Mechanical separation of meat was found to result in a high degree of muscle fiber destruction, which releases intracellular fluids rich in nutrients and of low acidity that supports bacterial growth. Furthermore, the potential rise in temperature during mechanical separation could also support bacterial growth (EFSA, 2013). This makes the mechanically separated meat substantially more perishable than fish fillets.

Considering the results of the microbiological analysis, mechanically separated meat utilized from rest raw materials of carp processing is not suitable for raw, chilled products, due to its rapid deterioration and the presence of pathogenic bacteria. Freezing of the fish paste immediately after processing and the pre heat-treatment of the hamburger patties prepared from the MSCM is recommended, in order to get a microbiologically stable product. Heat treatment at  $90^\circ\text{C}$  for 10 minutes that was applied to the samples is recommended for cooked-chilled products with an extended shelf-life of more than 10 days (Can, 2011).

## 4.2 Experiment two - Effects of mixing MSCM with meat in different ratios

In order to evaluate the effects of mixing mechanically separated carp meat in different ratios with turkey and pork meat for hamburger patties, pH, color, water holding capacity and the hardness of the samples were tested. Furthermore, a sensory evaluation was performed, in order to determine how the addition of pork and turkey meat changes the consumer acceptance of the product recipes. The combination of sensory and instrumental tests allowed to identify the actual product properties contributing to the scores of the sensory panel. This could give further information to see what needs to be changed, in order to achieve better consumer acceptance for the product.

### 4.2.1 pH measurement

The results of the pH measurement are shown on Figure 8. The highest pH was measured in the control samples, containing 100% fish meat. With the increase of the non-fish ingredients, the pH of the sample decreased. The pH of all samples was found to be significantly different from each other.

As it has been reported by several authors, fresh fish meat's pH is generally close to neutral. The pH of fish mince was reported to be around 6.5-6.8 (Asgharzadeh et al., 2010; Gelman & Benjamin, 1989; Haq et al., 2013). After death, the pH of muscle decreases, based on the amount of glycogen present, which is converted anaerobically to lactic acid during the pre-rigor period. Generally, fish present relatively lower residual glycogen levels than mammals (Palmeira et al., 2016). This is in agreement with the pH of 5.3-5.8 (Furtado et al., 2019) reported in pork meat. In turkey thigh meat, the pH of 5.8-6.5 was recorded (Barbut, 2007).

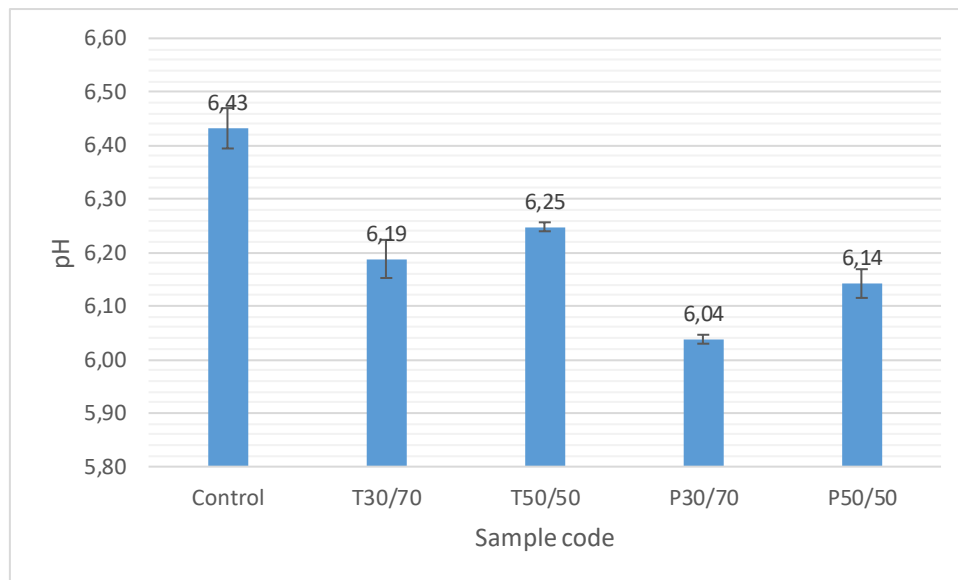


Figure 8: pH of the raw hamburger patties prepared from the mixture of mechanically separated carp meat with pork and turkey meat; P30/70: 30-70% mixture of pork and carp meat; P50/50: 50-50% mixture of pork and carp meat; T30/70: 30-70% mixture of turkey and carp meat; T50/50: 50-50% mixture of turkey and carp meat; Control: 100% carp meat.

The pH values measured in the control samples are slightly lower than the ones reported in the literature. Especially, because mechanically separated fish tends to have higher pH due to its higher moisture content compared to fish fillets (Palmeira et al., 2016). Possible explanation for the lower pH could be the poor slaughtering technology of the fish, as high exposure to stress before death could result in lower pH (Lyu et al., 2015). Furthermore, the reduction in pH can also be attributed to the addition of salt to the mixtures.

#### 4.2.2 Water holding capacity

The water holding capacity of the raw burger patties is shown in Figure 9.

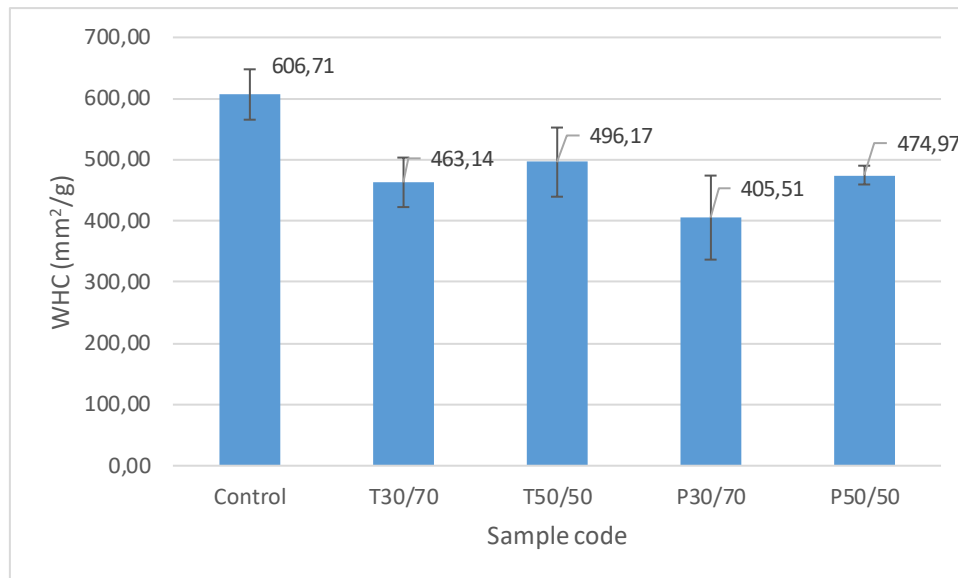


Figure 9: Water holding capacity of the raw hamburger patties prepared from the mixture of mechanically separated carp meat with pork or turkey meat, expressed as mm<sup>2</sup> wet strain left by 1 g meat; P30/70: 30-70% mixture of pork and carp meat; P50/50: 50-50% mixture of pork and carp meat; T30/70: 30-70% mixture of turkey and carp meat; T50/50: 50-50% mixture of turkey and carp meat; Control: 100% carp meat.

Water holding capacity (WHC) of the raw samples is an important property of the hamburger patties, since it indicates the expected water loss during the heat treatment. Compared to the control sample, all samples released less water by the end of the five minute pressing time. Significant difference ( $p < 0.05$ ) was found between the control and the other samples. Furthermore, the 30-70% mixture of carp and pork meat left significantly smaller water stain than the other samples. The pH of the muscle plays an important role in the number and distribution of charged groups on the protein molecules. At the isoelectric point the net charge is zero and therefore WHC is at its lowest (Offer & Trinick, 1983). Although the samples with the higher pH were expected to have better WHC, the opposite effect has been observed.

The sample with the highest pork meat concentration released the least moisture, which can be explained as while the pork and turkey meat was minced, resulting in a more intact meat structure, the fish meat was pressed through a perforated drum with much smaller holes, resulting in a finer structure. Due to the poor microbiological quality of the MSCM and the schedule of the experiments, the raw materials had to be frozen until the further experiments. This frozen storage could induce the freeze denaturation of the fish proteins, further reducing the WHC.

Another reason for the result above can be that beside the pH of the meat, the protein content can also have an influence on the water holding capacity. Mechanically separated meats were found to be relatively low in protein both in quantity and quality as it contains

more collagen and less myofibrillar proteins than minced meat. These can negatively influence overall protein functionality by decreasing the ability to retain water during processing and storage, to emulsify lipids and to form a stable gel during cooking (Froning & Mckee, 2001).

Further explanation of this phenomenon could be the difference in the toughness of the samples. Due to the softness of the control sample, the deformation caused by the 500g weight was bigger than that of the much tougher pork meat. Due to the deformation, the meat sample came into contact with more of the filter paper, increasing the size of the wet strain. This might lead to the underestimation of the WHC.

#### 4.2.3 Warner-Bratzler test

Texture is a very important property of food products. The results of the Warner-Bratzler test are shown in Figure 10. As the work required to cut through the samples followed the same trend as the maximum force required for the cut, only the recorded maximum forces are discussed in this project. The maximum force values give information about the hardness of the samples.

All samples were found significantly harder than the control sample. In general, the hardness was increasing with the decrease of fish content in the mixture. Samples containing higher percentage of pork meat were found significantly harder than the mixtures of turkey and fish.

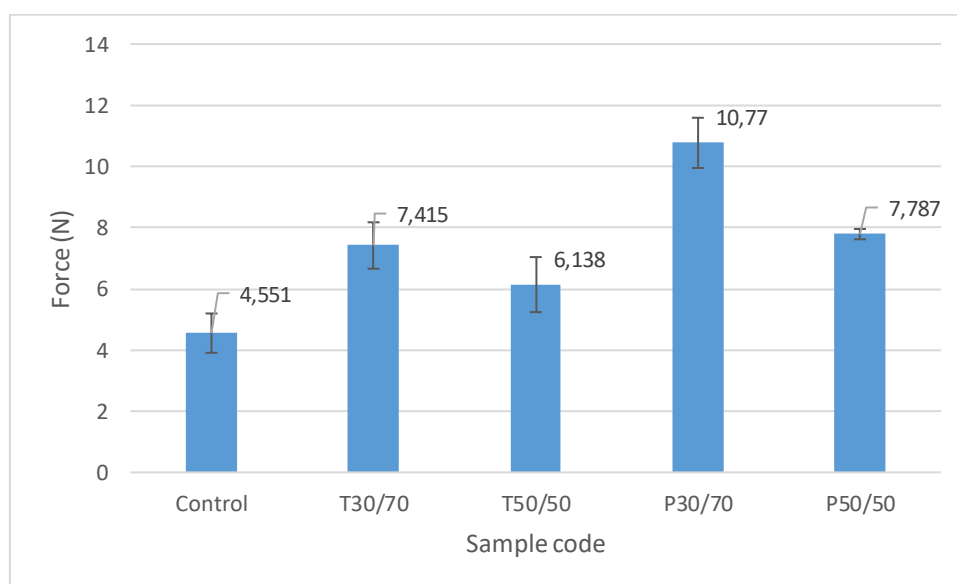


Figure 10: Hardness of the heat treated hamburger patties prepared from the mixture of mechanically separated carp meat with pork or turkey meat, expressed as the maximum force required to cut through the samples; P30/70: 30-70% mixture of pork and carp meat; P50/50: 50-50% mixture of pork and carp meat; T30/70: 30-70% mixture of turkey and carp meat; T50/50: 50-50% mixture of turkey and carp meat; Control: 100% carp meat.

As the results of the Warner-Bratzler test were following similar trends as the filter paper test performed to measure WHC of the samples, the results support the assumption that the hardness of mixtures could affect the outcomes of the filter paper test.

Both the literature (see section 1.9) and the measured data supports the hypothesis that the addition of pork or poultry meat increases the hardness of the hamburger patties. As the soft texture of fish is a common downside for the consumer acceptance of fish burger products, this increase could have the desired effect for a product targeting an audience less familiar with the properties of 100% fish products.

#### 4.2.4 Color measurement

The CIELAB color coordinates recorded on the surface of heat treated hamburger patties are shown in Table 6.

Table 6: CIELAB color coordinates of the heat treated hamburger patties prepared from the mixture of mechanically separated carp meat with pork or turkey meat; P30/70: 30-70% mixture of pork and carp meat; P50/50: 50-50% mixture of pork and carp meat; T30/70: 30-70% mixture of turkey and carp meat; T50/50: 50-50% mixture of turkey and carp meat; Control: 100% carp meat.

Sample code	CIELAB color coordinates		
	L*	a*	b*
<b>Control</b>	62.08±0.60 <sup>a</sup>	3.01±0.33 <sup>a</sup>	10.39±0.78 <sup>a</sup>
<b>T30/70</b>	57.83±1.69 <sup>c</sup>	5.22±0.46 <sup>d</sup>	10.07±1.43 <sup>a</sup>
<b>T50/50</b>	58.50±0.82 <sup>c</sup>	4.76±0.29 <sup>c</sup>	9.96±0.45 <sup>a</sup>
<b>P30/70</b>	63.69±0.95 <sup>b</sup>	4.17±0.10 <sup>b</sup>	9.95±0.62 <sup>a</sup>
<b>P50/50</b>	60.30±1.30 <sup>b</sup>	3.88±0.25 <sup>b</sup>	8.41±0.56 <sup>b</sup>

\* Mean value ± standard deviation of five individual measurements.

\* Data bearing different superscripts in the same column differ significantly (p<0.05).

Overall, all mixtures were found to be significantly different in the L\* black-white and a\*green-red color scales. All samples, but the 30-70 % mixture of pork and fish, were found to be darker than the control sample, as it is indicated by the lower L\* values.

On the green-red color scale, all samples were found to be significantly redder than the control sample. The a\* values did increase with the decrease of the fish content. The addition of turkey meat was found to increase the redness of the patties. In the b\* values, no difference was found between the samples. Only the 50-50 pork sample had a slightly less yellow color.



The  $\Delta E^*_{ab}$  color differences were calculated from the  $L^*$ ,  $a^*$  and  $b^*$  values, expressing if the color difference between the samples is detectable by human eye. The calculated results are shown on Table 7.

Table 7: The  $\Delta E^*_{ab}$  color difference between the different mixtures the heat treated hamburger patties prepared from the mixture of mechanically separated carp meat with pork or turkey meat; P30/70: 30-70% mixture of pork and carp meat; P50/50: 50-50% mixture of pork and carp meat; T30/70: 30-70% mixture of turkey and carp meat; T50/50: 50-50% mixture of turkey and carp meat; Control: 100% carp meat.

Sample code	$\Delta E^*_{ab}$ color difference				
	Control	T30/70	T50/50	P30/70	P50/50
<b>Control</b>	-	4.79	4.01	2.03	2.79
<b>T30/70</b>	4.79	-	0.82	5.95	3.26
<b>T50/50</b>	4.01	0.82	-	5.22	2.53
<b>P30/70</b>	2.03	5.95	5.22	-	3.73
<b>P50/50</b>	2.79	3.25	2.53	3.73	-

As the results show, there are noticeable or clearly visible difference between the samples, except of the mixtures of turkey and fish where the difference is barely noticeable. The biggest difference was found between turkey and pork meat, due to the differences in redness and lightness of the patties.

#### 4.2.5 Sensory evaluation

The sensory evaluation of the hamburger patties was performed by 54 panelists. The panelists were asked to evaluate on a scale of 1-5, how much they like fish. Based on the answers, the results were separated into two groups. Panelist answering between 1 and 3 were placed in the non-fish liking group and the ones answering with 4 or 5 were placed in the fish liking group. The results of the sensory evaluation are shown in Table 8.

In general, all mixtures achieved satisfactory scores in all attributes. In the non-fish liking group, no significant difference was found between the different mixtures, other than that the 50-50% mixtures of turkey and fish meat received a better score on their general appearance. In the fish liking group, the general appearance of 50-50 turkey and fish mixture, the odor of 30-70 pork and fish mixture and the taste of both 30-70 pork and turkey mixtures with fish received significantly lower score than the other samples.

Table 8: Sensory scores of the heat treated hamburger patties prepared from the mixture of mechanically separated carp meat with pork or turkey meat; P30/70: 30-70% mixture of pork and carp meat; P50/50: 50-50% mixture of pork and carp meat; T30/70: 30-70% mixture of turkey and carp meat; T50/50: 50-50% mixture of turkey and carp meat; Control: 100% carp meat.

Sample code	Non-fish liking (1-3) group				
	General appearance	Color	Texture	Odor	Taste
Control	2.96±1.19 <sup>a</sup>	3.39±0.94 <sup>a</sup>	3.35±1.11 <sup>a</sup>	3.43±0.84 <sup>a</sup>	3.30±1.11 <sup>a</sup>
T30/70	3.61±0.94 <sup>ab</sup>	3.26±0.01 <sup>a</sup>	3.70±0.76 <sup>a</sup>	3.48±1.08 <sup>a</sup>	3.74±0.92 <sup>a</sup>
T50/50	3.65±1.19 <sup>b</sup>	3.65±0.88 <sup>a</sup>	3.83±1.89 <sup>a</sup>	3.57±1.08 <sup>a</sup>	3.70±1.11 <sup>a</sup>
P30/70	3.48±0.85 <sup>ab</sup>	3.30±0.88 <sup>a</sup>	3.26±0.86 <sup>a</sup>	2.96±1.07 <sup>a</sup>	3.09±0.85 <sup>a</sup>
P50/50	3.39±0.94 <sup>ab</sup>	3.61±0.23 <sup>a</sup>	3.43±0.90 <sup>a</sup>	3.30±0.82 <sup>a</sup>	3.57±1.11 <sup>a</sup>
Sample code	Fish liking (4-5) group				
	General appearance	Color	Texture	Odor	Taste
Control	3.94±1.09 <sup>a</sup>	3.77±0.80 <sup>a</sup>	4.00±1.13 <sup>a</sup>	4.07±0.96 <sup>a</sup>	4.19±1.08 <sup>a</sup>
T30/70	3.26±1.00 <sup>b</sup>	3.19±0.95 <sup>b</sup>	3.65±1.11 <sup>a</sup>	3.55±0.85 <sup>a</sup>	3.52±1.03 <sup>b</sup>
T50/50	3.71±0.82 <sup>ab</sup>	3.68±0.91 <sup>a</sup>	3.77±0.88 <sup>a</sup>	3.84±0.86 <sup>a</sup>	3.87±0.81 <sup>ab</sup>
P30/70	3.58±0.89 <sup>ab</sup>	3.61±0.80 <sup>ab</sup>	3.84±0.86 <sup>a</sup>	3.07±1.03 <sup>b</sup>	3.65±0.95 <sup>b</sup>
P50/50	3.81±0.91 <sup>a</sup>	3.77±0.96 <sup>a</sup>	4.00±0.81 <sup>a</sup>	3.65±1.02 <sup>a</sup>	3.84±0.93 <sup>ab</sup>

\* Mean value ± standard deviation of 54 individual measurements.

\* Data bearing different superscripts in the same column differ significantly ( $p < 0.05$ ).

The comparison of the two groups showed that there is significant difference between the scores of General appearance, Texture, Odor and Taste. Although there are no major differences between the scores of the initial mixtures within the groups, the members of the non-fish liking group gave generally lower scores for all samples compared to the fish liking group. Although substantial differences were revealed in the hardness and color of the different mixtures in the previous instrumental tests, these differences could not be observed in the results of the sensory evaluation.

The panelists were asked to put the samples in order, based on the intensity of their fish taste and odour. The answers of the panelists are summarized in Table 9. As it was expected, most of the panelists placed the control sample in the first place, as it contains 100% fish meat. The second most intense in fish taste were found to be the two 50-50% mixtures. The least intense fish taste was experienced in the 30/70 % samples. At the fourth place, slightly more panelists decided on the samples containing pork meat. The

50-50 mixture of pork and fish was found to be the sample with the second least intensive fish taste. It might be possible that the more characteristic taste of pork is able to compensate the fish taste more effectively than turkey meat.

*Table 9: Ranking of the heat treated hamburger patties prepared from the mixture of mechanically separated carp meat with pork or turkey meat based on the intensity of fish taste and odor; P30/70: 30-70% mixture of pork and carp meat; P50/50: 50-50% mixture of pork and carp meat; T30/70: 30-70% mixture of turkey and carp meat; T50/50: 50-50% mixture of turkey and carp meat; Control: 100% carp meat.*

Sample code	Intensity of fish taste / odor				
	1 most intense	2	3	4	5 least intense
<b>Control</b>	37	3	3	5	6
<b>T30/70</b>	5	10	8	11	20
<b>T50/50</b>	3	18	19	10	4
<b>P30/70</b>	5	7	11	13	18
<b>P50/50</b>	4	16	13	15	6

Overall, every mixture received a satisfactory score in all categories. While the ranking of intensity of fish taste and odor shows that the panelists identified the differences in the fish content of samples, it did not influence the sensory scores in the non-fish liking group. However, in the fish liking group, samples with higher fish content received significantly better scores than the 30-70% mixture of pork, turkey and fish.

#### 4.3 Experiment three - Effects of different mixing ratios on shelf-life

The eight day storage test was aiming to determine the shelf-life of the product and to evaluate whether the applied heat treatment sufficiently eliminated the high microbial count and pathogenic microorganisms detected in the mechanically separated carp meat. The same microbiological properties were analyzed as in case of the MSCM. The analysis was performed on the 0<sup>th</sup>, 3<sup>rd</sup>, 6<sup>th</sup> and 8<sup>th</sup> days of chilled storage. Unfortunately, some of the vacuum bags, in which the samples were packaged before the heat treatment got damaged during the storage. The results of microbiological analysis performed on the hamburger patties with an undamaged packaging are shown in Table 10.

As the results show, the heat treatment at 90°C for 10 minutes did successfully eliminate the high microbial count and *Listeria monocytogenes* detected in the raw material. No bacterial growth was observed on any of the undamaged samples during the eight day storage.

Table 10: Effects of the mixing ratio and storage time on the microbiological properties of the undamaged heat treated hamburger patties prepared from the mixture of mechanically separated carp meat with pork or turkey meat; P30/70: 30-70% mixture of pork and carp meat; P50/50: 50-50% mixture of pork and carp meat; T30/70: 30-70% mixture of turkey and carp meat; T50/50: 50-50% mixture of turkey and carp meat; Control: 100% carp meat.

Sample code	Number of undamaged samples	Total Bacteria (count/g)	<i>Listeria monocytogenes</i> (presence in 25g)	<i>Listeria monocytogenes</i> (count / g)	Sulphite-reducing <i>Clostridia</i> (count / g)	<i>Staphylococcus aureus</i> (count / g)	<i>E.coli</i> (count / g)	<i>Enterobacteriaceae</i> (count / g)	<i>Salmonella ssp.</i> (presence in 25 g)
<b>Day 0</b>									
Control	2	<10	negative	<10	<10	<10	<10	<10	negative
T30/70	3	<10	negative	<10	<10	<10	<10	<10	negative
T50/50	1	<10	negative	<10	<10	<10	<10	<10	negative
P30/70	1	<10	negative	<10	<10	<10	<10	<10	negative
P50/50	2	<10	negative	<10	<10	<10	<10	<10	negative
<b>Day 3</b>									
Control	3	<10	negative	<10	<10	<10	<10	<10	negative
T30/70	2	<10	negative	<10	<10	<10	<10	<10	negative
P30/70	3	<10	negative	<10	<10	<10	<10	<10	negative
P50/50	3	<10	negative	<10	<10	<10	<10	<10	negative
<b>Day 6.</b>									
Control	3	<10	negative	<10	<10	<10	<10	<10	negative
T30/70	3	<10	negative	<10	<10	<10	<10	<10	negative
T50/50	1	<10	negative	<10	<10	<10	<10	<10	negative
P30/70	2	<10	negative	<10	<10	<10	<10	<10	negative
P50/50	3	<10	negative	<10	<10	<10	<10	<10	negative
<b>Day 8.</b>									
Control	3	<10	negative	<10	<10	<10	<10	<10	negative
T30/70	1	<10	negative	<10	<10	<10	<10	<10	negative
T50/50	3	<10	negative	<10	<10	<10	<10	<10	negative
P30/70	3	<10	negative	<10	<10	<10	<10	<10	negative
P50/50	3	<10	negative	<10	<10	<10	<10	<10	negative

\* mean value of the undamaged samples from three individual measurements.

While the undamaged samples were found to be microbiologically stable during the eight days of storage, substantial growth of bacteria was observed in the damaged samples. The results of microbiological analysis performed on the hamburger patties with damaged packaging are shown in Table 11.

Table 11: Effects of the mixing ratio and storage time on the microbiological properties of the damaged heat treated hamburger patties prepared from the mixture of mechanically separated carp meat with pork or turkey meat; P30/70: 30-70% mixture of pork and carp meat; P50/50: 50-50% mixture of pork and carp meat; T30/70: 30-70% mixture of turkey and carp meat; T50/50: 50-50% mixture of turkey and carp meat; Control: 100% carp meat.

Sample code	Total Bacteria (count /g)	Listeria monocytogenes (presence in 25g)	Listeria monocytogenes (count / g)	Sulphite-reducing Clostridia (count / g)	Staphylococcus aureus (count / g)	E.coli (count / g)	Enterobacteriaceae (count / g)	Salmonella ssp. (presence in 25 g)
<b>Day 0</b>								
Control 1.	2,0*10 <sup>1</sup>	negative	<10	<10	<10	<10	<10	negative
T50/10 1.	1,3*10 <sup>5</sup>	negative	<10	<10	<10	<10	5,1*10 <sup>2</sup>	negative
T50/50 2.	1,0*10 <sup>1</sup>	negative	<10	<10	<10	<10	<10	negative
P50/50 1.	2,0*10 <sup>1</sup>	negative	<10	<10	<10	<10	<10	negative
<b>Day 3</b>								
T50/50 1.	3,1*10 <sup>2</sup>	negative	<10	<10	<10	<10	<10	negative
T50/50 2.	5,8*10 <sup>5</sup>	negative	<10	<10	<10	<10	4,5*10 <sup>2</sup>	negative
T50/50 3.	9,8*10 <sup>6</sup>	negative	<10	<10	<10	<10	3,2*10 <sup>5</sup>	negative
T30/70 1.	3,8*10 <sup>6</sup>	negative	<10	<10	<10	<10	1,7*10 <sup>4</sup>	negative
<b>Day 6.</b>								
T50/50 1.	2,8*10 <sup>7</sup>	negative	<10	<10	<10	<10	8,4*10 <sup>3</sup>	negative
T50/50 2.	3,0*10 <sup>7</sup>	negative	<10	<10	<10	<10	1,5*10 <sup>4</sup>	negative
P30/70 1.	2,4*10 <sup>3</sup>	negative	<10	<10	<10	<10	<10	negative
<b>Day 8.</b>								
P30/70 1.	5,8*10 <sup>7</sup>	negative	<10	<10	<10	<10	2,0*10 <sup>5</sup>	negative
P30/70 2.	4,0*10 <sup>1</sup>	negative	<10	<10	<10	<10	<10	negative

The diversity of bacterial counts observed at the hamburger patties with damaged packaging might be explained by the bags losing their vacuum at different times of the storage. There is a six log difference in the total bacterial counts of the damaged 30-70 % mixture of pork and carp samples. Assumably the sample with the TBC of 5.8\*10<sup>7</sup> lost

the vacuum at the start of storage, while the one with  $4.0 \times 10^1$  leaked at the end of the storage period.

Overall, the results of the storage test indicate that the microbiological shelf-life of the vacuum packaged, cooked and chilled hamburger patties is longer than eight days. However, for the better approximation of the shelf life, a longer storage test would be beneficial. Although the samples with undamaged packaging did not show microbiological growth during the storage, the damaged samples which are assumed to have gotten damaged at the start of the tests, reached high microbial counts (respectively  $3.8-9.8 \times 10^6$ ) after three days. This makes it crucial to improve the packaging technology of the product, because about 20% of the vacuum bags lost vacuum during storage. Considering that the product is targeted for children, the microbiological safety is even more crucial. Beside the improvement of the packaging, frozen storage could further improve the safety of the product, which requires the further optimization of the recipe, as the limiting factors of shelf life are the chemical changes occurring during frozen storage.

## 4.0 Conclusion

The main objective of this study was to evaluate the viability of mechanically separated meat obtained from rest raw materials of carp processing as raw material for chilled hamburger patties and to investigate the effects of the different mixing ratios of this raw material with pork and turkey meat on the quality and consumer acceptance of the product recipes. The project included the chemical and microbiological analysis of the mechanically separated carp meat and determination of the effects of different recipes on the texture, water holding capacity and color. Furthermore, the microbiological stability of the products was evaluated.

The results show that mechanically separated meat recovered entirely from carp bones and cutoffs is characterized by low protein content (about 11.6-12.32%) and high fat content (about 22.04-22.84%). Two different batches of raw materials were investigated and significant difference has been found between all parameters, including their fatty acid composition. The notable difference between their calcium content is a good indicator of several conditions of the separation process. The considerable amount of calcium in the fish pulp could be a result of the pressure applied being too high during the separation and the composition of rest raw materials. The difference between the

calcium content of the two batches seems to be proportional with the concentration of the other parameters. The assumably higher proportion of fish bones instead of cutoffs and belly flaps resulted in higher fat and lower protein content.

The fatty acid composition of carp fat is in accordance with the literature reporting oleic acid as the main unsaturated fatty acid and palmitic acid as the dominant saturated fatty acid, in this case contributing to 43.77-47.44 % and 20.04-22.84 % of the total fatty acids. The dominant polyunsaturated fatty acids were found to be linoleic acid: C18: 2n-6  $\alpha$ -linolenic acid: C18:3n-3 and eicosapentaenoic acid: C 20:5 n-3 at 5.70%, 1.94% and 1.16% respectively in the first batch. In the second batch, the eicosapentaenoic acid content was significantly lower (0.46 %) and the linolenic acid was higher (6.51 %). All these results are well explained by the cereal based diet and the insufficient production of more polyunsaturated n-3 fatty acids in the carp's organism. Although the mechanically separated carp meat had high fat content, it was found to be an unreliable source of EPA and DHA, considering their generally low amount and the differences in their concentration in the two batches.

The detection of *Listeria monocytogenes* and the high 5-6 log CFU/g total bacterial count after 2-3 days of chilled storage shows the microbiological instability of mechanically separated carp meat and probably also the poor hygienic conditions during processing, therefore it is necessary to freeze the raw materials. It was concluded that the microbiological properties of the MSCM make it reasonable to utilize it in a pre-heat-treated chilled product.

The texture analysis supported the hypothesis that the addition of pork or poultry meat could improve the texture of the product by increasing its hardness. Significantly higher force was required to cut through both pork and turkey mixtures compared to the 100% fish samples. The recipes containing pork meat were found to be the hardest from all the mixtures. The same trend was identified at the measurement of the water holding capacity. Applying force on the control sample resulted in a substantially bigger meat juice stain than in the case of the other mixtures. The water holding capacity of the mixtures containing the least fish was found to be the highest. The differences in hardness could result in the underestimation of WHC, as the lower hardness might have resulted in bigger deformation from the force applied, increasing the contact surface between the filter paper and the sample and resulting in a bigger meat stain area. However, the results



of the WHC measurement could also be explained by the high degree of muscle fiber destruction during the separation of fish meat.

During the consumer evaluation, all mixtures got satisfactory ratings in the categories of general appearance, texture, odor, color and taste. The average ratings ranged between 3 and 4 on the scale of 5. Overall, the panelists who were less comfortable with the smell, taste and consistency of fish, gave lower ratings on these categories compared to the panelist who do like fish. The mixtures of 30-70% turkey or pork and carp meat were chosen to have the least intense fish taste and odor. Although the instrumental analysis of hardness and color shown major differences between the mixtures, no significant difference was found between the sensory scores of color and texture. Based on the sensory results, the 50-50% mixtures of turkey or pork and fish would be the most suitable recipe for the audience targeted by the project as it received high sensory scores in both groups, despite being ranked high based on the intensity of fish taste and odor. However, the 100% carp burger could also be a viable product for another target audience.

During the eight day storage test, no microbiological growth was experienced on the hamburger patties packaged in intact vacuum bags. The heat treatment of 90°C for 10 minutes was found to be effective. However, about 20% of the samples lost vacuum during the storage. The damage on the packaging resulted in the rapid spoilage of the samples, reaching  $3.8-9.8 \cdot 10^6$  total bacterial count after three days.

Everything considered, mechanically separated meat prepared entirely from rest raw materials of carp was proven to be a low quality raw material, due to its low concentration of proteins and essential n-3 PUFA, high content of fractured bones and poor microbiological properties. However, mixing it with pork or turkey meat resulted in improved water holding capacity and hardness. The main objective of the project was partly fulfilled, as a product acceptable for consumers was developed from cheap rest raw materials. However, the nutritional quality and packaging of the hamburger patties require further research in order to make a healthier and safer product.

## 5.0 Future research

In this study, the mechanically separated carp meat was purchased from one of the biggest Hungarian carp aquaculture producers. However, they could not supply enough

raw materials on demand, as during the off season, there was not enough carp processed to have enough rest raw materials. Since about 30% of the yearly carp processing happens in December, the frozen storage of the mechanically separated carp meat would be crucial if the developed product is intended to be produced during the whole year. Therefore, the utilization the rest raw materials into a surimi like frozen product which can be further applied to hamburger patties, should be considered. Using the washing steps characteristic for surimi production could result in improved water holding capacity, microbiological properties and milder fish taste, which was the objective of this study.

Considering the poor quality of the MSCM meat from rest raw materials, it would be beneficial to add a certain amount of fish fillet into the mince, improving the nutritional quality. A future study could investigate the effects of different ratios of MSCM from rest materials and fillets on the nutritional and sensory quality.

The storage test of the hamburger patties resulted in numerous damaged products. The rapid spoilage of the product in case of a leakage on the vacuum bag makes it necessary to improve the packaging technology. Optimizing the recipes on frozen storage would result in a safer product, which can be a focus of some future research and development.

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