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THE EFFECTS OF SELECTED PHOSPHATE SALTS AND HYDROCOLLOIDS ON THE TEXTURAL PROPERTIES OF MEAT PRODUCTS

VLIV VYBRANÝCH FOSFOREČNANOVÝCH SOLÍ A HYDROKOLOIDŮ NA TEXTURNÍ VLASTNOSTI MASNÝCH VÝROBKŮ

DOCTORAL THESIS

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ABSTRACT

The main aim of this study was to investigate the effects of different concentrations and types of selected phosphate salts and hydrocolloids (i.e. carrageenans) on the textural properties of meat batters made from Mechanically Deboned Poultry Meat (further only MDPM).

For this purpose, three independent studies were proposed. Firstly, the effect of different concentrations and types of selected phosphate salts on the textural properties of meat batters was analyzed. Nine types of phosphates (sodium and potassium salts of mono-, di-, tri- and polyphosphates) in the concentration range of 0-0.45% (w/w) – namely, Monosodium Phosphate (MSP), Disodium Phosphate (DSP), Trisodium Phosphate (TSP), Tetrasodium Diphosphate (TSPP), Disodium Diphosphate (SAPP), Sodium Tripolyphosphate (PSTP), Sodium Hexametaphosphate (SHMP), Tripotassium Phosphate (TKP) and Tetrapotassium Diphosphate (TKPP), with a concentration step of 0.05% were used for sample manufacture. The pH values and textural parameters like hardness, cohesiveness, adhesiveness and gumminess were determined. The results indicated that individual phosphate salt types influenced the textural samples´ textural parameters in different ways. The concentration of phosphate salts added significantly affected the change in pH values and also the textural properties of the MDPM batters. Increases in the hardness and gumminess of different samples were observed at the phosphate concentration range of 0.20-0.35%.

In the second study, selected binary phosphate salt mixtures were also tested. The three different types of phosphate chosen were TSPP, SHMP and SAPP, at the concentration of 0.25%, and with the percentage ratios of 100:0; 90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90; 0:100. Similar to the first study, the pH values and same textural parameters were also determined. The results of the second study showed that Binary Phosphate SAPP and SHMP had a strong effect on hardness, and also showed the maximum adhesiveness value reported, and with an average value of 0.3, almost reached the maximum value for cohesiveness found using TSPP and SHMP (≈0.3).

Finally, the impact of hydrocolloids on the model samples´ textural parameters was also studied. Two types of carrageenans (κ- and ι-carrageenans) were used in the concentration range of 0-0.5% (w/w), with a concentration step of 0.1%. The pH values and textural parameters were also evaluated - similar to the previous studies. The results indicated that the use of carrageenans affected the textural properties - especially hardness values. At concentration of ≈ 0.4% and ≈ 0.2% respectively for κ-carrageenan and ι-carrageenan, these showed the highest hardness value. In addition, carrageenans did not influence the pH values of meat batters.

Overall, the study demonstrates the beneficial effect of phosphates and hydrocolloids in the influence of meat batters texture-made from MDPM and also points to a good potential use of phosphates, as well as hydrocolloids, in the development of any new product in the Meat Products Processing industry.

Keywords: deboned poultry meat, batters, phosphate, hydrocolloid, textural parameters

ABSTRAKT

Cílem dizertační práce byla studie vlivu různých typů fosforečnanů a jejich koncentrací a dále hydrokoloidů (karagenanů) na texturní parametry modelových mělněných masných výrobků vyrobených z mechanicky separovaného drůbežího masa (MDPM).

Celkem byly provedeny tři studie. Nejprve byly jednotlivě testovány sodné a draselné soli fosforečnanů, a to v různých koncentracích a hodnocen jejich vliv na vybrané texturní parametry. Celkem bylo použito 9 typů fosforečnanových solí (sodné nebo draselné soli mono-, di-, tri- anebo polyfosforečnanů) v koncentracích 0-0,45% (w/w) s koncentračním krokem 0,05%: dihydrogenfosforečnan sodný (MSP), hydrogenfosforečnan sodný (DSP), fosforečnan sodný (TSP), difosforečnan sodný (TSPP), dihydrogendifosforečnan sodný (SAPP), trifosforečnan sodný (PSTP), polyfosforečnan sodný (SHMP), fosforečnan draselný (TKP) a difosforečnan draselný (TKPP). Sledovány byly hodnoty pH modelových vzorků a dále vybrané texturní parametery (pevnost, soudržnost, lepivost a gumovitost). Na základě výsledků je možné konstatovat, že jednotlivé fosforečnany ovlivňují sledované ukazatele vzorků různým způsobem. Koncentrace přidávaných fosforečnanů také významně ovlivnily změny pH modelových vzorků i sledované texturní parametry výrobků. Zvýšení pevnosti a gumovitosti modelových mělněných masných produktů bylo obvykle pozorováno při koncentraci fosforečnanů 0,20–0,35%.

Ve druhé fázi byly testovány binární směsi vybraných sodných solí fosforečnanů. Pro tuto studii byly určeny TSPP, SHMP a SAPP v celkové koncentraci 0,25%. Binární směsi byly testovány v následujících procentuelních poměrech: 100:0; 90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90; 0:100. Hodnoty pH i texturních parametrů byly určovány stejným způsobem jako v 1. fázi dizertační práce. Měnící se poměry binární směsi složení z SAPP a SHMP vykazovaly významný vliv na studovanou matrici.

Poslední fází byla testace vlivu hydrokoloidů na texturní parametry modelových vzorků. Pro studii byly vybrány dva karagenany a to: κ- karagenan a ι-karagenan, které byly použity v koncentracích 0–0,5% (s krokem po 0,1%). Hodnoty pH i texturních parametrů byly určovány stejným způsobem jako v předcházejících fázích dizertační práce. Z výsledků bylo zjištěno, že použití karagenanů podstatně ovlivní pevnost vzorků. Při koncentracích κ-karagenanu ≈0,4% a ι-karagenanu ≈0,2% byla detekována maximální hodnota pevnosti modelových výrobků. Použití karagenanů podstatně neovlivnilo hodnoty pH vzorků.

Provedené studie poukázaly na zlepšující efekt vybraných fosforečnanů a hydrokoloidů na texturní parametry mělněných masných výrobků vyrobených z MDPM, a také na potenciál těchto přídatných látek při vývoji nových masných výrobků.

Klíčová slova: mechanicky separované drůbeží maso, mělněné masné výrobky, fosforečnany, hydrokoloidy, texturní parametry.

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1. INTRODUCTION

Meat consumption is an important part of the diet nutritional contribution and energy for human activities. As defined by the Codex Alimentarius, meat correspond to all the parts of an animal that are intended or have been judged as safe and suitable for human consumption [1]. Meat and meat products provide a high quality sources of protein, fat, vitamins and minerals such as iron, zinc, calcium and phosphorus, which are necessary for the human growth [2]. Moreover, meat has all the essential amino acids which contributes to improve the health for the consumers and also offers a variety of positive properties and a choice of tastes and textures. In addition, meat is a very versatile culinary product and has become a vital element of European cuisine and culture. According to the statistical and economic information of the EU in 2008, the consumption of poultry meat in EU per head per year was approximately 23 kg and 24 kg for the years 2004 and 2008 respectively [3; 4].

Table 1. Meat market in the world [5] (million tons)

World balance	**2009**	**2010**
Production	283.6	290.8
Bovine meat	65.0	65.0
Poultry meat	93.6	98.1
Pig meat	106.3	109.2
Ovine meat	12.9	13.0
Trade	25.2	27.4
Bovine meat	7.2	7.6
Poultry meat	11.1	12.1
Pig meat	5.8	6.6
Ovine meat	0.9	0.8

Poultry meat is a relatively cheap source of animal protein compared to other meats and also counts with the consumer preferences in food preparation [6]. Hence, in recent years, consumption of poultry meat has raised as shown in Table 1 where the poultry meat consumption average per capita is approximately 13.6 kg and 14.2 kg per head per year in 2009 and 2010 respectively. This increase can be partly a result of its price competitiveness and

due to consumer's concern associated with other meats. In 2011, for the first time in history, the world's production of poultry meat exceeded 100 million tons. Countries such as USA, China and Brazil owned 22, 17 and 16% of the total production respectively. The European Union shared 12% followed by Mexico (4%) and India (3%). Due to the growth of the global human population, it is expected that by the year 2020, the world production of poultry meat will approach 122.5 million metric tons and that by 2030 the global market's composition is predicted to change so that poultry meat will be positioned as the world's most popular meat. The Asia – Pacific region is predicted to contribute largely to the demand, which will increase up to 56% of the total meat demand from 2010 to 2020 while European countries will only increase around 7%. An additional increase for a number of Asian countries as China, India and Japan will present the higher demand of poultry meat with an increase of over 30, 80 and 15% respectively. According to the European Commission report on prospects for agricultural markets, it is predicted that for the coming years, EU-27 poultry meat production will reach around 12.2 million tons in 2015 and then grow to 12.47 million metric tons in 2020. Currently, the uptake of poultry meat by European Union consumers is also expected to grow from 23.4 kg per person per year to 27.7 kg by the year 2020. [7; 8]

In EU, the main poultry meat producing countries are France, UK, Spain, Germany, Italy, Poland, and Netherlands mainly with products as broiler, turkeys, ducks and "spent hens". Especially, broiler meat is the most important type of meat within poultry in all EU countries [9].

Therefore, in the process of manufacturing poultry meat, some parts of meat are still on necks and carcasses after filtering broiler meat. In order to be able to use all the meat, machines have been developed to extract them [10]. The meat remaining on carcasses and necks on poultry constitute about 12-24% of the total meat, which represents a non-negligible amount [11].

The product obtained by separating the meat from the bones is called mechanically deboned poultry meat (MDPM) and can be considered as a by-product of the poultry processing industry. It is produced from the deboning and cutting of parts with lower commercial value, such as back, neck, feet, and head skins and bones of poultry. To produce MDPM, manufacturers use the specially designed machine by which the meat slides away from the bones. In fact, MDPM has good nutritional and functional properties and is suitable to manufacture as a poultry meat product. Negrão et al. [12] reported that mechanically deboned chicken meat contained all the essential amino acids as shown in Table 5 and its biological values did not differ significantly compared to fresh chicken breast meat and casein. However, MDPM is currently only used as an ingredient playing a role as a protein source improving textural properties. Practically, it is also considered as a replacement of raw meat by economic factors which could help to reduce the product cost in the manufacture of different meat products such as sausages, bolognas, or salamis [13].

Thermal processing is applied to produce the ready-to-eat products in the manufacturing of meat products. After this treatment, their sensory values, textural properties and/or water holding capacity (WHC) could negatively change leading to losses. To improve the texture of meat products, salt, phosphates and/or alkaline and/or hydrocolloids (gums, dextrose and/or carrageenans) have been used [14; 15; 16]. Although alkaline (NaOH or NH_4OH) has also been used to adjust the pH leading to an increment of WHC, its contribution is not significant compared to phosphates [17; 18]. Thus, many researches have published papers about the properties of meat after processing [17; 18; 19; 20; 21; 22; 23; 24; 25; 26]. Unfortunately, each author used different condition for the phases such as raw material and dry matter content. Hence, the results could be compared with difficulties. Furthermore, above mentioned researches have tended to focus only on pork and beef rather than on poultry meat including MDPM [27]. Actually, no systematic information about the effects of phosphates and hydrocolloids addition on textural properties of MDPM is available.

In relation to Asian countries, lower domestic production and a trend to increase the consumption of fish over poultry are promoting extra Japanese imports. Whereas, in Korea, the strong consumer demand combined with a tariff-free quota import contributed to raise the imports to 50,000 metric tons last year [28]. Europe increased the imports under the circumstances of higher domestic consumption. In addition, higher production costs contributed to the poor ability to compete against imports. At the present, being a nation in the Asian region, Vietnam has not MDPM. Vietnam local consumers, like those in other Asian countries, prefer poultry meat. The consumption of poultry meat in Vietnam has been growing every year. According to the statistical and economic information of the Vietnam Department of Agricultural, the poultry production per head per year is about 3.96, 4.32, 4.56, for the years 2002, 2004 and 2006 respectively [29]. In addition, the number of frozen poultry meat imported in 2011 was 93,800 tons [30], which raised an average of the consumption approximately to 4.82 kg per head per year, but the major poultry production is frozen broiler meat or whole. However, the bones and the other waste have been mainly use for pet or in other industrial sectors. For these reasons, MDPM would be a potential material for manufacturing the meat products in Vietnam.

The present dissertation consists of three main parts: introduction, theory and phase.

The first chapter gives the overview of the research area of using phosphate salts and hydrocolloids in meat products, while the second chapter presents the main aim of this dissertation.

The theoretical section contains the overview description and theoretical knowledge about the meat and meat products, phosphates and hydrocolloids.

The phase section describes the design of phases, analysis methods using to determine parameters of chemical composition of meat, and textural properties as well as statistical methods.

2. SCOPE OF THE STUDY

The present study is a part of the project aiming to the improvement of textural parameters of meat products made from MDPM. Overall, a better understanding of the interactions of phosphates, hydrocolloids and MDPM is important in the development of any new product. Therefore, the aim of the present work was to study:

- the effect of selected phosphate salts (sodium and potassium salts of mono-, di-, tri- and polyphosphates) with different levels of concentrations on textural properties of meat batters;
- the effect of binary mixtures of selected phosphate salts on textural properties of meat batters;
- the effect of selected hydrocolloids (κ- and ι-carrageenans) with different levels of concentrations on textural properties of meat batters;
- the levels of pH of mechanically deboned poultry meat batters (with and without phosphate salts);
- the textural parameters of mechanically deboned poultry meat batters including hardness, adhesiveness, cohesiveness and gumminess values;
- the results of the data obtained from phases and focus on statistical evaluation of the results obtained.

3. REVIEW OF THE LITERATURE

3.1. Meat and meat products

3.1.1. Meat

Meat is a food stuff containing good nutrients and all the essential amino acids, fat, minerals and vitamins (often analyzed as ash) such as iron, zinc and vitamins B, especially vitamin B_{12} (cobalamin). Therefore, the different types of food products made from meat have been manufactured and supplied as human food. Moreover, because meat has a good taste, meat products are mainly presented in meals every day with exception of vegetarians.

Composition of meat changes and depends on the position of meat as well as the weight and type of animal. Table 2 shows the water, protein, fat and ash contents in different meats such as beef, pork, chicken and venison and other food sources. Therefore, meat is a very good nutrition source. Unfortunately, meat is also an appropriate environment of many microorganisms. In meat, there exist the suitable elements for the growth of bacteria such as carbon, nitrogen, minerals, moisture and pH. Generally, meat chemical composition comprises 56-72% water, 15-22% protein, 5-34% fat and 3.5% other substances such as carbohydrates, dissolved nitrogen substances, minerals and vitamins [31].

Table 2. The approximate chemical composition of meats and other food sources per 100 g [32]

Product	Water	Protein	Fat	Ash
Beef (lean)	75.0	22.3	1.8	1.2
Beef carcass	54.7	16.5	28.0	0.8
Pork (lean)	75.1	22.8	1.2	1.0
Pork carcass	41.1	11.2	47.0	0.6
Veal (lean)	76.4	21.3	0.8	1.2
Chicken	75.0	22.8	0.9	1.2
Venison (deer)	75.7	21.4	1.3	1.2
Beef fat (subcutaneous)	4.0	1.5	94.0	0.1
Pork fat (back fat)	7.7	2.9	88.7	0.7
Milk (pasteurized)	87.6	3.2	3.5	0.7
Egg (boiled)	74.6	12.1	11.2	1.0
Bread (rye)	38.5	6.4	1.0	0.2
Potatoes (cooked)	78.0	1.9	0.1	0.5

Water

Meat is mainly composed of a high percentage of water. The majority of water in muscle is held within the structure of muscle itself or within myofibril. Water can be divided into three types in muscle as bound, entrapped (immobilized) and free water. The content of bound water held closely to protein is a very small portion of the total water in muscle cells. Therefore, water significantly affect to the structure and quality of meat not only after slaughtering but also during the storage time. In addition, the sensory and textural properties of meat products are also affected. Moreover, water is a good media for the reactions occurring inside the meat, and also a suitable environment for microorganism growth.

Protein

Nutritionally, the meat protein is probably the most important constituent of meat. Meat protein is the second largest component after the water. Protein in muscle meat is classified into three protein types as follow (Fig. 1): [33]

- Myofibril proteins: salt soluble, are proteins with long chain such as actin, titin and myosin.
- Sarcoplasmic proteins: water soluble or soluble at very low salt concentrations, containing an amount of glycosomes and myoglobin.
- Structure proteins (connective/muscle tissue): insoluble by the impact of salt solution, are mainly composed of collagen and eslatin.

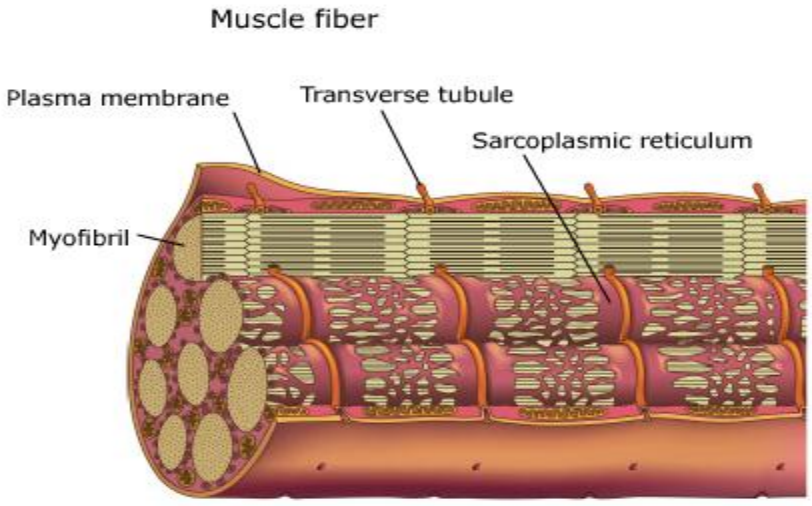

Figure 1. Structure of muscle fiber [34].

Expressed in percentage, muscle protein consists of 55-60% myofibrils protein, around 30% sarcoplasmic protein and around 10-15% connective tissue.

Actin, myosin, tropomyosin, troponin and actinin, as illustrated in Fig. 2, are components of myofibril protein in which actin and myosin are respectively around 42% and 16%.

Myosin is the largest component in myofibril and can be extracted from meat by salt solutions of moderate ionic strength. This myosin extracts gels on heating, emulsifying and binding pieces of meat together and to other components in meat products [35].

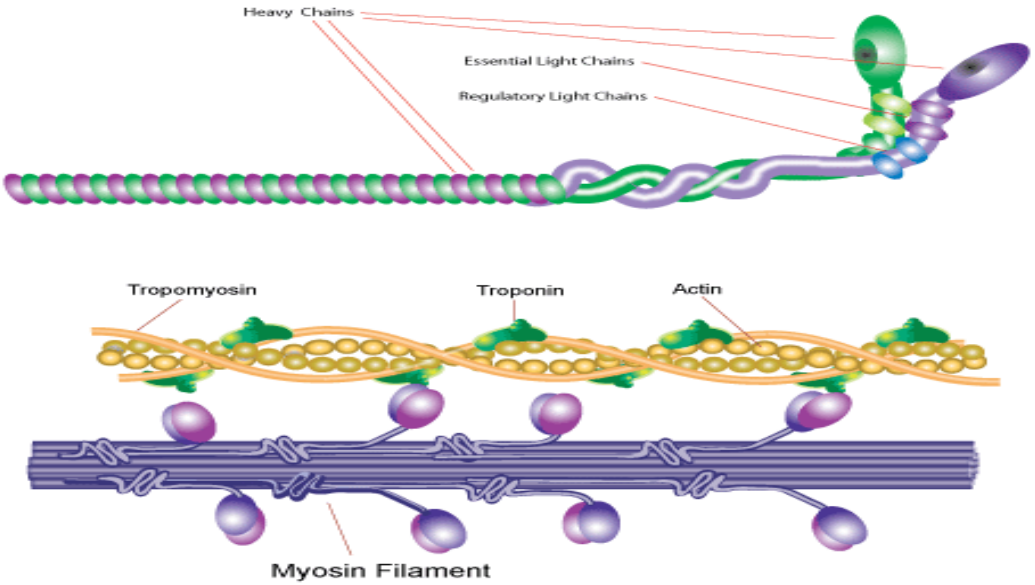

Figure 2. Structure of myosin [36].

The nutritional value of meat is mainly due to the protein content which differs according to the location in the animal body. Typically, loin lean and round lean contain the highest protein content [16]. Protein of muscle meat is a perfect protein due to the fact that contains all the essential amino acids [33].

The solubility of meat protein is one of the most important factors affecting to the water holding ability of meat and meat products. The correlation between protein and solubility is related to mechanical properties such as tenderness or hardness of meat during the processing and storage [37].

Fat

Fat, or more correctly lipid, is also one of the most important parts in meat. Fat is a source of energy providing a double energy value than that of carbohydrate or protein. In technology and science of meat, fat usually mean fatty tissue [16].

Fats are divided into three major groups (as illustrated in Fig. 3):

- Intramuscular fat: fat between the muscle fibers and fiber bundles

- Intermuscular fat: between individual muscles.
- Subcutaneous or depot fat: under the skin.

Fat tissue content depends on the type of animal and position of meat. For instant, fat content of beef meat in round, brisket, neck and flank is 5%, 18%, 8% and 17%, respectively [16].

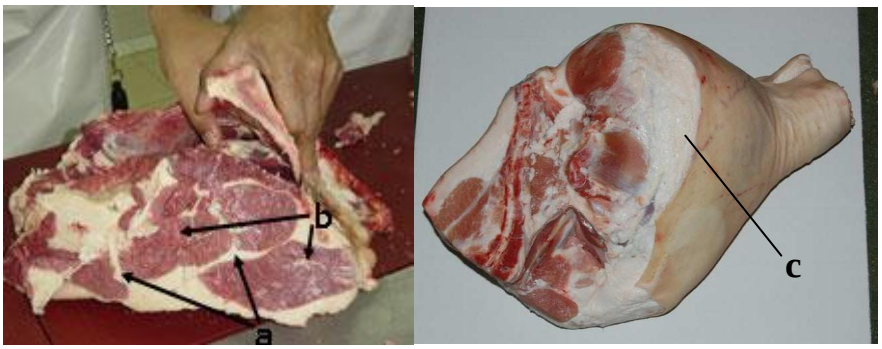

Figure 3. Illustration of meat fat: (a) Intermuscular fat; (b) Intramuscular fat and (c) Subcutaneous fat [32].

The composition of fatty acids of meats and other oils is shown in Table 3.

Table 3. Fatty acid composition of some fats and oils (as a percentage of the total fatty acids) [38]

	Lamb	**Beef**	**Pork**	**Chicken**	**Salmon**	**Maize oil**
Saturated fatty acids	53	45	40	35	21	13
Saturated fatty acids	47	55	60	65	79	87
Ratio (saturated/unsaturated acids)	1.1	0.8	0.7	0.6	0.3	0.2
Hardness of fat	Hard	→				Soft

Minerals and vitamins

Meat contains many different types of vitamins such as riboflavin, niacin, pantothenic acid, α-tocopherol and pyridoxin (vitamin B_6) as shown in Table 4. In particular, meat is excellent source of bio-available vitamin B_{12}. In addition, meat is the richest source of the minerals iron and zinc; and red meat is also a good source of selenium. Other minerals are also present in meat such as copper, potassium, sodium, phosphorus and calcium.

Table 4. Composition of minerals and vitamins (per 100 g) of lean meat [39]

Components	Beef	Veal	Lamb
Thiamin (mg)	0.04	0.06	0.16
Riboflavin (mg)	0.18	0.20	0.25
Niacin (mg)	5.0	16.0	8.0
Pyridoxine (mg)	0.52	0.8	0.8
Cobalamin (g)	2.5	1.6	2.8
Pantothenic acid (mg)	0.35	1.50	1.33
Vitamin A (µg)	<5	<5	7.8
β-caroten (µg)	10	<5	<5
α-tocopherol (mg)	0.63	0.50	0.20
Sodium (mg)	51	51	71
Potassium (mg)	363	362	365
Calcium (mg)	4.5	6.5	6.6
Iron (mg)	1.8	1.1	3.3
Zinc (mg)	4.6	4.2	3.9
Magnesium (mg)	25	26	28
Phosphorus (mg)	215	260	290
Copper (mg)	0.12	0.08	0.22
Selenium (µg)	17	<10	<10

3.1.2. Meat products

According to the Regulation (EC) 853/2004 [40], meat products is defined as processed products resulting from the processing of meat or from the further processing of such processed products, so that the cut surface shows that the product has no longer the characteristics of fresh meat.

Many different meat products have been currently manufactured in several countries around the world with the different product names and characteristics. However, some products also have many similarities in the processing and technology. Hence, based on the processing technologies used and taking into account the treatment of raw materials and the individual processing steps, meat products can be divided into six groups as presented in Fig 4.

The following text is the summarized definitions of the classified meat products presented in the Fig. 4:

- Fresh processed meat products: are meat mixtures composed of comminuted muscle meat with varying quantities of animal fat. Examples of these products are hamburger, fried sausage, kebab and chicken nuggets.

- Cured meat cuts: are made of entire pieces of muscle meat and can be sub-divided into two groups, cured-raw meats and cured –cooked meats. The

meat pieces are treated with nitrite salt. Examples of products of this type are raw cured beef, raw ham, cooked ham and reconstituted products.

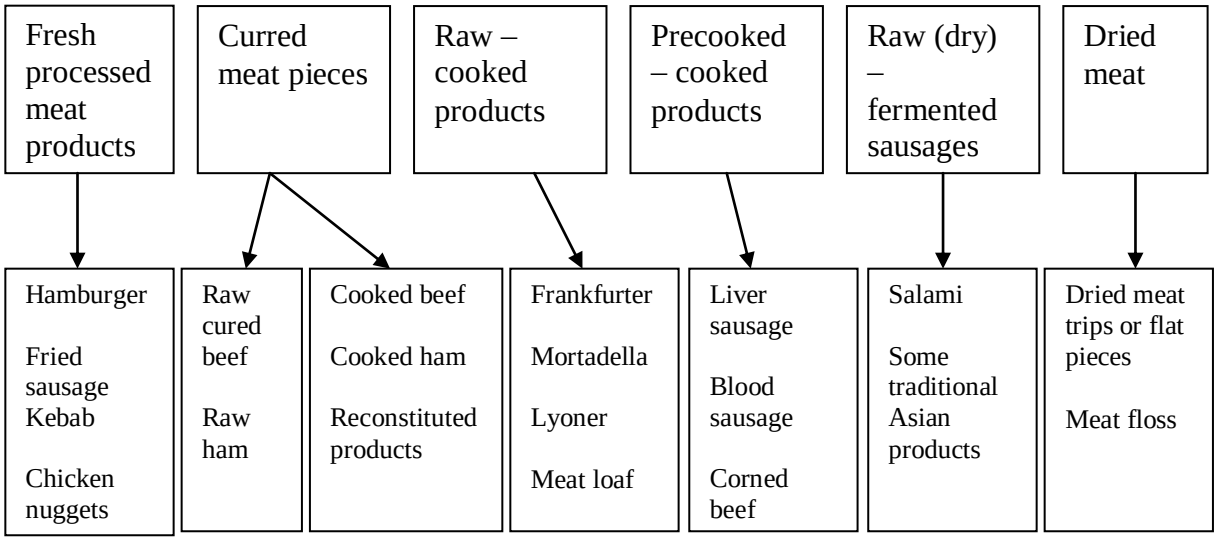

Figure 4. Meat products grouped according to the processing technology applied [32].

- Raw-cooked meat products: are composed of muscle meat, fat and non-meat ingredients which are processed raw, i.e. uncooked by comminuting and mixing. The resulting viscous mix/batter is portioned in sausages or otherwise and thereafter submitted to heat treatment.

- Precooked-cooked meat products: contain mixed mixtures of lower-grade muscle trimmings, fatty tissues, head meat, and other by-products. Based on heat treatment, there are two subgroup divided as followed: the first heat treatment is the precooking of raw meat materials; and the second heat treatment is the cooking of the finished products.

- Raw-fermented sausages: are uncooked meat products and consist of coarse mixtures of lean meats and fatty tissues combined with salts, nitrite, sugar and other ingredients filled into casing and followed by a fermentation processes.

- Dried meat products: are the products of the simple dehydration or drying of lean meat. Many of the nutritional properties of meat, especially the protein content, remain unchanged through drying.

3.2. Mechanically deboned poultry meat (MDPM)

Mechanically deboned meat or mechanically separated meat is common names used for meat that results from a process in which the meat is separated

from the bones by the machine. Mechanically separated meat is defined by the Regulation (EC) 853/2004 [40] and (EU) 1169/2011 [41] as the product obtained by removing meat from flesh-bearing bones after boning or from poultry carcasses, using mechanically means resulting in the loss or modification of the muscle fiber structure [40; 41]. Other names that have been used for MDPM include comminuted, finely comminuted and ground poultry. Overall, mechanically deboned poultry meat can be considered as the by-product of the poultry meat processing industry. MDPM produced from necks, backs and other bones started in the late 1950s [13]. The schematic view of the manufacturing of mechanically deboned meat is shown in Fig. 5.

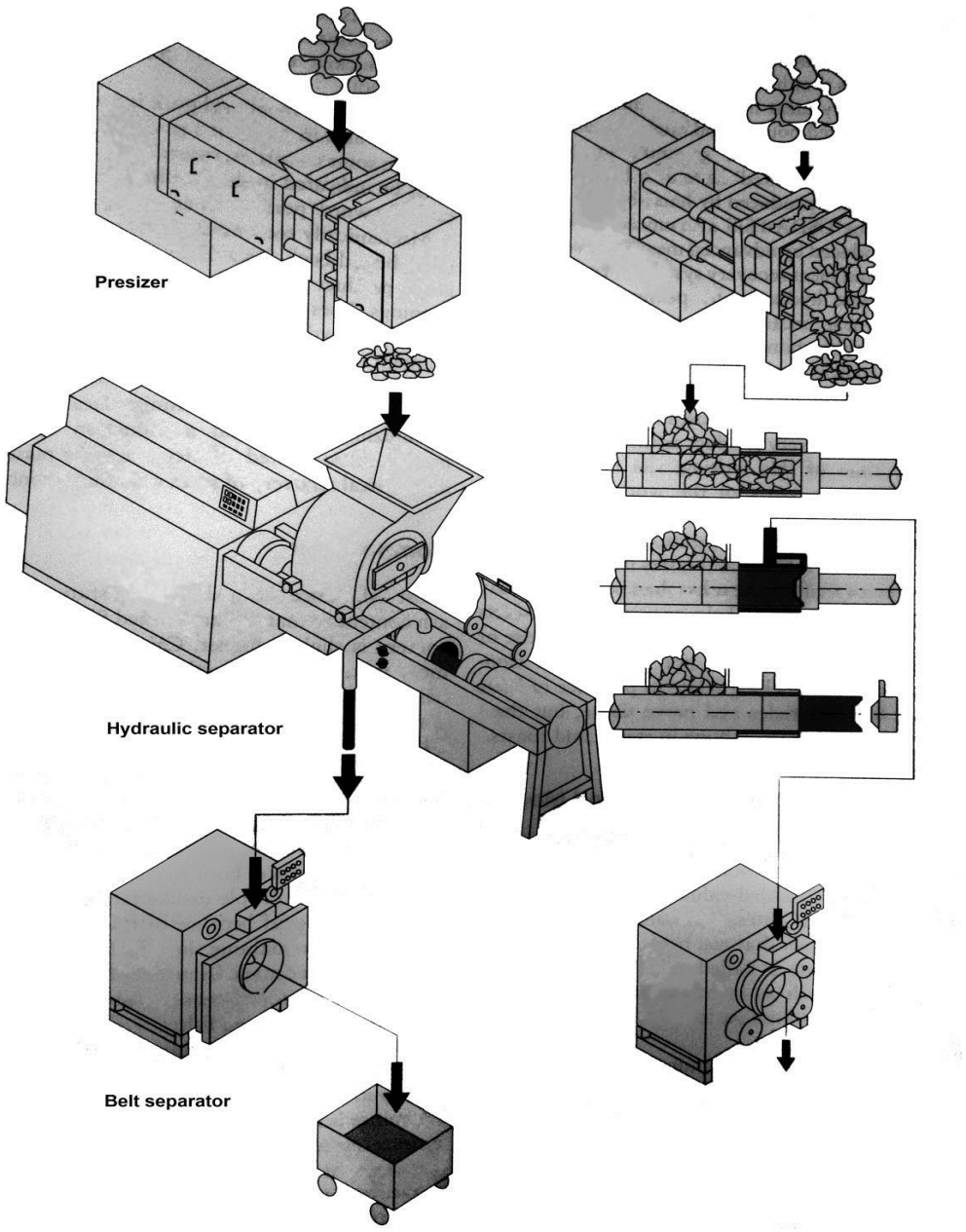

Figure 5. Schematic view of the steps involved in mechanical deboning of meat using a presizer, a hydraulically powered press and a belt-drum separator [13].

As shown in Fig. 5, the steps of manufacturing mechanically deboned meat includes presizing, pressing and desinewing. Presizing consists of dividing the bones into sections 10-15 mm in length. Bone sections are then pressed at high pressure in a position-like like device with holes in the walls and the pressing head. When bones compress, meat is pushed off the bone separately, through filters and away from the machine. Compressed bone is ejected from chamber and another batch of presized bone enters. Finally, deboned meat is transferred to a desinewing step where it passes between a belt and a drum with holes 1.0-1.3 mm in diameter; and sinew, cartilage and bone particles are also removed [13].

The chemical composition of MDPM is highly dependent on factors such as the age of the bird, type, the proportion of bone, meat, fat and skin in the material being deboned. Table 5 shows the different of composition of MPDM with and without skin removed.

Table 5. Composition of hand-boned and mechanically deboned poultry [13]

Nutrient	**Hand-boned, no skin**		**Mechanically deboned**			
	Breast	**Leg**	**Broiler backs and necks**		**Mature hens**	
			With skin	Without skin	With skin	Without skin
Water (g)	74.8	76.1	62.7	69.3	69.8	70.9
Protein (g)	23.1	20.1	11.4	13.8	20.4	20.4
Fat (g)	1.2	3.8	24.7	15.5	9.1	7.5
Ash (g)	1.0	0.9	1.0	1.0	1.3	1.3
Calcium (mg)	11	11	118	133	112	130
Iron (mg)	0.7	1.0	1.6	1.7	1.3	1.3
Cholesterol (mg)	58	80	140	120	122	110

In the study on biological evaluation of mechanically deboned chicken meat protein quality, Negrão et al. [12] reported that mechanically deboned chicken meat contained higher concentration of fat and lower concentrations of moisture and protein when compared to fresh chicken breast meat. These researchers also determined and showed a comparison between amino acids composition of mechanically defatted mechanically deboned chicken meat (MDCM) and fresh chicken breast meat (FCBM) and are presented in Table 6.

Table 6. Essential amino acid composition (mg/g protein) of MDCM and FCBM [12]

Essential amino acids	MDCM powder	FCBM powder
Histidine	17.4	30.9
Isoleucine	29.6	45.5
Leucine	58.7	86.4
Lysine	8.2	88.9
Methionine + Cysteine	24.4	36.7
Phenylalanine + Tyrosine	48.8	72.6
Treonine	31.2	49.5
Tryptophan	ND	ND
Valine	33.3	48.3

ND: not determined.

(Tyrosine and cysteine in the above are not essential amino acids)

At the present, MDPM has been widely used to increase the economic value which mainly used for pet food before. Being an additional source of high quality protein, MDPM has been recently used to manufacture the different meat products as an ingredient or as a replace material. Thus, the effective of using agricultural resources has risen significantly. Until 1995, MDPM was labeled as chicken or turkey when used as an ingredient in manufacture of poultry meat products such as frankfurters in the United States [13].

In the study of Field [13], he also reported that MDPM exceeded about 318 million tons annually in the United States in which approximately 182 million were used for sausages such as frankfurters and bologna, and approximately 136 million tons were used in products such as chicken patties, nuggets and poultry rolls.

3.3. Phosphates and hydrocolloids

The use of food additives has become more prominent in recent years due to the increased production of prepared, processed and convenient foods [14]. Additives are used for technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result in it or its by-products becoming directly or indirectly a component of such foods [42]. Thus, food additives are widely used and essentially in food manufacture industries.

3.3.1. Phosphates

Structure of phosphate

Phosphates, one of the main food additives, also called food grade phosphates, are the salts of phosphoric acid and sodium or potassium containing molecules like those in which the central phosphorus atom is surrounded by four oxygen atoms. The oxygen atoms spatially occupy a structure resembling a tetrahedron with the oxygen atoms at the corners.

Depending on the number of P atoms in molecule, the usual name will change as follows: (i) one phosphorus atom $(PO_4)^{3-}$ monophosphates (formerly orthophosphates); (ii) two phosphorus atoms $(P_2O_7)^{4-}$ diphosphates (formerly pyrophosphates); (iii) three phosphorus atoms $(P_3O_{10})^{5-}$ tripolyphosphates; and more than three phosphorus atoms $(P_nO_{3n+1})^{(n+2)-}$ polyphosphates (Fig.6) [43].

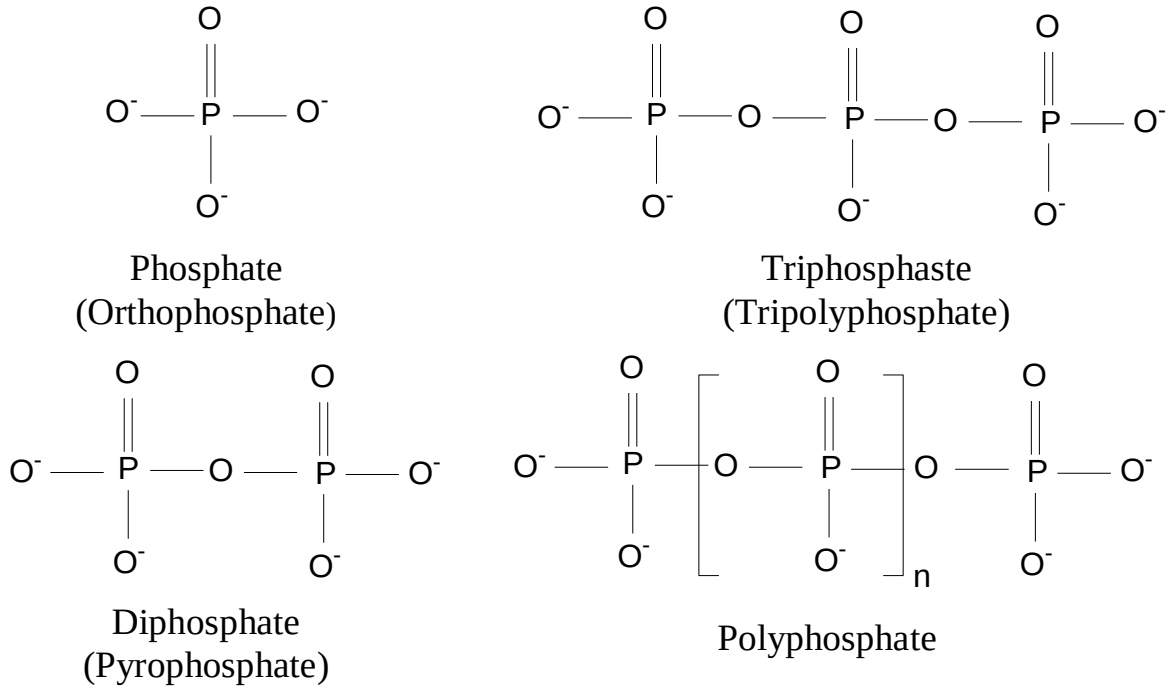

Figure 6. Linear polyphosphate ions [44].

Metaphosphates are cyclic compounds having the general $(HPO_3)_n$ which may also be expressed $(P_nO_{3n})^{n-}$. The term ultraphosphate includes any phosphate having a tridimesional structure. The latter group of phosphates are of the general form P_nO_{3n+x}, where $1 \geq x \leq n/2$ [45]. Only chain phosphates (linear) are permitted to be used in food processing industries. Ring phosphates are mainly used in the other industries such as those for water treatment, metal cleaning and detergent productions [33].

Table 7. The list of phosphates commonly used in meat products and some properties of phosphates [a]

Common names	Abbreviation	Formulas	pH (1% solution)	Solubility (g/100g H_2O)	E number [b]	$\%P_2O_5$ [c]
Sodium monophosphate						
Monosodium phosphate	MSP	NaH_2PO_4	4.4	85.0 (20°C)	E 339(i)	59.2%
Disodium phosphate	DSP	Na_2HPO_4	8.8	7.7 (20°C)	E 339(ii)	50.0%
Trisodium phosphate	TSP	Na_3PO_4	12.0	13 (20°C)	E 339(iii)	43.3%
Sodium diphosphate (tetrasodium pyrophosphate)	TSPP	$Na_4P_2O_7$	10.2	6 (20°C)	E 450(iii)	53.4%
Disodium diphosphate (sodium acid pyrophosphate)	SAPP	$Na_2H_2P_2O_7$	4.2	12.0 (20°C)	E450(i)	64.0%
Sodium tripolyphosphate (pentasodium phosphate)	STPP	$Na_5P_3O_{10}$	9.8	15.0 (20°C)	E 451(i)	57.9%
Sodium hexametaphosphate [d] (Graham's salt)	SHMP	$(NaPO_3)_n$		High soluble	E 452(i)	69.6%
		n = 10-15	6.2			
		n = 50-100	7.0			
Potassium monophosphate						
Monopotassium phosphate	MKP	KH_2PO_4	4.4	20.0 (20°C)	E 340(i)	52.1%
Dipotassium phosphate	DKP	K_2HPO_4	9.5	120.0 (20°C)	E 340(ii)	40.8%
Tripotasium phosphate	TKP	K_3PO_4	12.0	51.0 (20°C)	E 340(iii)	33.4%
Potassium diphosphate (tetrapotassium pyrophosphate)	TKKP	$K_4P_2O_7$	10.4	180.0 (20°C)	E 450(v)	43.0%
Potassium tripolyphosphate	KTPP	$K_5P_3O_{10}$	9.6	178.0 (20°C)	E 451(ii)	47.5%

[a] Adapted from Lampila et al. [46].

[b] Adapted from Council Directive No 95/2/EC [42].

[c] $\%P_2O_5$ was calculated by the P_2O_5 content of a phosphate and is expressed as a percentage.

[d] Modified from Molins [45].

Some functional properties of phosphates

The selected properties such as the formula, pH, solubility, E-code (for food additives) and relative content of P_2O_5 (in %) are presented in Table 7. Nearly all phosphates, as well as their blends rise in pH inside the meat product due to their high pH value (Table 7). The rising of pH increases the net negative charges in the muscle leading to enhance the water binding capacity of proteins because strong electrostatics and repulsive forces create large gaps between actin and myosin and larger amounts of added water can be bound [17; 21; 46; 47].

Mixtures of monophosphates (MSP, DSP and TSP) are excellent buffers; diphosphates could also be signed as buffers, but chains longer than two phosphorus atoms are not good buffers at all [45; 46]. Buffering property helps the meat to retain and protect fresh color by changing the pH of meat after slaughtering [46].

Phosphates have properties of strong metal ion chelating or sequestering, that is, the capability to form complexes with monovalent or polyvalent metal cations. Due to long chain structure, the chelating or sequestering ability of polyphosphates is greater than that of orthorphosphates. In addition, complexes of phosphates are followed as level of strong to weak: polyphosphate > pyrophosphate > orthophosphate. Longer chain polyphosphates are more effective chelators of calcium, but not of magnesium than are pyrophosphate or orthophosphate at pH < 8 [45]. Moreover, the binding of metal ions could reduce the oxidative rancidity [33; 45; 46; 48; 49].

Binding of phosphates with Ca^{2+}, Mg^{2+} (cross-bridges in actomyosin complex which are present in meat) forming a complex contribute to separate actin and myosin after rigor mortis. Hence, the above mentioned process will also enhance the water holding capacity of meat and meat products, improve the degree of tenderness and color of meat.

Phosphates as polyelectrolytes are able to change the ionic charges distribution. Consequently, the addition of phosphate increases the ionic strength of the meat causing a more severe degree of swelling of the muscle fibers and activation of protein. Enhanced levels of activated and swollen protein support the immobilization of the water added to meat products and the emulsification of fat [26; 33; 50; 51; 52]. Salt enhances water binding but cannot be used in high amounts because of the effects that has on the taste and risk of diseases [53; 54]. Thus, the addition of salts together with phosphates at the same time to a meat product will make the muscular protein to become soluble and solubilized, or activated; and the solubilized protein can immobilize higher content of water as well as emulsify a large amount of fat by increasing the ionic strength [19; 37; 46; 48; 54; 55; 56; 57; 58].

Phosphates are also slightly bacteriostatic on some gram-positive bacteria when used as acidulants or in combination with other food ingredients such as

nisin, EDTA, NaCl, nitrites, erythorbate, etc; can inhibit gram-positive bacteria such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus* sp., *Micrococcus luteus*, *Corynebacterium glutamicum*; and have a little effect on gram-negative bacteria such as *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Escherichia coli* [33; 46; 59; 60; 61; 62; 63; 64].

The solubility of phosphates must be considered because every phosphate has a different value (see in Table 7). Hence, phosphates are typically dissolved at room-temperature in water before adding salt and then chilled before use [33; 46; 53]. The most functional phosphates are diphosphates (especially tetrasodium diphosphate - TSPP) because they act on the actomyosin complex of meat protein right away and have a high pH value. The buffer capacity, binding of meat ions, active component on the protein of meat and solubility in cold water of phosphate salts are shown as a model in Fig 7 [33].

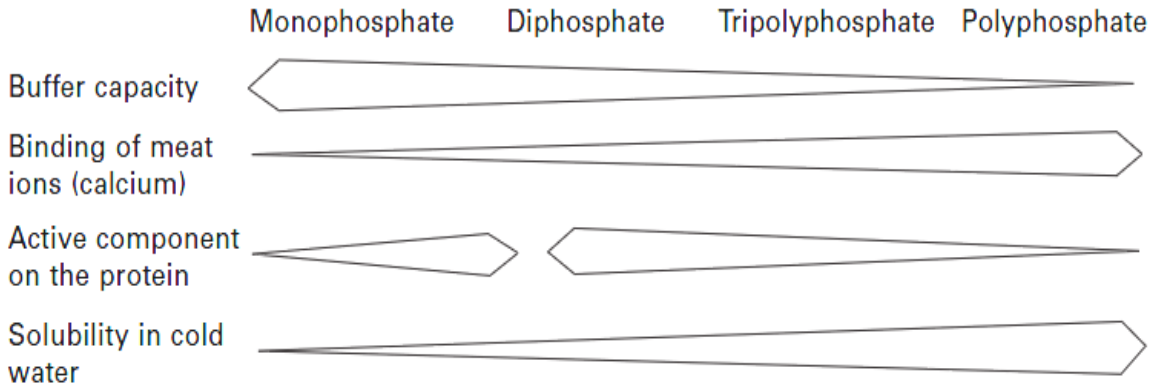

Figure 7. Properties of different phosphates [33].

The use of TSPP results in higher protein solubility which induces good water-binding ability of proteins in comparison with the application of polyphosphates [37; 45]. However, solubility of TSPP is low (as shown in Table 7). Therefore, longer-chain phosphates such as STTP and SHMP are commonly mixed with TSPP to use them as a blend to improve and optimize solubility and functionality in a variety of meat product formulations [50; 53; 55]. Sensory properties of products should be taken into account while choosing appropriate phosphate mixture content. Phosphate flavor is usually considered as unpleasant. The concentration of 0.3 to 0.5% could lead to products with unacceptable bitter taste [16; 33].

Moreover, polyphosphates can be hydrolyzed to other phosphate forms during the cooking time as well as the action of microorganisms in phosphate treated meat products. However, the hydrolysis of polyphosphate would be not a problem if the meat products treated with phosphate are cooked immediately after treatment.

Influence of phosphates on health

Food phosphates, used in meat and meat products, must be manufactured according to good manufacturing practices (GMP). The U.S. Food and Drug Administration have classified the food phosphates as generally recognized as safe (GRAS) when used in accordance with GMP [65]. Phosphates are not permitted in fresh meat but could be added to meat preparations, minced meat and meat products [40]. The maximum permitted level of phosphates in meat and meat products according to European legislation is 5 g/kg as phosphorus peroxide (P_2O_5) individually or in combination to the finished product [42]. According to FAO/WHO food standards, the maximum permitted level of phosphates (singly or in combination) is: (i) 2200 mg/kg as phosphorus (approximately 5041 mg/kg expressed in P_2O_5) in the finished product as frozen processed poultry meat and game products, in whole pieces or cuts and in processed comminuted meat, poultry and game products [66]; (ii) 3000 mg/kg as P_2O_5 in the finished product as luncheon meat [67], in cooked cured ham [68], in cooked cured pork shoulder [69] and in cooked cured chopped meat [70].

Phosphorus is responsible for many biological properties and functions. It is present in DNA, RNA, enzymes, etc. and especially co-exists with calcium and magnesium forms in bones. Generally, phosphorus is needed for the growth, maintenance and repair of all tissues and cells of living organisms. According to Institute of Medicine recommendation, the recommended dietary intakes (RDIs) of phosphorus depend especially on the age of people and/or some special status: (i) 0 to six months, 100 mg/day; (ii) seven to 12 months, 275 mg/day; (iii) one to three years, 460 mg/day; (iv) four to eight years, 500 mg/day; (v) nine to 18 years, 1,250 mg; (vi) adults (> 19 years), 700 mg/day; (vii) pregnant or lactating women 14 to 18 years, 1,250 mg/day and older than 18 years 700 mg/day [71]. Several studies which focused on the effect of the addition of phosphates on consumer health have been published and these studies have given contradictory results. The kidneys easily control the blood phosphorus level and efficiently excrete any excess of phosphorus; hence, up to now, there is no evidence that higher phosphate intakes are detrimental to bone health or to bone calcium excretion in the urine in healthy adults not having problems with kidneys [72; 73]. However, in the study of Huttunen et al. [74] with adult rats, excessive intake of dietary phosphate without the company of calcium caused rise in concentration of serum parathyroid hormone and hindered mineral deposition into cortical bone, leading to lower bone mineral density. Generally, to avoid potential adverse risks on health, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes [71] has recommended a tolerable upper intake levels (ULs) for adults, 4 g per day of phosphorus.

3.3.2. Hydrocolloids

Hydrocolloids are the range of polysaccharides and proteins which have been widely used in a variety of industrial sectors to perform a number of functions including thickening and gelling aqueous solutions, stabilizing foams, emulsions and dispersions, inhibiting ice and sugar crystal formation and the controlled release of flavors [75]. Based on classification of Imeson [76], hydrocolloids are divided by their origin as follows: (i) botanical: *trees:* cellulose; *tree gum exudates:* gum Arabic, gum karaya, gum ghatti, gum tragacanth; *plants:* starch, pectin, cellulose; *seeds:* guar gum, locust bean gum, Tara gum, tamarind gum; *tubers:* Konjac mannan; (ii) algal: *red seaweeds:* agar, carrageenan; *brown seaweeds:* alginate; (iii) microbial: xanthan gum, curdlan, dextran, gellan gum, cellulose; (iv) animal: gelatin, caseinate, whey protein, chitosan.

Hydrocolloids with the functions as viscosity, stability, suspension and gelation have been recently used as food additives or ingredients in many different food products such as reduced or low-fat, dairy products and some meat products [77; 78; 79; 80; 81].

The functional properties of hydrocolloids are mainly viscosity, stability, suspension and gelation [76]. Viscosity is probably one of the most widely used properties of hydrocolloids. With this function, hydrocolloids are often applied to manufacture reduced-fat products or replace the fat or oil to give a product with similar properties to the full-fat food. In addition, hydrocolloids are also used for fruit juice and table syrups, particularly low-calorie syrups. To prevent separation in emulsion as well as control ice crystal formation in frozen food, hydrocolloids are also used for stabilization purpose. Some hydrocolloids create solutions with a yield point that will keep particles immobilized in suspension. Moreover, one of the key texturizing aspects of hydrocolloids is the ability to gel and solidify fluid products. Typical gelling agents are such as pectin, gelatin, carrageenan and agar. The food industry has a myriad of gelling applications ranging from soft, elastic gels to hard and brittle gels. In general, hydrocolloids used meat products by having several functions as followed: [33]

- Hydrocolloids help to reduce cooking loss and increase yield by forming a gel or acting as a thickener.
- The formation of gel assists in obtaining texture in meat products.
- A higher yield results in a more succulent product.
- Hydrocolloids assist against syneresis in the finished product.
- Hydrocolloids do not interfere with the activation of protein within meat products.

Carrageenans

Carrageenans are a naturally occurring carbohydrate polymer consisting of potassium, sodium, magnesium, calcium and ammonium sulfate ester of D-galactose and 3,6 anhydro-D-galactose copolymers, and is widely used in food, pharmaceutical, cosmetics and industrial products [82]. Carrageenans, one of the important commercial hydrocolloids, a natural carbonhydrate, are extracted from the raw material red seaweeds. This particular type of seaweeds is common in the Atlantic Ocean near Britain, Europe and North America. The seaweed is boiled to extract the carrageenan. Carrageenan is widely used as a food additive in the food processing industry due to its gelling, thickening and stabilizing properties.

There are several carrageenans, differing in their chemical structure and properties lead to the difference in their application. The three main types of commercial carrageenans, namely ι-, κ- and λ- carrageenans have been widely used in food productions. The structure of carrageenans differs in the proportion and location of 3,6-anhydro-D-galactose and ester sulfate content presented in Fig. 8 and Table 8.

Table 8. Differences between the three types of carrageenans [33]

Carrageenans	Gel strength	Viscosity	Synerisis	Elasticity
κ-	High	Low	High	Low
ι-	Medium	Medium	Medium	Medium
λ-	No gel	High	Low	High

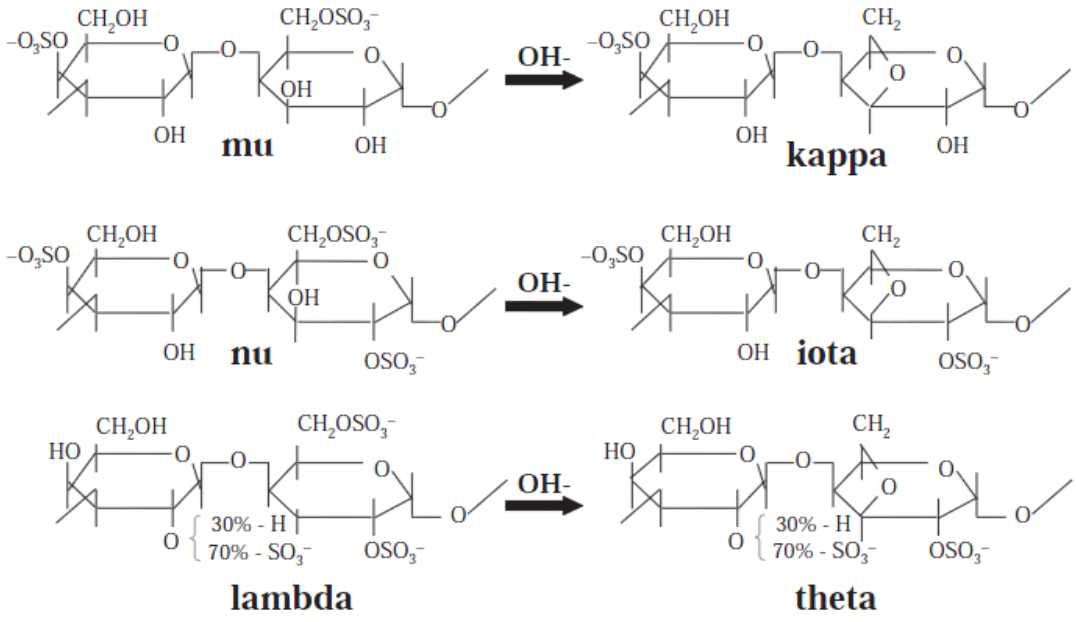

Figure 8. Structure of primary carrageenans [76].

Table 9. Summary of carrageenans properties [76]

Solubility	**Kappa**	**Iota**	**Lambda**
Hot (80°C) water	Soluble	Soluble	Soluble
Cold (20°C) water	Na^+ salt soluble. Limited swelling of K^+, Ca^{2+} salts	Na^+ salt soluble with Ca^{2+} salt gives thioxotropic swollen particles	All salts soluble
Hot (80°C) milk	Soluble	Soluble	Soluble
Cold (20°C) milk	Insoluble	Insoluble	Thickens
Cold milk (TSPP added)	Thickens or gels	Thickens or gels	Increased thickening or gelling
50% sugar solutions	Soluble hot	Insoluble	Soluble
10% salt solutions	Insoluble	Soluble hot	Soluble hot
Gelation			
Effect of cations	Strongest gel with K^+	Strongest gel with Ca^{2+}	Non-gelling
Gel texture	Brittle	Elastic	-
Syneresis	Yes	No	-
Hysteresis	10-20°C	5-10°C	-
Freeze-thaw stable	No	Yes	Yes
Synergy with locust bean gum	Yes	No	No
Synergy with Konjac flour	Yes	No	No
Synergy with starch	No	Yes	No
Shear-reversible	No	Yes	Yes
Stability in acid	Hydrolysis, accelerated by heat, low pH, and time Gels are stable.		Hydrolysis
Protein reactivity	Specific reaction with κ- casein	Strong protein interaction in acid	

Variation in the components of carrageenan molecules affect to the functional properties such as the gel strength, texture, solubility, melting temperature, syneresis and interaction with other hydrocolloids and ingredients. The functional properties of carrageenans are also summarized and presented in Table 9. As shown in Table 9, all carrageenans are soluble in hot water, only sodium salts of κ- and ι- and all salts of lambda are soluble in cold water. All carrageenans are soluble in hot milk, but in cold milk only λ-carrageenan has solubility, producing a thickening effect via protein interactions, this being enhanced by the presence of phosphate [76]. Depending on the type, carrageenans are used to produce a wide range of gelling and thickening effects. The mechanism of gel formation of carrageenans is presented in Fig. 9. Gel I is elastic gel formed by ι-carrageenan, gel II is brittle gel formed by κ-carrageenan.

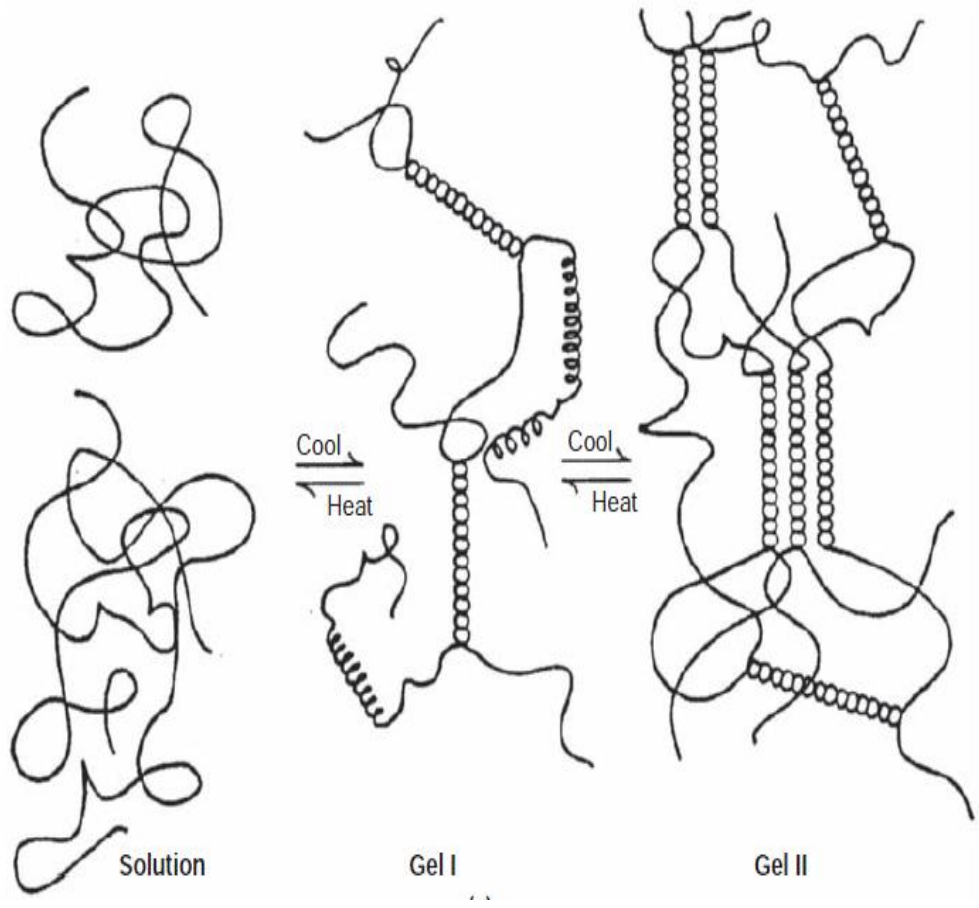

Figure 9. Carrageenan gelation mechanism [76].

Iota carrageenan: gel formed on cooling in the presence of salts. Molecules undergo a coil-helix transition followed by aggregation of helices (Fig 10). The presence of salts reduces electrostatic repulsion between chains promoting aggregation.

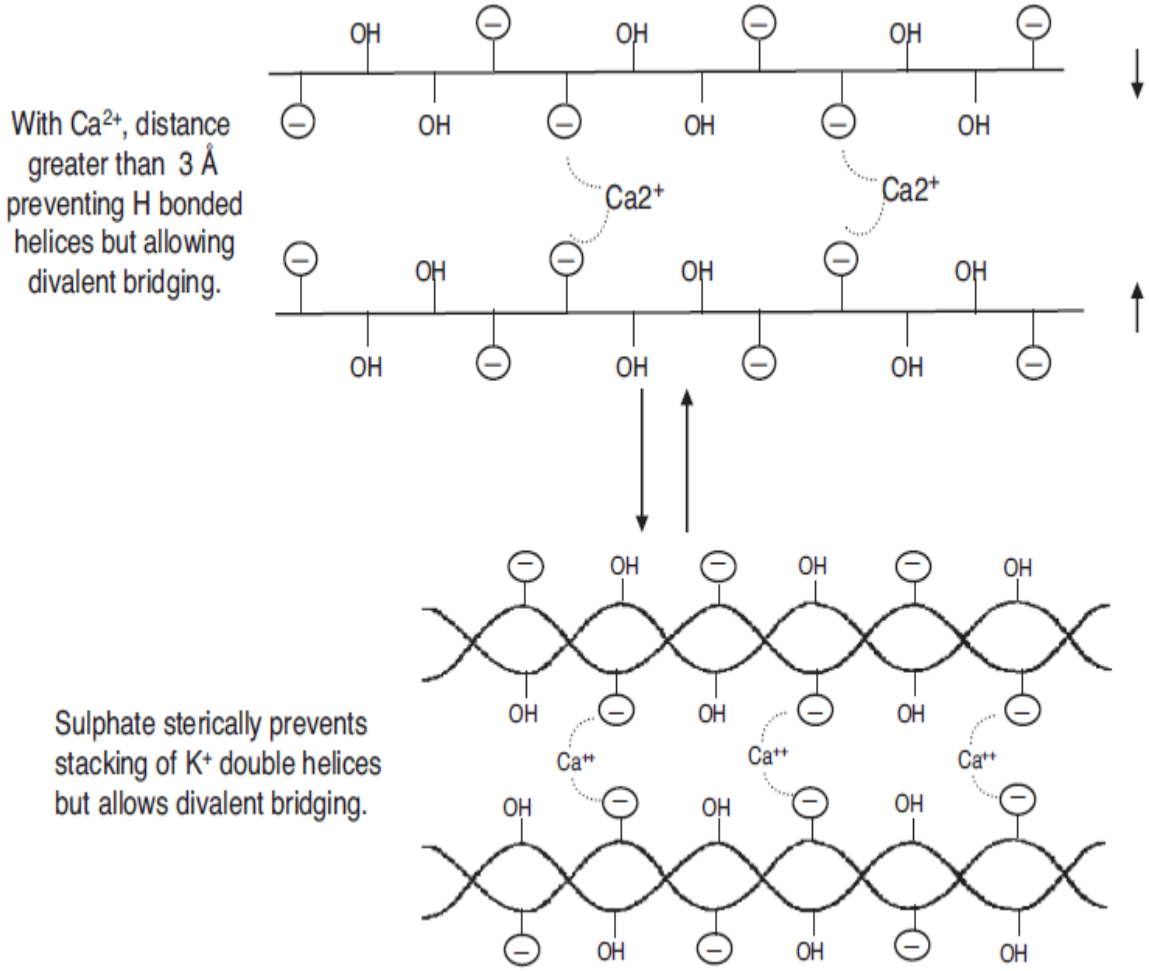

Figure 10. Gel-I mechanism of ι-carrageenan [76].

*Kappa carrageenan: g*el formed on cooling in the presence of salts notably potassium salts (Fig. 11). Molecules undergo a coil helix transition followed by aggregation of helices. Potassium ions bind specifically to the helices. Similar to the case of ι-carrageenan, the presence of salts also reduces electrostatic repulsion between chains promoting aggregation.

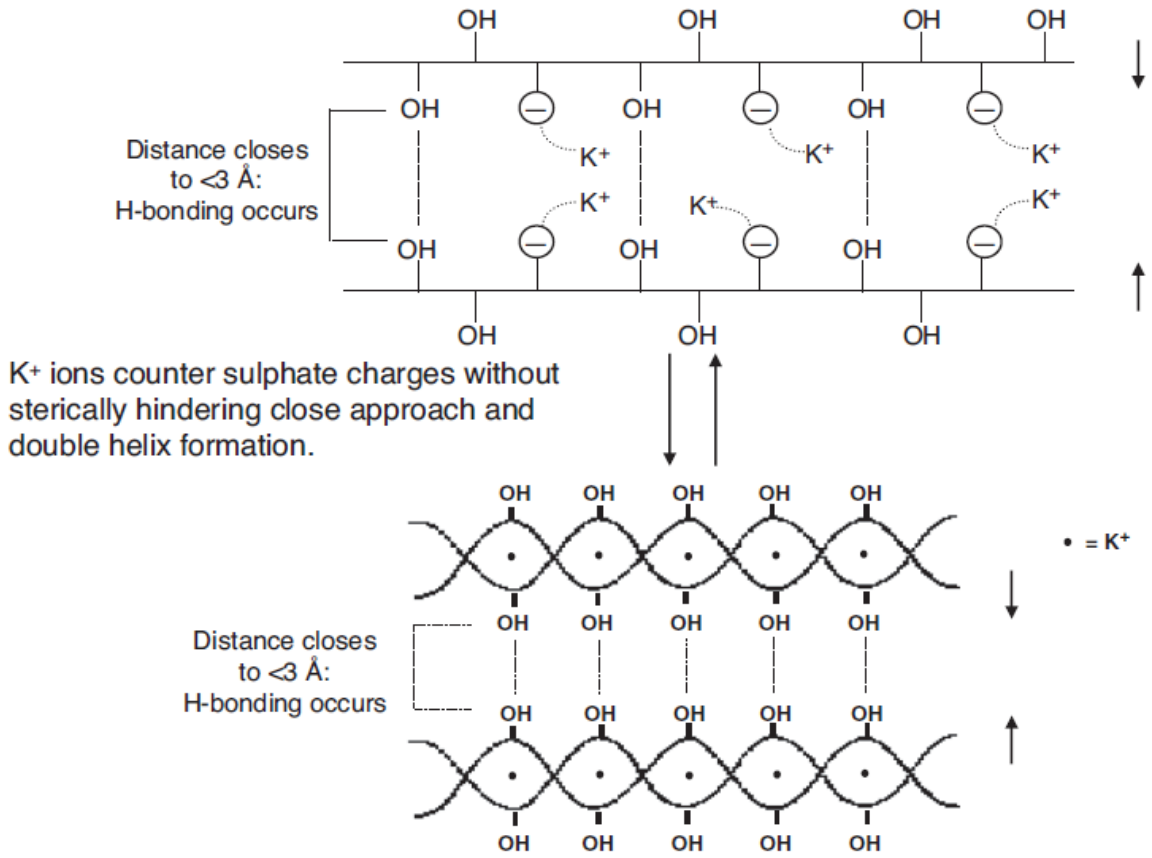

Figure 11. Gel-II mechanism of κ-carrageenan [76].

The hydration and gelation temperatures are strongly dependent on the salts associated with the carrageenan or added separately to the solution. An example of the effect of hydration temperature on viscosity of κ-carrageenan in water and in 2% sodium chloride is shown in Fig. 12.

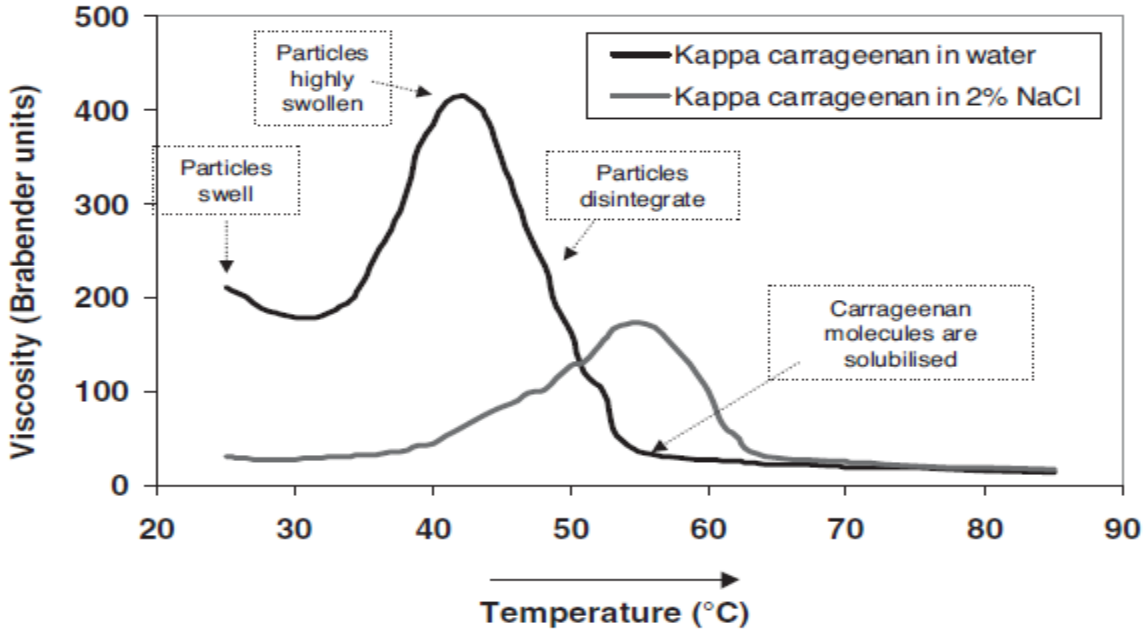

Figure 12. Hydration profile of κ-carrageenan in water and in 2% salt solution [76].

Overall, in the meat products processing industry, added carrageenans must be fully dissolved at the temperature around 70°C. The ionic composition of a food system is important for effective utilization of the carrageenans. Also as shown in Table 9, κ- carrageenan links to potassium ions to stabilize the junction zones within the characteristically firm, brittle gel, whereas ι carrageenan combined to calcium ions in order to bridge between adjacent chains to give typically soft, elastic gels. Combination of κ- and ι-carrageenans give gel strengths and textures intermediate to the two extremes and in line with the ratio used, as shown in Fig. 13. [76]. Table 9 also shows that the κ- carrageenan gels have high syneresis levels, iota gels is no syneresis. This synerisis property is directly related to freeze-thaw stability, where freezing further irreversibly tightens the kappa gel structure, but has no influence on the ι-carrageenan gels, which fully recovers when thawed [76].

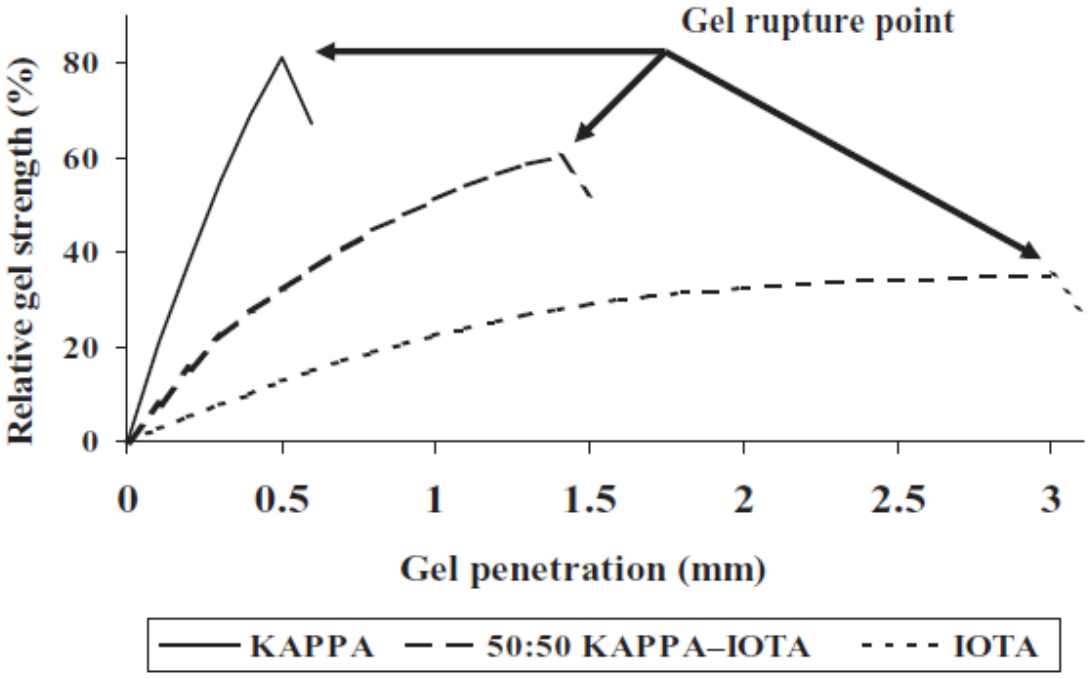

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Figure 13. Gel properties of pure and blended κ- and ι-carrageenans [76].

Carrageenan particles not only have a high affinity for water, but also have structural ‘memory’ [76]. Specific application of this water-binding property is the use of carrageenan in delicatessen meats, such as turkey breast and ham. The carrageenan is dispersed in brine before pumping into or tumbling with meat.

In the processing meat products, the brine extracts protein from the meat but the carrageenans only hydrate. When the meat is cooked at the high temperature, the carrageenans remain hydrated parts and continue to bind water, but the proteins form the gels, trapping the carrageenan particles in the gel matrix. Purge losses are minimised for improved cooked yield and moisture is retained

for improved eating qualities [76]. When the product is cooled to the ambient temperature, a gel or network which holds water within will be formed. This structure maintains products integrity during high speed slicing operation and binds moisture in the products throughout shelf life. Moreover, during cooling of a meat product containing carrageenans, mechanical forces such as squeezing of the products should be avoided to allow the gel to set properly.

The high reactivity of carrageenans with protein is caused by the strong electrostatic interaction between the negative charged ester sulfate groups in the carrageenans with a high positive charged protein in meat. On the other hand, another form of interaction is through the ester sulfate groups in the carrageenan molecules with carboxylic residues of amino acids extracted from meat protein. Hence, the reactivity with the protein is dependent on many factors such as the concentration of carrageenans, the type of proteins, the temperature, the pH and the isoelectric point of meat protein. In general, the use of hydrocolloids especially carragenans, has currently been growing to improve the textural properties of meat products has currently been growing. In the manufacture of meat products, carrageenans enhance the quality and/or increases the cooked yield of poultry, ham and sausage products [76]. In addition, carrageenans improve moisture retention, cooking yields, slicing properties, mouthfeel and succulence of canned, cooked and sliced meat products.

Influence of carrageenans on health

Similar to phosphates, carrageenans are also considered as GRAS when using in food products by FDA [65]. Moreover, it has ADI value “not specific” by the Join Expert Committee on Food Additives (JECFA) of the United Nation’s Food and Agriculture Organization (FAO) and the World Health Organization (WHO) [83]. In EU, carrageenans are known as E407 for refined carrageenan and E407a for semi refined. Many researches on the effect of using carrageenans on health have been studied. However, the results of these studies are contradictory. In 2006, Weiner et al. reported that no evidence was obtained carrageenan affect to health. In his study, carrageenan with a relatively high percentage of low molecular weight tail did not have any adverse toxicological effects when administered to rats at up to 50.000 ppm in the diet for 90 days. On the other hand, Tobacman with her studies in 1997 and 2001 stated that carrageenans were a cause of a range cancer, especially gastrointestinal ones, and other illnesses [84; 85; 86]. Thus, although carrageenans have natural origins, they should be used in accordance with good manufacturing practice, at a level not higher than the necessary to achieve the desired technological effect [42].

3.3.3. Effects of phosphates and hydrocolloids on selected properties of meat products

Bendall [19] evaluated the effect of 0.25 and 0.50% of diphosphate in 1% sodium chloride solution (overall concentrations) on the volume increase of the mince rabbit muscle. The addition of: (i) 1% sodium chloride solution led to the volume increase of 120.0 ± 6.0%; (ii) 1% sodium chloride solution/0.25% diphosphate led to the volume increase of 151.0 ± 14.0%; and (iii) 1% sodium chloride solution/0.5% diphosphate led to the volume increase of 164.0 ± 14.0% (expressed as the percentage of untreated fresh muscle). The cooked volumes were 171.0 ± 4.0% (1% sodium chloride solution), 189.0 ± 8.0% (1% sodium chloride solution/0.25% diphosphate) and 199.0 ± 6.0% (1% sodium chloride solution/0.5% diphosphate).

Restructured meat products are small pieces of meat reformed into steaks, chops and/or roast-like meat products. Minced, flaked, diced or mechanically recovered meat may be used to produce restructured meat [87]. Schwartz and Mandigo [20] studied the effect of salt, STPP, and storage on the restructured pork. The results indicated that the combination of salt and STPP (0.75 and 0.125%, respectively) on restructured pork after four weeks storage at -23°C, improved color, aroma, flavor, eating texture, cooking loss, and increased water holding capacity and juiciness rating.

Wierbicki and Howker [88] studied the effect of NaCl, phosphates (STPP, equivalent amounts of TSPP – expressed in % P_2O_5) and other curing ingredients on the shrinkage of lean pork meat and the quality of smoked processed ham. NaCl (1 to 10%), STPP (0.15 to 0.90%), equivalent amounts of TSPP (expressed in % P_2O_5), 0.015% $NaNO_2$, 0.06% $NaNO_3$, 0.0275% sodium ascorbate and 0.0275% sodium erythorbate were used in this study. The results showed that the curing ingredients $NaNO_2$, $NaNO_3$, sodium ascorbate and sodium erythorbate have little effect on meat shrinkage; the addition of either 0.3% STPP or 0.217% TSPP with 3% salt decreased the meat shrinkage to 5% and no significant effect on the meat shrink was observed by increasing the addition of STPP above 0.3%. Cut-and-formed smoked, cured ham containing 3% salt, either 0.3% STPP or 0.217% TSPP and other curing ingredients was as acceptable as the ham with either 0.5% STPP or 0.362% TSPP. Therefore, in cured hams, STPP could be used in 0.3% concentration.

Anjaneyulu et al. [55] studied the effect of the additions of NaCl, polyphosphates and their blends on the physicochemical properties of buffalo meat and patties. In this study, along with 2% NaCl, concentrations of phosphates (TSPP, STPP, SHMP, sodium acid diphosphate (SAPP)) and their blends at 0.3, 0.5 and 0.7% were evaluated. The results indicated that the order of effect of phosphates and their blends at all concentrations was TSPP > STPP > SHMP. The individual usage of SAPP and SHMP had significantly little effects on the improvementof the quality of meat such as the increase of pH,

WHC, emulsifying capacity, extractability of salt soluble proteins, color of ground meat, decreased cooking loss, improved emulsion stability, enhanced yield, texture and moisture retention of cooked patties. Blends containing two phosphates: 90% TSPP + 10% SHMP and 75% TSPP + 25% STPP were relatively more effective. And a phosphate blend consisting of 65.0% TSPP, 17.5% STPP and 17.5% SAPP was equally effective like that of TSPP in improving the functionality of hot and chilled meat and had the advantage of reducing the amount of sodium up to 3%. Again, in 1990, Anjaneyulu et al. [17] studied the effect of the blends of phosphate on the functional properties and yield of buffalo meat patties. Samples in this study included phosphate blends of 0.5% (including 65.0% TSPP, 17.5% STPP, and 17.5% SAPP) + NaCl 2%, NaOH 0.5% (used to adjust the pH to equal that of the phosphate treatment) + NaCl 2% and control without either NaCl or added polyphosphate. The results showed improved emulsifying capacity; increased emulsion stability, yield of patties and WHC; and reduced cook-cool loss and shrinkage of patties as the consequence of the treatments in the following sequence: phosphate blends > NaOH pH adjustment > control. This cofirmed that the effect of polyphosphate is not only due to a pH effect.

Moiseev and Cornforth [18] studied the effect of NaOH and STPP on bind strength and sensory characteristic of restructured beef rolls. Various levels of added water (0, 5 and 10%) and three types of ingredients were used: (i) 1% NaCl (control); (ii) 1%NaCl + 0.375% STTP and (iii) 1% NaCl + 0.07% NaOH. The results show that with either 5 or 10% added water, there were no differences in the juiciness of NaOH and STPP rolls, but both were juicier than controls. However, STPP rolls with 20% added water had higher juiciness score than either NaOH rolls or controls. The overall acceptability of STPP rolls was higher than NaOH rolls at 5 and 20% added water, but at 10% added water there was no significant difference in the acceptability of NaOH and STPP rolls. The strength of water-binding and cooked yield of samples was improved as follows: STPP > NaOH > control. These results confirmed that STPP did not only increase the pH value but also strongly increased the extraction of protein in meat.

Hunter L*a*b* color reflectance measurement system is one of the color measurement methods using to determine color of meat. In this measurement system, the L* value (0 and 100) represents the difference between white and black; the a* value represents the green to red tone; the b* value represents the blue to the yellow tone. Both of the values (a* and b*) have no specific numerical limits. Positive a* is green, negative a* is red; positive b* is yellow, negative b* is blue. [33; 89; 90]

Lee et al. [91] studied the effect of sodium phytate (SPT), TSPP, and STPP on physico-chemical characteristics of restructured beef. The four samples which included: (i) 1% NaCl (control); (ii) 1% NaCl + 0.5% TSPP; (iii) 1% NaCl + 0.5% STPP; and (iv) 1% NaCl + 0.5% SPT were studied. The results showed

that the SPT, TSPP, and STPP increased pH in raw beef stored for one day at 4°C and in the cooked beef. In the raw beef, salt-soluble protein level was as follows: STTP > SPT > TSPP > control. In the cooked beef, increase of bind strength, cook yield, moisture level was as follows: STPP > TSPP > SPT > control. SPT, TSPP, and STPP decreased L* value and b* value; and increased a* value in the raw beef but had no effect on the color values in the cooked beef.

Sheard et al. [22] studied the injection of polyphosphate solutions into pork to improve juiciness and tenderness after cooking. Two injection levels (5 and 10%) and three concentrations of STPP (0, 3 and 5%) were used in 64 pork loin samples to assess the influence of STPP injection on the eating quality of pork steaks cooked by grilling to a centre temperature of 72.5 or 80.0°C. The results of sensory evaluation in this study showed that pork steaks containing 5% STPP, injection level 10% and cooked to 80°C were tendered, but as juicy as steaks without STPP.

Torley et al. [92] studied the effect of ionic strength, polyphosphates type, pH, cooking temperature and preblending on the functional properties of normal and pale, soft, exudative (PSE) pork. With TSPP (0.35%) and STPP (0.37%), it was noted that the ionic strength, pH and addition of polyphosphates had much smaller effects on the functional properties of PSE pork than in normal pork meat. Added polyphosphate only gave a lower cook loss though the texture was still inferior.

Capita et al. [93] studied the effect of trisodium phosphate solutions washing on the sensory evaluation of poultry meat. In this study, chicken thigh samples were dipped in TSP solutions (8, 10 and 12%) with the ratio 1:4 (w/v) at 20°C temperature for 15 min; after that, the samples were stored at 2°C until the sensory tests were performed; the sampling days were at day 0 (the day of slaughter, collection and treatment) for raw thighs and day seven of storage at 2°C for raw and cooked thighs. The results indicated that the scores for sensory quality evaluation of 10 and 12% sample were higher than those of the control sample in day 0: better smell and color (chicken thighs dipped in 10% TSP) and better color and overall acceptability (chicken thighs dipped in 12% TSP). However, there were no significant differences between the sensory characteristics of control or treated raw samples after seven days storage apart from the color, flavor and overall acceptability of thighs dipped in 12% which were rated significantly lower than the control sample. These results suggested that TSP solutions have good potential as dips to sanitize chicken carcasses.

Puolanne et al. [21] studied the combinatory effects of sodium chloride and raw meat pH on WHC in cooked sausage with and without added phosphate. In this study, beef and pork with varying natural post-rigor pH value ranges (pork: 5.50 to 6.12 and beef: 5.60 to 6.48) were used as mixtures, and 0.5 to 2.5% NaCl was used with or without added commercial sausage phosphate (2.5 g/kg determined as P_2O_5). The results showed that high pH value and added salt increased WHC in pork and beef meat. The pH-value of raw meat materials for

the maximum water-holding was 6.3. The maximum in water holding was reached with 2.5% NaCl in all pH-values, both with and without added phosphate. When phosphate was added, the pH value of sausage increased approximately 0.5 to 0.7 units. On the other hand, when salt was added, pH value decreased about 0.1 pH unit per 1% NaCl. The same water-holding as with 2.5% NaCl in pH 5.7 reached with 1.5% NaCl in pH 6.1 with increased pH of the batter. In sausages with a reduced content of NaCl, the pH of the batter should increased by using high-pH meat mixtures and/or pH-raising phosphates in order to reach a higher and enough level of water-holding.

Hsu and Chung [23] studied the effect of κ-carrageenan, salt, phosphate, and fat on the qualities of low fat emulsified meatballs (Kung-wans). κ-carrageenan (0 to 2%), salt (1 to 3%), polyphosphate (mixture of sodium polyphosphate and sodium diphosphate, 1:1 ratio, w/w, 0.0 to 0.4%) and pork-back fat (0 to 10%) were used in this study. The results indicated that fat addition (0 to 10%) did not have a significant effect on the measured qualities of low fat Kung-wans. κ-carrageenan addition affected significantly the product cooking yield, hardness, adhesion, chewiness, gumminess and viscosity. Polyphosphate addition showed significant effects on product cooking yield, diameter, lipid content, adhesion, viscosity and a* value (Hunter system - mentioned earlier). The salt content had significant effects on product cooking yield, diameter, lipid content, cohesiveness, brittleness, gumminess and viscosity. The combination of salt and polyphosphates had significant effects on the product's texture and overall acceptance. Additional levels of salt, polyphosphates and κ-carrageenan at around 2.7, 0.17 and 2% respectively, generated products that were more acceptable.

The combination of dextrose and tripolyphosphate with 2% salt to improve tenderness of lamb carcasses was studied by Murphy and Zerby [94]. In this study, each carcass was randomly assigned to one of the following: (i) deionized water (H_2O); (ii) 2% NaCl (S); (iii) 3% dextrose (D); (iv) 0.5% STPP (P); (v) 2% NaCl + 3% dextrose (SD); (vi) 2% NaCl + 0.5% STPP (SP); (vii) 0.5% STPP + 3% dextrose (PD), and (viii) 2% NaCl + 0.5% STPP + 3% dextrose (SPD). The results showed that the use of SD, SP and SPD solutions all improved tenderness, decreased cook loss and increased ultimate pH when compared with the others and had no adverse effects on microbiological growth when stored at 0 to 4°C for six days. Meanwhile, a sample of S solution moderately decreased cook loss, but H_2O, P and D solutions did not; and the use of H_2O, P, D, and S solutions also slightly improved tenderness, but increased the growth of microorganisms.

Fernández-López et al. [48] studied the effect of NaCl, STPP and pH on the color properties of pork meat . The effect of different pH values (4, 5, and 6), different concentrations of NaCl (none, 1.5, and 3%) and of STPP (none, 0.15, and 0.3%) were used in this study. For the pH levels (4, 5, and 6), either 1 M of lactic acid or 1 M of NaOH was added to the pork meat. The results indicated

that when increasing the addition NaCl or STPP, WHC rose, lightness (L*) fell but a* and b* value rose compared to control (without either NaCl or STTP); WHC of samples with added STTP was higher than those with added NaCl. On the other hand, pH value fell with an increased NaCl while it rose with an increased STTP. A decrease in the pH of meat raised L* and b* value but decreased a* value and WHC. However, a lower pH and the addition of NaCl or STPP led to an increase in the metmyoglobin percentage.

The effect of enhancement with phosphates at different injection rates along with 2% NaCl on color, quality, and sensory characteristics of beef was studied by Baublits et al. (2005a, b). In these studies, varying phosphates such as STPP, SHMP, and TSPP at the concentrations 0.2 and 0.4% with rates of injection (12 and 18%) along with 2% NaCl were used. The results indicated that STPP was the most effective phosphate type for maintaining the color of beef in concentration 0.4% at the rate of injection 18% [24]. SHMP, STPP, and TSPP were all evaluated as causing more tenderness and juiciness ($P < 0.05$) by sensory panelists in steaks than the enhancement done only with sodium chloride 2%, but STTP or TSPP in 0.4% with the injection rate 18% can improve sensory tenderness perceptions without decreasing product yields [95]. With the same conditions mentioned earlier, Baublits et al. (2006) studied the effect of enhancement with the variant of phosphate types, concentrations, and injection rates without sodium chloride on color, quality and sensory characteristics of beef. When the samples were without sodium chloride, all the three samples with phosphate types maintained higher L* values than untreated steaks (CNT) through five days-of-display, and SHMP had higher L* values than STPP and TSPP through seven days-of-display; but steaks enhanced with TSPP had higher a* values than CNT on days five and seven of display, whereas SHMP or STPP enhanced steaks generally had similar a* values as CNT after three days of display; no differences were observed between 12 or 18% injection rates. Thus, only steaks enhanced with TSPP were redder, more vivid, and had higher oxymyoglobin proportions with 0.4% concentration [96]. On the other hand, the three phosphate types (SHMP, STPP and TSPP) with different concentrations did not improve sensory tenderness or juiciness compared to untreated muscles, but enhancement at an 18% pump rate improved overall tenderness. These results showed that phosphates enhancement independent of sodium chloride which generally did not improve water retention, cooked yields and palatability compared to untreated samples [97].

Sen et al. [98] studied the effect of chilling, polyphosphate and bicarbonate on quality characteristics of broiler breast meat. The phase with pre-chill and post-chill breast meat, treated with: (i) 3% TSPP; (ii) 3% sodium bicarbonate + 2% NaCl; (iii) 2% NaCl alone (control) was carried out; and the treated samples were stored at 4°C for 24 h. The result of the treatment with phosphate and bicarbonate plus NaCl increased pH in both the pre- and post-chill groups; and treated breasts exhibited lower L* and higher a* value (that is, appeared redder)

than controls. However, the sample treated with TSPP had a smaller effect than the sample treated with sodium bicarbonate plus NaCl.

Ünal et al. [99] investigated the effects of temperature on phosphate diffusion mechanism in meat samples dipped in different concentrations of STPP (0 to 6%) at different temperatures (18 to 36°C). The results indicated that when the concentration of STPP solutions increased, the phosphate concentration in the beef samples also rose, and the diffusion was found to be strongly temperature dependent, that is, the increment in temperature caused an increase in the diffusion.

Barbut and Somboonpanyakul [100] studied the effect of crude Malva nut gum (CMG) and phosphate on yield, texture, color, and microstructure of mechanically deboned chicken meat batters. In this study, mixtures of CMG (none, 0.2 and 0.6%) and STPP (none and 0.5%) were used. The results indicated that the batters with CMG or STPP or mixture of them all decreased cook and fat losses compared with the control batter. Hardness values using the mixture of CMG and STPP were higher than those of the control batter; and hardness values of using CMG or STPP were lower than those of the control batter. The batter with 0.5% STPP and the batters with a mixture of CMG and STPP had higher springiness compared with batters with CMG alone or control sample. Increasing the CMG level to 0.6% reduced the lightness and redness of the cooked products.

Erdogdu et al. [25] studied the effects of processing conditions (cooking time, STPP concentration and dipping time) on cooking losses and textural properties of red meats. For this study, meat pieces (2 × 2 × 2 cm in size) were dipped in different concentrations of STPP solutions (2 to 6%) for 10 to 30 min, and were cooked in boiling water for 5 to 15 min. The results indicated that an increase in STPP concentration increased cohesiveness; an increase in cooking time resulted in higher hardness, gumminess, chewiness and cook losses, while an increase in dipping times decreased the cook losses and hardness. These results also showed that STPP concentration, STPP dipping and cooking times had significant effects on the changes of textural properties and cook losses of red meat.

Somboonpanyakul et al. [101] evaluated the effect of Malva nut gum (CMG) addition to poultry breast meat batters formulated with different salt levels and phosphate. The treatments which consisted of salt (0, 1, 2 and 3%), CMG (none and 0.2%) and STTP (none and 0.5%) were studied. The results showed that the cooked batter with 2% NaCl and 0.5% phosphate presented the highest values for all of the textural parameters. However, the cohesiveness and chewiness were reduced by the addition of 0.2% CMG. Frankfurters with 0.2% CMG had low cooking loss and better textural properties than the frankfurters without CMG. However, frankfurters' lightness and redness were reduced due to the addition of CMG.

Shu Qin et al. [26] studied the influence of marinating with polyphosphate on Simmental beef shear value and ultrastructure. Polyphosphates were used to marinate beef at 5% disodium dihydrogen diphosphate (DSPP), 3% TSPP, 3% SHMP and 3% STPP for one to three days. By increasing the concentration and marinating time, the tenderizing effect of polyphosphates on meat samples changed as follows: TSPP ≈ SHMP > STPP > DSPP > control. The addition of polyphosphates decreased shear force significantly in comparison with controls. After marinating for three days, DSPP significantly increased the soluble collagen content compared with the other polyphosphates. TSPP and SHMP both disrupted the myofibril structure completely and myofibril bundles collapsed together. STPP disrupted the myofibril structure as well. TSPP dissolved the perimysium into collagen fibers and collagen fibrils which arranged loosely and looked like dispersed silk. The perimysium was separated into collagen fibers and collagen fibrils by STPP and SHMP, but the collagen fibrils were in close contact with each other. These results showed that polyphosphates can make the soluble protein in meat to increase binding water and improve tenderness of meat.

In general, with several functions, especially functions such as the adjustment of pH, buffer properties, sequestering of selected cations, charging the ionic charges distributions, changing the ionic strength of environment and /or bacteriostatic effects, phosphates have been widely used in meat products [16; 33; 43; 45; 102; 103; 104]. Although alkaline has also been used to adjust pH leading to increased WHC, its contribution was not significant compared with phosphates [17; 18; 105]. Many types of phosphates and their mixtures at different concentrations and in combinations with other substances also were examined in meat and meat products. The effects of the combination of phosphates and hydrocolloids were studied as well [23; 101; 105]. The results of these studies reported that the use of phosphates increased water holding capacity [21; 47; 53; 105], improved color properties of meat products [48; 95; 96; 97]. Additionally, the individual use of phosphate as well as the combination of phosphate and hydrocolloid were also observed in improvement of textural properties of meat products as bind strength, emulsifying capacity, emulsion stability, yield of patties, tenderness, juiciness and decreased cooking-loss, shear force [17; 22; 23; 25; 26; 47; 100; 101; 106; 107; 108]. Practically, the researchers have nearly tended to focus only on pork, buffalo and beef rather than on poultry meat. Hydrocolloids have rarely been used in the above studies as well [23; 80; 109; 110].

4. DESIGN OF PHASES

4.1. Phase I

For the purpose of the study, the nine following phosphates were chosen to use as follows: monosodium phosphate (MSP), disodium phosphate (DSP), trisodium phosphate (TSP), tetrasodium diphosphate (TSPP), disodium diphosphate (SAPP), sodium tripolyphosphate (PSTP), sodium hexametaphosphate (SHMP), tripotassium phosphate (TKP) and tetrapotassium diphosphate (TKPP).

The aim of the Phase I was to evaluate the effect of different salts of phosphates (sodium and potassium salts of monophosphate, diphosphate, triphosphate and/or polyphosphates) and its concentrations on textural properties of model meat products. For the phase I, in order to obtain model samples, MDPM (525 gram), ice water (176-183 gram), salt (mixture of NaCl and $NaNO_2$ in ratio of 500:1; 14 gram) and selected phosphates were used. The formulation of the phase I is shown in Table 10.

Table 10. Formulation for phase I (g)

Formulation	Meat	Salt	Phosphates	Ice-water
control	525	14	0	176
0.05%	525	14	0.300	177
0.10%	525	14	0.600	177
0.15%	525	14	0.900	178
0.20%	525	14	1.200	179
0.25%	525	14	1.500	180
0.30%	525	14	1.800	180
0.35%	525	14	2.100	181
0.40%	525	14	2.400	182
0.45%	525	14	2.700	183

For the first study, the nine different types of phosphates were used in the concentration range of 0-0.45% (w/w) with a concentration step of 0.05%, where 0% represented the control sample.

The addition of phosphate was compensated using water (1–7 g) for keeping the dry matter content constant (the target dry matter content of control and also model samples was 30–31% w/w).

The pH value of phosphate in solution 1% is shown in Table 11.

Table 11. pH-values of selected phosphates in 1% solution at room-temperature used in the study

Phosphates	**pH (solution 1%)**
Monosodium phosphate (MSP)	4.82 ± 0.01
Disodium phosphate (DSP)	9.62 ± 0.01
Trisodium phosphate (TSP)	12.61 ± 0.01
Tetrasodium diphosphate (TSPP)	10.34 ± 0.04
Disodium diphosphate (SAPP)	4.81 ± 0.01
Sodium tripolyphosphate (PSTP)	6.44 ± 0.02
Sodium hexametaphosphate (SHMP)	10.06 ± 0.01
Tripotassium phosphate (TKP)	12.46 ± 0.01
Tetrapotassium diphosphate (TKPP)	10.53 ± 0.02

(Each value is the mean of three determination ± standard deviation)

The dry matter content, fat content, true protein (the sum of amino acid contents determinated using Amino Acid Analyzer) and pH value of raw MDPM were analyzed.

All the above raw materials were finely stirred by the stirrer Vorwek Thermomix TM31-1 instrument (Vorwerk & Co., GmbH, Wuppertal, Germany) at a low speed (approximately 100 rpm for the first minute and 300 rpm for two minutes) at temperature lower than 12^{o}C to form homogeneously emulsified mixtures in laboratory room in the Faculty of Technology. These mixtures were stuffed into glass cans (diameter 8.0 cm, height 7.0 cm), closed with screw lids, then through thermal treatment processing (the temperature was controlled at 70±1^{o}C) for 15 minutes. After heating, the treated samples were cooled in an ice water tub for 30 minutes to achieve the endpoint product temperature of 25^{o}C. Finally, the samples were stored at 6±1^{o}C in the fridge for 7 days, and then they were removed on the seventh day of storage to analyze their textural parameters. Treatment for each type of phosphates was performed three times for statistical purpose (including the control sample).

4.2. Phase II

The aim of the Phase II was to evaluate the effect of binary mixtures of selected sodium and/or potassium salts of phosphates on textural properties of model meat products. Phosphates selected after obtaining the results of Phase I were used for the second study including tetrasodium pyrophosphate, disodium diphosphate and sodium hexametaphosphate. For the whole Phase II, the same concentration of total phosphates was maintained. The manufacture was

according to the same protocol as in Phase I. The formulation of phase II is shown in Table 12.

Table 12. Formulation for phase II (g)

Formulation	Meat	Salt	Binary Phosphates *	Ice-water
control	525	14	0	176
100:0	525	14	1.5	180
90:10	525	14	1.5	180
80:20	525	14	1.5	180
70:30	525	14	1.5	180
60:40	525	14	1.5	180
50:50	525	14	1.5	180
40:60	525	14	1.5	180
30:70	525	14	1.5	180
20:80	525	14	1.5	180
10:90	525	14	1.5	180
0:100	525	14	1.5	180

* Three salts of phosphates chosen for phase II were tetrasodium pyrophosphate, disodium diphosphate and sodium hexametaphosphate. Thus, three binary mixtures in 11 percentage ratios (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100) were applied. The concentration of binary phosphates used in Phase II was 0.25%.

4.3. Phase III

The aim of Phase III was to evaluate the effect of different carrageenans (κ- and ι- carrageenans) on textural properties of model meat products (without phosphates). Individual carrageenans were used at concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5% (w/w), respectively. The control samples without any carrageenans were also prepared. The manufacture was realized according to the same protocol as in Phase I and II.

The formulation of phase III is shown in Table 13.

Table 13. Formulation for phase III (g)

Formulation	Meat	Salt	Carrageenan	Ice-water
control	525	14	0	176
0.1%	525	14	0.715	177
0.2%	525	14	1.430	179
0.3%	525	14	2.145	180
0.4%	525	14	2.860	182
0.5%	525	14	3.575	183

5. ANALYSIS METHODS

5.1. Chemical analysis

According to AOAC [111], dry matter content was determined in raw mechanically deboned poultry meat by the difference before and after oven drying at 102°C for 16 h.

Crude lipid content was measured by drying the test samples in a 102°C oven for 6h, then extracting the lipid with ether in Soxhlet extractor for 4 h.

The sum of amino acid contents was determined by using Amino Acid Analyzer. Three samples of MDPM (0.100–0.110g) were accurately weighed into screw-capped test tubes (washed in chromosulphuric acid for 24 h) with Teflon caps (20 ml, Labicom, Olomouc, Czech Republic). Fifteen milliliters of 6 $mol \cdot l^{-1}$ HCl were added to the tubes, which were purged by argon for 1 min. Then the tubes were placed in a thermoblock (Labicom, Olomouc, Czech Republic) heated at 110±1°C and hydrolyzed for 24 h. The temperature of the thermoblock was independently controlled by using a thermometer drowned in a test tube filled with silicone oil (the test tube was placed in the thermoblock). After a 16h oxidation (with the mixture of 30% [v/v] hydrogen peroxide and 98% [v/v] formic acid in the ratio 1:9 [v/v]), sulfur amino acids as cysteine and methionine were hydrolyzed in the same way. After the hydrolysis, the test tubes were cooled down to 20°C. Hydrochloric acid was evaporated and the ropy residue was diluted in loading buffer (as shown in Table 14) in a 25 ml volumetric flask. The mixture was filtered through a 0.45 mm filter and loaded into an analyzer. [112]

Table 14. Composition (g) of used sodium citrate buffers used for a final volume of 1L [112]

Reagent	**Buffer**				
	A	**B**	**C**	**D**	**Loading buffer**
Citric acid monohydrate	11.11	10.00	7.53	0	14.00
Sodium citrate dehydrate	4.04	5.60	9.06	19.60	0
Sodium chloride	9.29	8.36	18.0	52.60	11.50
Boric acid	0	0	0	2.05	0
Sodium azide	0.10	0.10	0.10	0.10	0.10
Sodium hydroxide	0	0	0	0.50	0
Thiodiglycol (ml)	2.50	2.50	2.50	0	5.00

Liberated amino acids were determined by using ion-exchange chromatography. During the acid hydrolysis, asparagine and glutamine were converted into aspartic and glutamic acid, respectively. The amount of a

hundred ml of the hydrolyzed extract in loading buffer was automatically injected into an Amino Acid Analyzer AAA400 (Ingos, Prague, Czech Republic) equipped with a column (370 mm x 3.7 mm, filled with an ion exchanger Ostion LG ANG – Ingos, Prague, Czech Republic), post-column ninhydrine derivatization and spectrophotometric detection (440 nm for proline and 570 nm for other amino acids). Amino acids eluted according to the use of a gradient (the composition of sodium citrate buffers is presented in Table 14): 0–5 min buffer A, 5–32 min buffer B, 32–44 min buffer C, 44–75 min buffer D. Then the column was regenerated by 0.2 $mol \cdot l^{-1}$ NaOH for 10 min and stabilized for further 17 min by buffer A. The temperature of column was set to 60°C (0–60 min and 90–102 min) and to 74°C (60–90 min), respectively. Sulfur amino acids were separated and quantified as cysteine acid and methionine sulfate. The buffer system and the process of ninhydrine reagent preparation [consisting of ninhydrine, methylcellosolve, acetate buffer (pH 5.5) and hydrindantine] had been recommended by the manufacturer of the analyzer. A flow rate was 0.3 $ml \cdot min^{-1}$ for buffers and 0.2 $ml \cdot min^{-1}$ for ninhydrine reagent. Each hydrolysate was analyzed in duplicate. A standard of 15 analyzed amino acids was obtained from Ingos, Prague, Czech Republic [112].

The pH value of material meat, homogenized meat mixes, meat products was measured directly with a glass electrode (pH Spear –Eutech Instrument).

Each sample was measured at least three times for the statiscal purpose.

5.2. Texture profile analysis

Texture profile analysis (TPA) has been widely used as an instrumental method, providing information on both the deformation and fracture properties of food.

The textural parameters of MDPM batters were determined using a texture analyzer TA.XTplus (Stable Micro Systems Ltd., Godalming, U.K.) with a load cell of 30 kg. The uniform cylindrical cores of the samples (diameter 3.5 cm, height 1.5 cm), considered as test specimens, were obtained from the middle portion of each batter using a cylindrical borer and a wire-cutting knife. Product cohesiveness, hardness, adhesiveness and gumminess were measured by compressing the cylinder plunger down on the sample specimens twice to 75% of its original height (pre-test speed 2.0 $mm \cdot s^{-1}$; test speed 0.5 $mm \cdot s^{-1}$; and time between two compressions 5.0 s). As showed in Fig. 14, the textural parameter determined in this study as follows:

Hardness was measured as force needed to attain a given deformation - the maximum force during the first compression; unit: N.

Cohesiveness, the strength of the internal bonds of batter, was the ratio of the positive force area during the second compression to that during the first compression; no unit.

Adhesiveness, the work needed to pull out the plunger from the sample, was the negative force area of the first compression cycle; unit: N·mm.

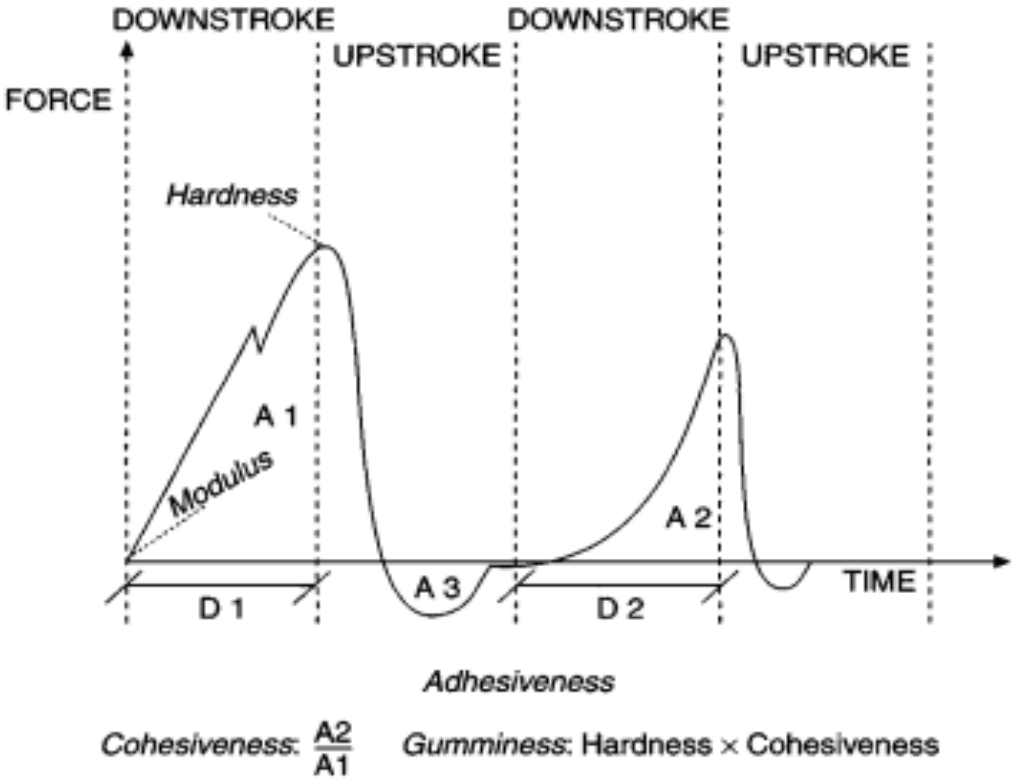

Figure 14. The model of TPA [113].

Gumminess, the energy required to disintegrate a batter so that it is ready for swallowing, was calculated as hardness × cohesiveness; unit: N.

Above textural parameters were obtained from the software Exponent Lite version 4.0.13.0 attached to the texture analyzer.

5.3. Statistical analysis

Homogeneity of pH in the individual samples was verified by Kruskall-Wallis and Wilcoxon tests (non-parametric variants of analysis of variants). The significance level used in the tests is 0.05. Unistat® 5.5 software (Unistat, London, UK) was used for the statistical evaluation. The same test was used for comparison of textural parameters of model samples with individual phosphates and/or hydrocolloids.

Polynomial regression was used in order to evaluate the global dependence of selected textural parameters (hardness, cohesiveness, adhesiveness and gumminess ratio – dependent variables) on covariates (the concentrations of phosphates, the concentrations of hydrocolloids and also the ratio of both phosphates in binary mixtures).

6. RESULTS AND DISCUSSION

6.1. Chemical analysis of raw material

Chemical analysis including the determination of pH value, fat, dry matter content and sum of amino acids were performed on the raw meat.

The results of chemical analysis are listed in Table 15 and Table 16.

Table 15. Chemical composition of MDPM

Measurements	**Value**
pH	6.35 ± 0.02
Dry matter %(w/w)	38.34 ± 0.27
Fat content %(w/w)	21.8 ± 0.9
True protein %(w/w)	14.0 ± 0.5

(Each value is the mean of three determination ± standard deviation)

Table 16. Amino acids composition of MDPM

Amino acids	**Value**
Asparagine	15.5 ± 0.8
Threonine	5.9 ± 0.1
Serine	19.7 ± 0.9
Glutamine	6.1 ± 0.1
Proline	6.9 ± 0.3
Alanine	8.2 ± 0.3
Valine	7.3 ± 0.1
Isoleucine	7.0 ± 0.1
Leucine	10.7 ± 0.1
Tyrosine	5.5 ± 0.1
Phenylalanine	5.8 ± 0.2
Histidine	6.8 ± 0.2
Lysine	11.8 ± 0.3
Arginine	10.8 ± 0.5
Cysteine	2.3 ± 0.1
Methionine	4.6 ± 0.2

(Each value is the mean of three determination ± standard deviation)

The proximate composition of MDPM was shown in Table 15 and 16. Data of proximate composition from our study were in agreement with those found by other authors [12; 13].

6.2. Effects of different types and concentrations of phosphate salts on textural properties of meat batter made from MDPM

6.2.1. Results

The values of pH of batters were significantly influenced by using of certain phosphate salts (Fig. 15). The pH-value of control samples was 6.36±0.03. With increasing concentrations of DSP, TSP, TKP, TSPP and TKPP the pH-values of samples were linearly rising ($P<0.05$). On the other hand, with increasing amounts of MSP, SAPP and SHMP the values of pH of products were linearly falling ($P<0.05$). The differences in pH-values between batters with sodium or potassium salts (of the same anions) were insignificant ($P\geq0.05$). For the concentration of phosphates salts 0.45% (w/w), the pH-values of samples were decreasing as follows:

$$TSP \approx TKP > DSP \approx TSPP \approx TKPP > PSTP > SHMP > MSP > SAPP$$

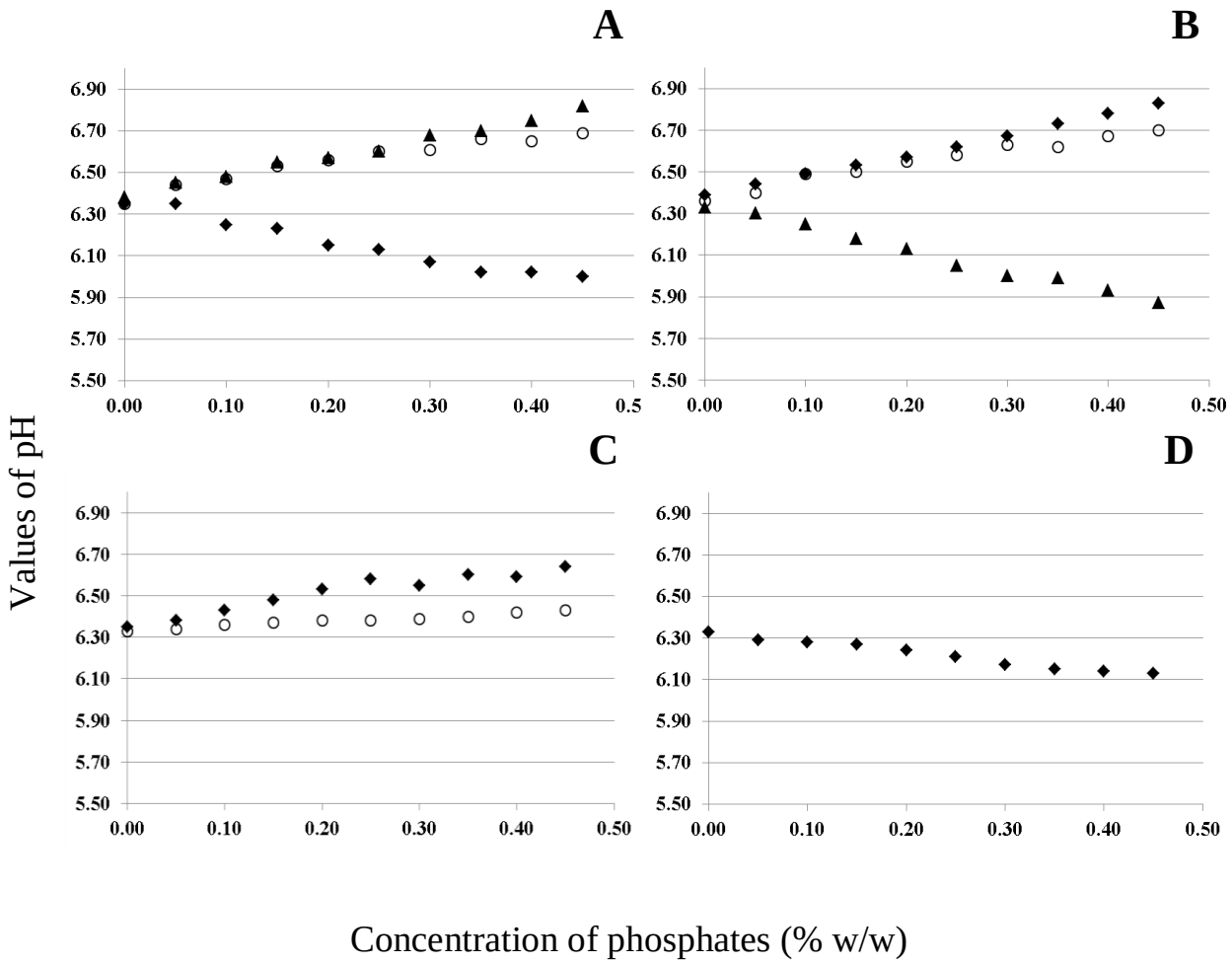

Figure 15. The dependence of pH-values on the type and concentration of sodium or potassium salts of phosphates (% w/w). Part A: monosodium phosphate (◆); disodium phosphate (○); trisodium phosphate (▲). Part B: tripotassium phosphate (◆); tetrasodium diphosphate (○); disodium diphosphate (▲). Part C: tetrapotassium diphosphate (◆); sodium triphosphate (○). Part D: sodium hexametaphosphate (◆).

The results of hardness and gumminess are shown in Figs. 16–19. All of the index of determinations of designed regression models were significant ($P<0.05$). The hardness and gumminess of control samples were in ranges of 90–95 N and 25–27 N, respectively. For sample SDP, the higher value reported for hardness was achieved when using a concentration of 0.25% and the lower using not only lower (0.05%) but also higher concentrations (0.45%). This described a fluctuating behavior. In the case of DSP samples, the higher hardness value was obtained using a concentration of 20%. The use of higher concentrations causes a decreased on the estimation of this parameter. For TSP samples there was not observed a significantly change of hardness using different concentrations. In general, hardness value decreased slightly when increasing the concentration. The analysis for TKP revealed that using lower concentrations (0.05%)

increased the hardness value, which decreased when increasing the amount of phosphate salt. Similarly as TSPP, using SAPP phosphate salt caused just a slightly change in hardness value. The lower value reported was 0.20% and the higher (88.5 N) was obtained at lower concentrations (0.05%). The behavior of hardness variation using TSPP can be described as fluctuating, since the hardness value starts to increases (114 N)in the presence of small amount of the phosphate salt (concentration of 0.10%) but with more addition suddenly drops (81.0 N). The lower hardness value found was 74.9 N using 0.40% of TSPP. Similarly, for TKPP the higher hardness value (100.9 N) was found at 0.10% of concentration and the lower (75.4 N) at concentration of 0.40%. For PSTP, considerably lower values of hardness were found, in comparison to other phosphate salts. The values were between 57.2 N and 80.5 N. It is evident from the data that higher concentrations of this phosphate salt had a strong influence on the hardness decay. The use of POLY caused also a fluctuating behavior, firstly, the hardness decreased with the addition of the phosphate salt but after the addition of more salt, (0.35%) it increased up to 86 N.

For gumminess analysis, the results showed that the use of SDP presented a fluctuating behavior, with lower gumminess values using lower (23.1%) and higher concentration (0.40%) of the salt. Similarly to hardness parameter, the higher gumminess value was obtained using 0.25% of the phosphate salt. When using DSP as phosphate salt, small amounts (concentration of 0.10%) caused an increase in gumminess (31.1 N) while higher concentrations caused a decreased on the value of this parameter (23.3 N using 0.45%). The use of TSP demonstrated that this salt had an effect in reducing the gumminess value. A concentration of 0.40% showed the lower value (19.4 N) and the higher value was obtained with 0.05% of salt. In the case of TKP, a fluctuating behavior was presented with higher values for gumminess using concentrations of 0.05 and 0.35% (28.2 and 26 N respectively). The increase in SAPP concentration caused a decrease in gumminess. While higher values were obtained with relatively lower concentration of phosphate salt (27 N with concentration of 0.10%), a lower value (19.3%) was presented at 0.35% concentration. The effect of TSPP was similar to the one presented using DSP. A higher gumminess value (36.2%) was reported for concentration of 0.10% and the lower value with concentration of 0.40% (18.2%). An opposite behavior to SDP was observed for TKPP phosphate. The higher value was found for concentration of 0.20% (31.1 N) and the lower (18.9 N) with higher concentration 0.40%. For PSTP, higher concentrations of phosphate caused a decreased on the value reported for gumminess. A slightly increase was observed using concentration of 0.10% (21.7 N) but in general lower values for this parameter were obtained. The lowest value was 14.7 N using the maximum possible concentration (0.45%). The effect of POLY was slightly similar to the one found for TKP, although not considerable difference was found using different amounts of phosphates, the effect of the increase in concentration on gumminess was similar for both salts. For POLY, values between 19.3 and 24.3 N were obtained. The samples SDP,

DSP, TSP, TKP, TSPP and TKPP presented at least one gumminess-value that was higher than the control sample.

In relation to the cohesiveness it was seen that using SDP as phosphate salt, the higher value was obtained using higher concentrations of salt (0.3 with concentration of 0.45%) which was indeed the highest value found from all the different phases. The use of DSP did not show a significant change on cohesiveness since the range was between 0.2 and 0.3. A different behavior was observed for TSP, where the increase in concentration caused a considerably decrease in cohesiveness values. The higher value found was 0.2 using concentration of 0.20%. In contrast, the use of TKP up to concentration of 0.35% showed a cohesiveness value of 0.3. For SAPP phosphate salt, a fluctuating behavior was observed with higher values of cohesiveness using relatively lower concentration (0.10%) and the values decayed while increasing the salt. In general, TSPP phosphate salt showed a similar behavior to DSP, with no significant variation in comparison to the standard. Slightly higher values were obtained using concentration of 0.35% (0.3). Using TKPP presented higher values using concentrations of 0.20 and 0.35% in comparison to other concentrations (0.3 and 0.2 respectively). When using PSTP as phosphate salt, the cohesiveness values started to decay. All the samples presented lower values in comparison to the control sample. The use of POLY did not showed a considerably effect in cohesiveness as only a slightly difference was found for the different concentrations.

In relation to the adhesiveness parameter, it was found a markedly variation when using SDP as phosphate salt. While the adhesiveness started to increase with the presence of the salt, it decreased for concentration values between 0.1 and 0.25%. The higher value was obtained with concentration of 0.30% (0.32 N·mm). In the case of DSP, the highest value of adhesiveness (0.4 N·mm) was found using the maximum concentration tested (0.45%). A similar effect was observed for TSP phosphate, obtaining a value of 0.4 N·mm. In fact, this value (with concentration of 0.45%) was the highest among all the phases. The use of TKPP showed a fluctuating behavior as the concentration raised up to 0.33 N·mm using 0.10% followed by increase and decrease of adhesiveness. For PSTP the higher value obtained was 0.42 N·mm using 0.40% of phosphate salt and in general it showed a considerable increase in comparison to the sample without phosphate. The addition of POLY as phosphate salt, a closer higher value was obtained (0.4 N·mm) with a concentration of 0.30%. All the phosphates tested presented at least one concentration that was higher in adhesiveness compared to the control sample.

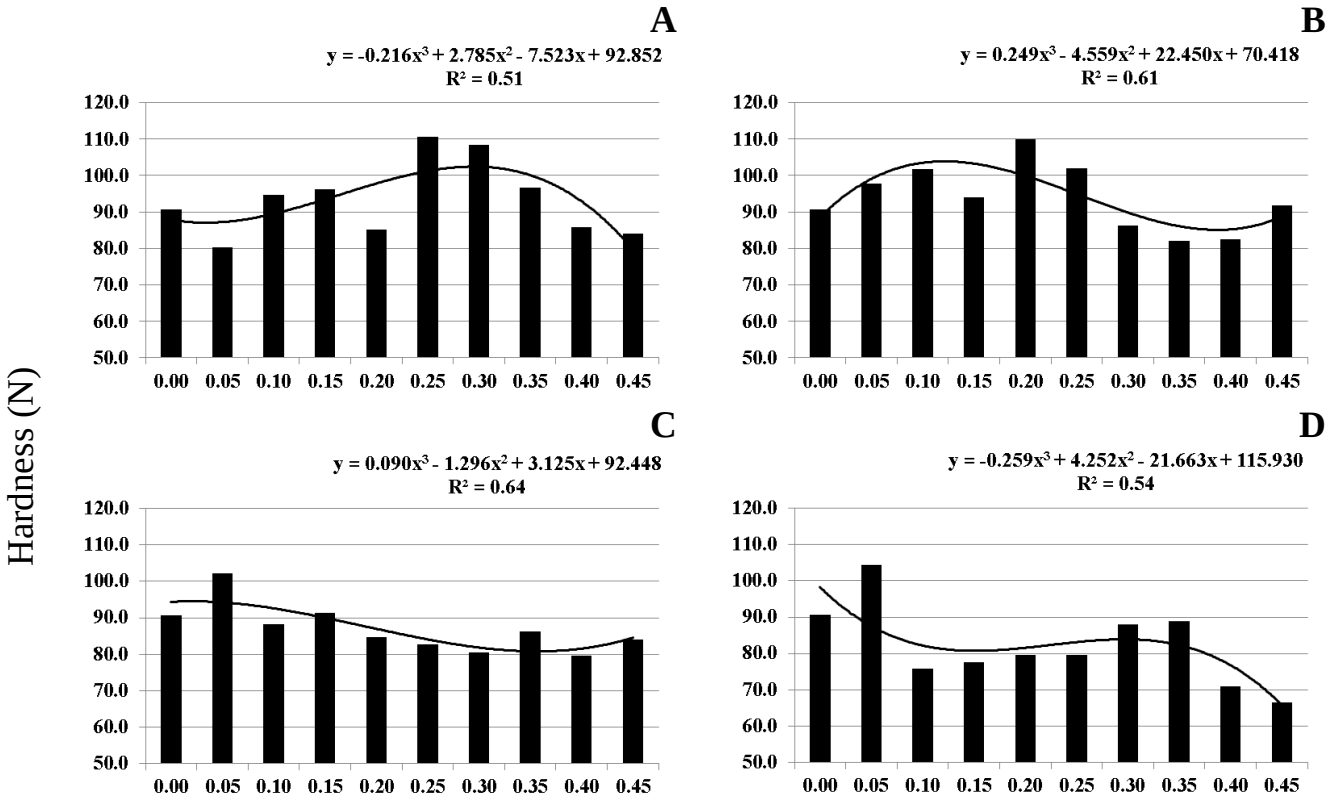

Figure 16. The dependence of hardness (N) on the type and concentration of sodium or potassium salts of phosphates (%, w/w). Part A – monosodium phosphate; Part B – disodium phosphate; Part C – trisodium phosphate; Part D – tripotassium phosphate. The results of regression analysis (the third order polynomial model and the index of determination of the model) were expressed.

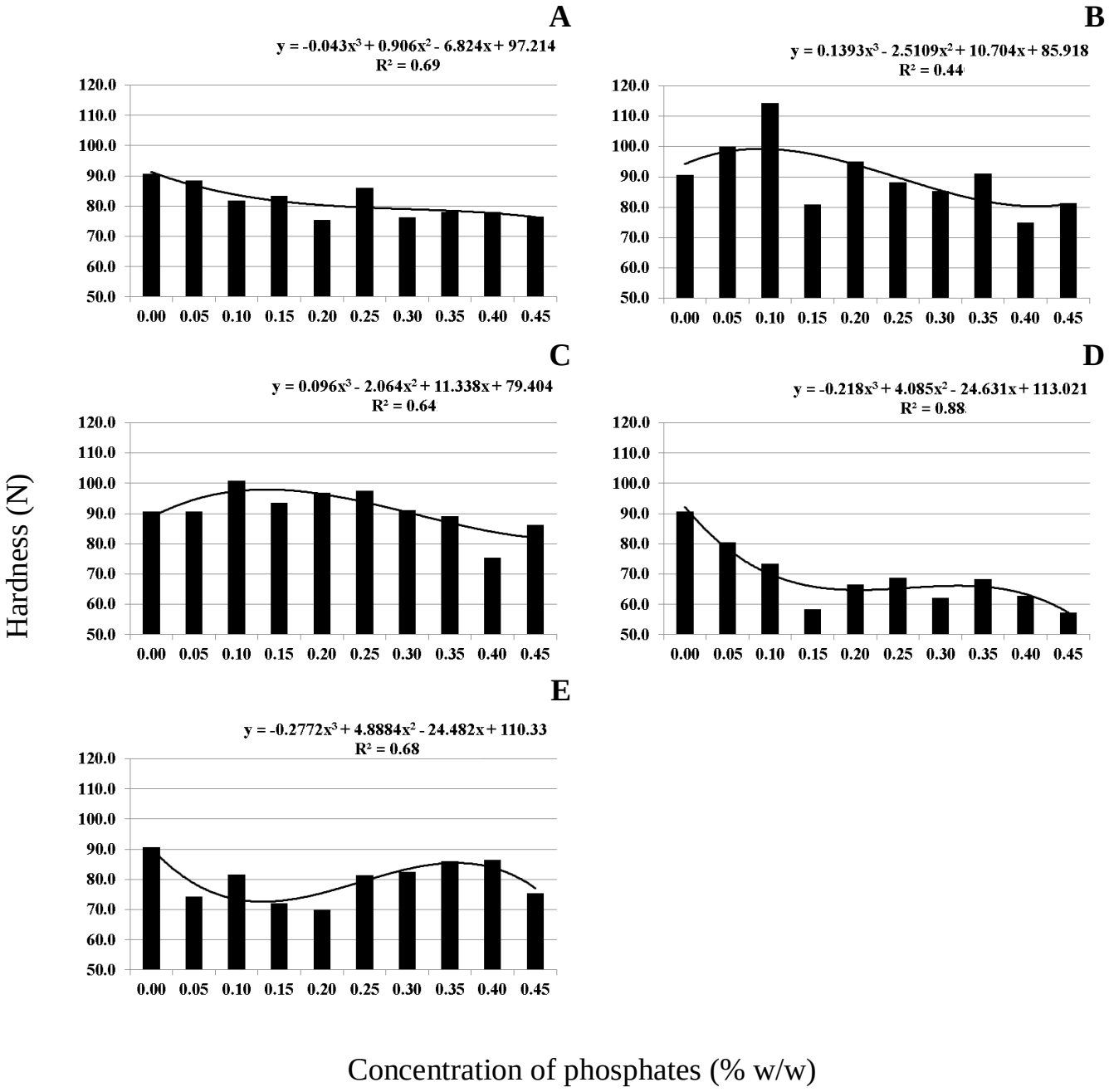

Figure 17. The dependence of hardness (N) on the type and concentration of sodium or potassium salts of phosphates (%, w/w). Part A – disodium diphosphate; Part B – tetrasodium diphosphate; Part C – tetrapotassium diphosphate; Part D – sodium triphosphate; Part E – sodium hexametaphosphate. The results of regression analysis (the third order polynomial model and the index of determination of the model) were expressed.

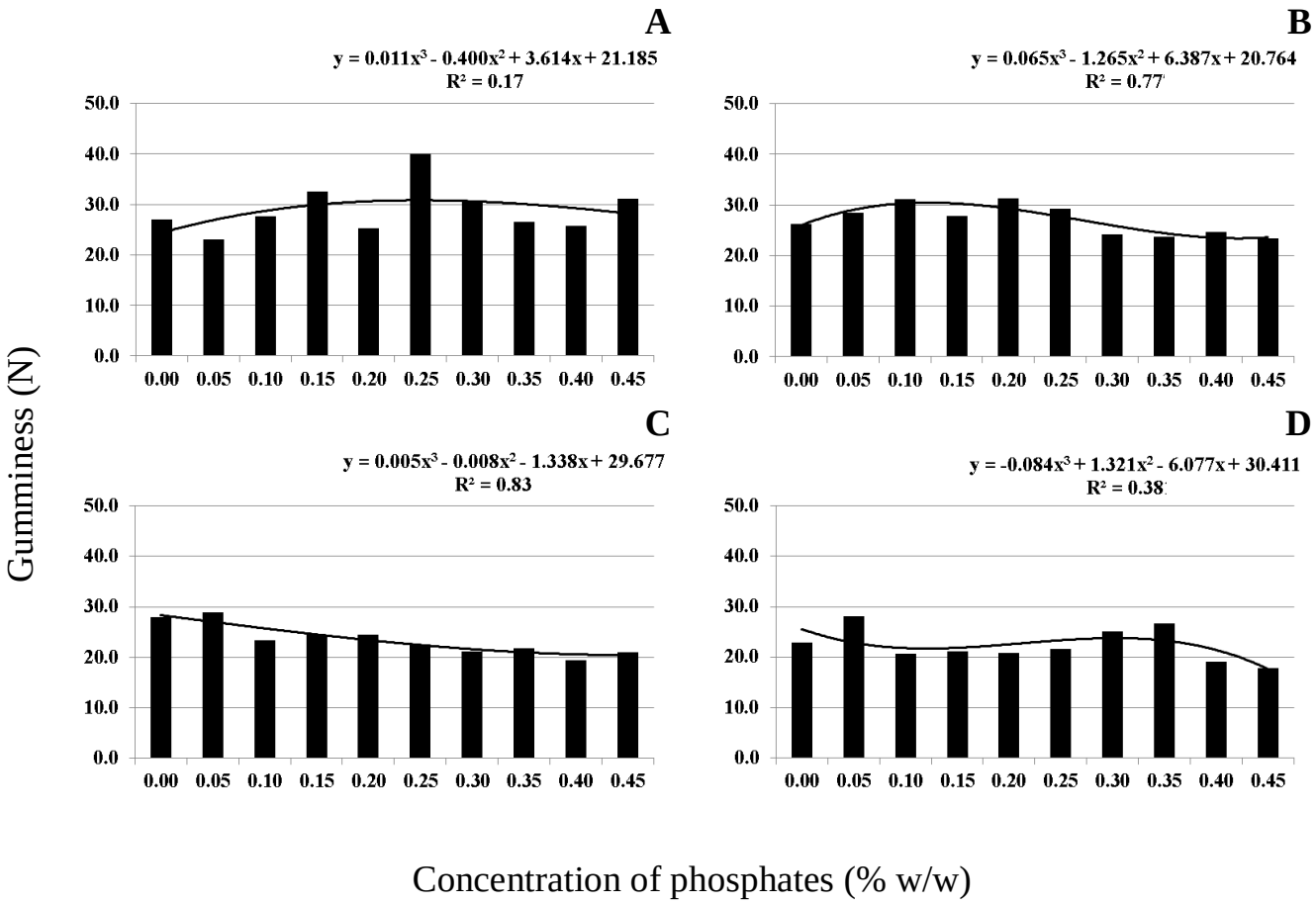

Figure 18. The dependence of gumminess (N) on the type and concentration of sodium or potassium salts of phosphates (%, w/w). Part A – monosodium phosphate; Part B – disodium phosphate; Part C – trisodium phosphate; Part D – tripotassium phosphate. The results of regression analysis (the third order polynomial model and the index of determination of the model) were expressed.

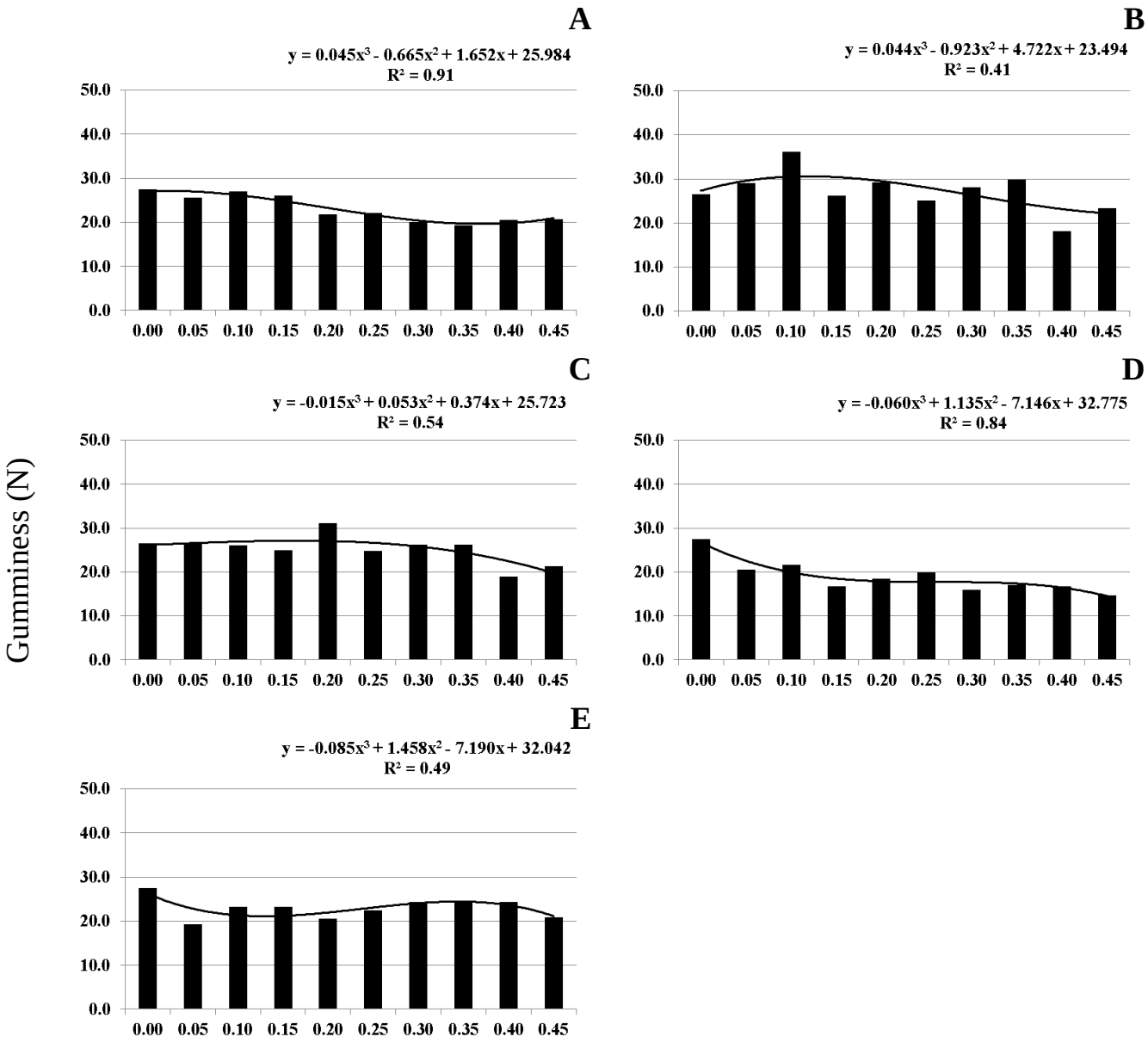

Figure 19. The dependence of gumminess (N) on the type and concentration of sodium or potassium salts of phosphates (%, w/w). Part A – disodium diphosphate; Part B – tetrasodium diphosphate; Part C – tetrapotassium diphosphate; Part D – sodium triphosphate; Part E – sodium hexametaphosphate. The results of regression analysis (the third order polynomial model and the index of determination of the model) were expressed.

In resume, the hardness and gumminess of samples with MSP and DSP were growing up to the concentration ranges of 0.25–0.35% and 0.20–0.25% (w/w), respectively. Higher concentrations caused a decrease in hardness and also gumminess measured. With increasing amounts of TSP, TKP, SAPP and PSTP (up to 0.45% w/w) the hardness and gumminess of batters is declining. Only exceptions were observed when the concentrations of TSP and TKP were ≈ 0.05% (w/w) when hardness and gumminess slightly rose.

The similar courses were regarded when TSPP and TKPP used. The hardness and gumminess of samples were growing up to 0.10% (w/w) and then with increasing amounts of TSPP or TKPP the decrease of the above mentioned textural parameters were recorded. The course of changes of batters with SHMP was different in comparison with the other phosphates used. The highest hardness and also gumminess were observed in elevated concentrations of phosphates (0.35–0.40% w/w). Systematic significant differences between hardness and gumminess of samples, where sodium and potassium salts (compared the products with the same anions, e. g. TSPP, TKPP) were used, were not observed ($P \geq 0.05$).

The changes of adhesiveness or cohesiveness of batters depending on the concentrations of individual phosphates salts were not significant ($P \geq 0.05$; data not shown). Cohesiveness and adhesiveness values of control samples were approximately in 0.2 (unitless) and 0.2 N·mm, respectively. Generally, slight decrease of cohesiveness of products was observed when phosphates salts were applied.

6.2.2. Discussion

The main purpose of this research was to study the effects of phosphates on the hardness, cohesiveness, adhesiveness and gumminess values of the MDPM batters. It is clear that phosphates have adjusting abilities of pH in meat products. On the other hand, the pH-values are not represented the alone impact on the textural parameters. The changes of pH were practically linear (Fig. 15). The dependence course of hardness or gumminess of samples with the most phosphates salts (practically without SAPP and PSTP) on concentration of phosphates showed that the local maximum (on the curves) existed (see Figs. 16–19). The dependences showed the third order polynomial course. The latter mentioned findings could be interpreted that optimal concentrations of individually phosphates should be found and pH-values are only a factor that could influence textural parameters. Myofibril proteins affect directly textural properties of meat products due to the presence of myosin. This result of the first study indicated that there was a network structure formed in the samples treated with phosphates, and the meat batters exhibited textural properties changed compared to the control sample. As mentioned in the literature review, the

texture of meat products could be influenced by many factors. Firstly, phosphates form a complex with divalent cations in meat protein as Ca^{2+} and Mg^{2+} to separate actin and myosin in myofibril protein. Combining along with sodium chloride, phosphates increase the extractability of muscle protein leading to the formation of the gel matrix [53]. The formation of gel structure is dependent on interfering with interaction between protein and protein. Hence, it can be considered that the gel network in meat batters is caused due to protein gelation. The decrease of pH of treated sample causes the denaturation of muscle protein, especially when pH reaches near isoelectronic point pH of meat protein about 5.5 [33]. By that time, proteins are closely which prevent the solubility of myosin and affect negatively the formation of gel network in meat batters. The protein denaturation is also dependence on cooking temperature [99]. Offer [114] also showed that protein denaturation was the result of the poor gelation in meat. According to the report of Acton and Dick [115], gels almost reach appreciable strength until the myosin tail portion has undergone helix-coil transformation and subsequent cross-linking and the complete myosin molecule is necessary for attaining appreciable continuity and strength in the protein matrix.

In addition, because of the different chemical and functional properties of phosphates, the hardness and gumminess of MDPM batters were significant different in this study. A decreasing in cohesiveness values has been associated to a reduction in the emulsification ability of meat products [77]. According to Molins [45], the most functional phosphates are diphosphates, especially TSPP, because they act on the actomyosin complex of the meat protein right away and have a high pH value. Baulblits et al. [24] showed that PSTP and TSPP in 0.4% improved sensory tenderness perceptions of beef without decreasing product yields. Erdogdu et al. [25] claimed that the polyphosphates decreasing protein-protein interaction, increasing protein solubility by enhancing water holding capacity and increasing denaturation temperature of proteins can be attributed to theirs effects on textural properties. Bartbut and Somboonpanyakul [100] also reported that the hardness value of the using 0.5% STPP in DPM batter was lower than that without phosphate batter but the cohesiveness value was higher.

6.3. Effects of binary phosphate salts on textural properties of meat batter made from MDPM

6.3.1. Results

As shown in Fig. 20, the pH values of meat batter treated with binary phosphates and with different ratios at concentration of 0.25% were significantly different. The changes of phosphate ratio in binary mixtures, and the pH values of the samples were linear. When increasing TSPP and SAPP respectively in binary phosphate TSPP: SHMP and SAPP: TSPP, the pH values of meat batters

increased linearly, whereas in binary phosphate of SAPP and SHMP pH values of meat batters decreased also linearly.

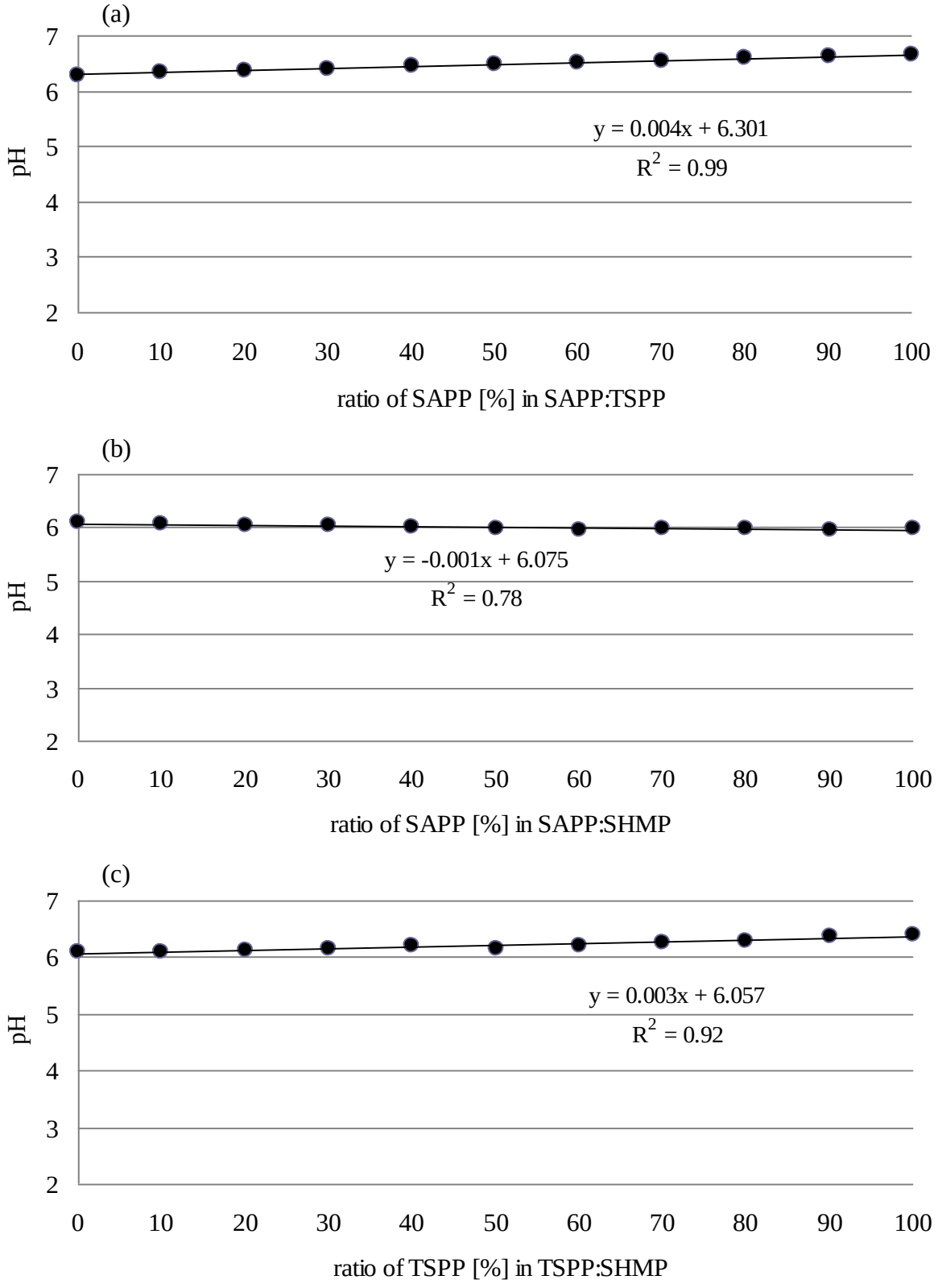

Figure 20. The dependence of pH values on binary phosphate with different ratios: (a) SAPP:TSPP, (b) SAPP:SHMP and (c) TSPP:SHMP.

The dependence of pH values on different ratios of three different binary phosphates (SAPP:SHMP, SAPP:TSPP, TSPP:SHMP) was presented in three different plots in Fig.20 (a,b,c). The first plot (Fig. 20a) showed that in the binary phosphate SAPP:TSPP, the pH value was proportional to the concentration of SAPP, as a result, higher values of SAPP increased the pH value. However, in the case of SAPP:SHMP as binary phosphate (Fig. 20b), the pH was proportional to the concentration of SHMP, as higher values (~ 6.1) were obtained with lower amount of SHMP. The decrease in this case was low, thus it could be considered as no significant. By a similar analysis, a binary phosphate of TSPP:SHMP with higher TSPP ratio raised the pH value (Fig. 20c).

Figs. 21-23 showed the dependence of hardness, cohesiveness and adhesiveness on the different ratios of binary phosphates. From the textural parameters of added binary phosphate of TSPP and SHMP, it was analyzed that the use of ratios of TSPP and SHMP (40:60 – 50:50) presented the high hardness values of meat batters, 10.3 N and 10.7 N, respectively. At concentration of TSPP and SHMP (40:60), adhesiveness and cohesiveness values were the highest, $-0.5 \cdot 10^{-2}$ and ~ -0.4, respectively. However, an increase in the ratio of TSPP in binary phosphate caused a fluctuating behavior in the response of hardness, adhesiveness and cohesiveness values of meat batter.

The use of binary phosphate of SAPP and TSPP showed the lowest values of hardness force, which were in the range of 4.4 - 9.9 N, compared to the other binaries. The samples treated with this mixture showed significant changes for hardness values and represented a maximum decrease at ratios of SAPP and TSPP (70:30-80:20) in relationship to the sample with only SAPP. It was similar to adhesiveness values, but not to cohesiveness values. The binary phosphate of SAPP and TSPP did not show influences in cohesiveness force as since the values measured remained almost constant.

In the case of binary phosphate of SAPP and SHMP, the higher values of hardness, adhesiveness and cohesiveness were obtained using binary phosphate with a SAPP and SHMP ratio of 20:80 and 40:60, respectively. It also was seen that higher ratios caused a decrease in the force values measured. The use of SAPP and SHMP as binary phosphate originated an increase in the response up to 11.7 N. This binary phosphate had a strongly positive influence on hardness force, since the maximum values reported increased nearly 8% in comparison to the samples with other binaries.

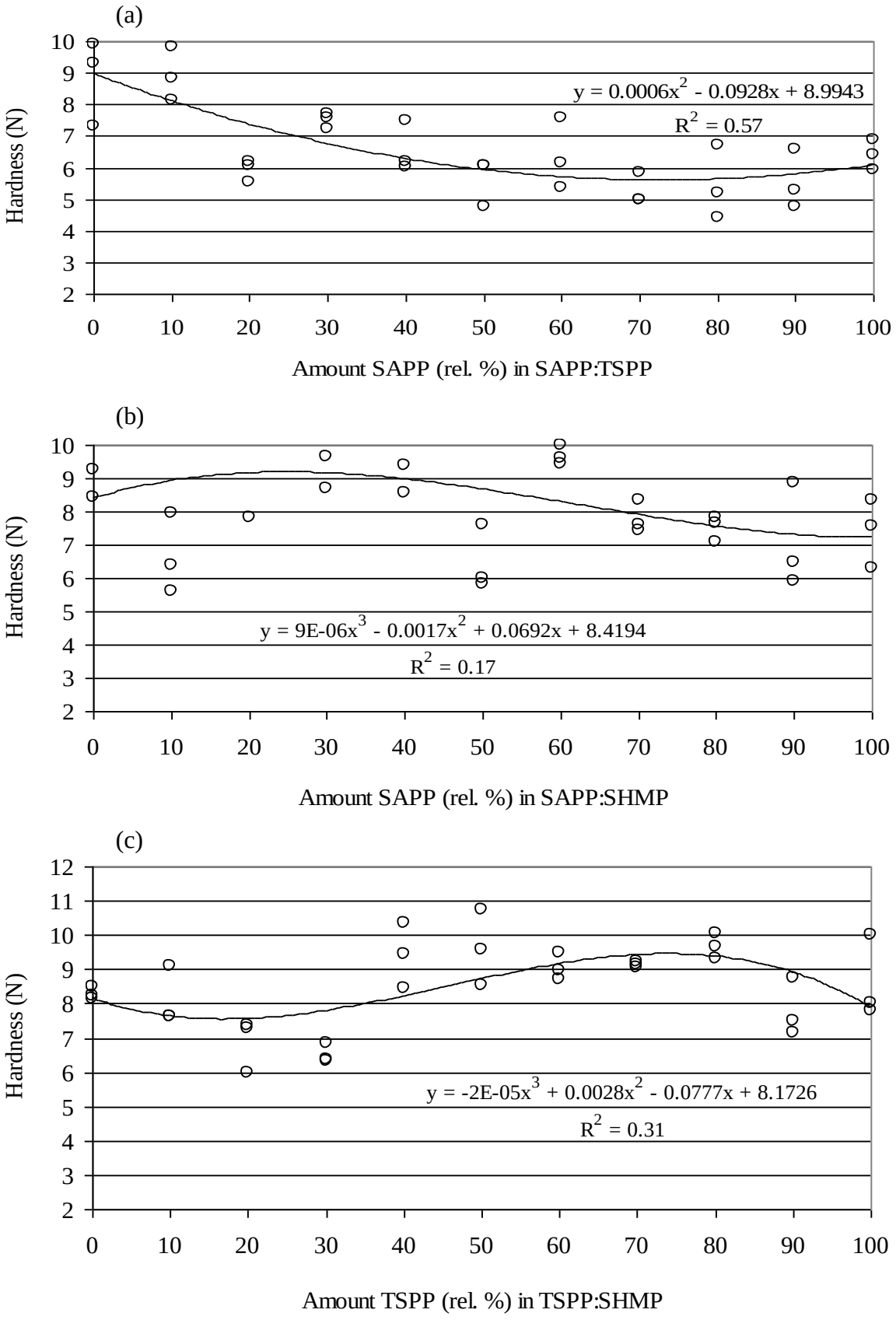

Figure 21. The dependence of hardness values on binary phosphate with different ratios: (a) SAPP:TSPP, (b) SAPP:SHMP and (c) TSPP:SHMP.

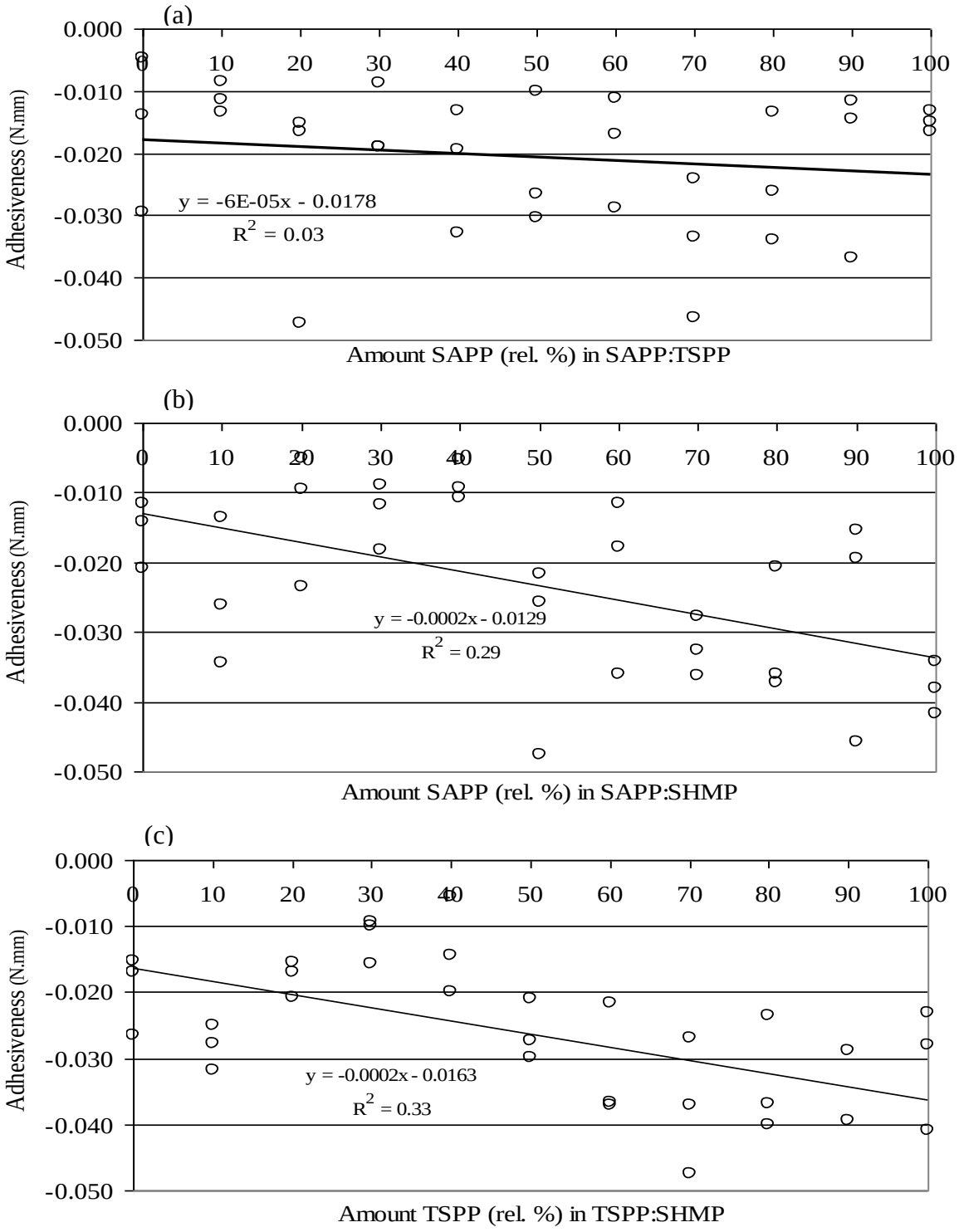

Figure 22. The dependence of adhesiveness values on binary phosphate with different ratios: (a) SAPP:TSPP, (b) SAPP:SHMP and (c) TSPP:SHMP.

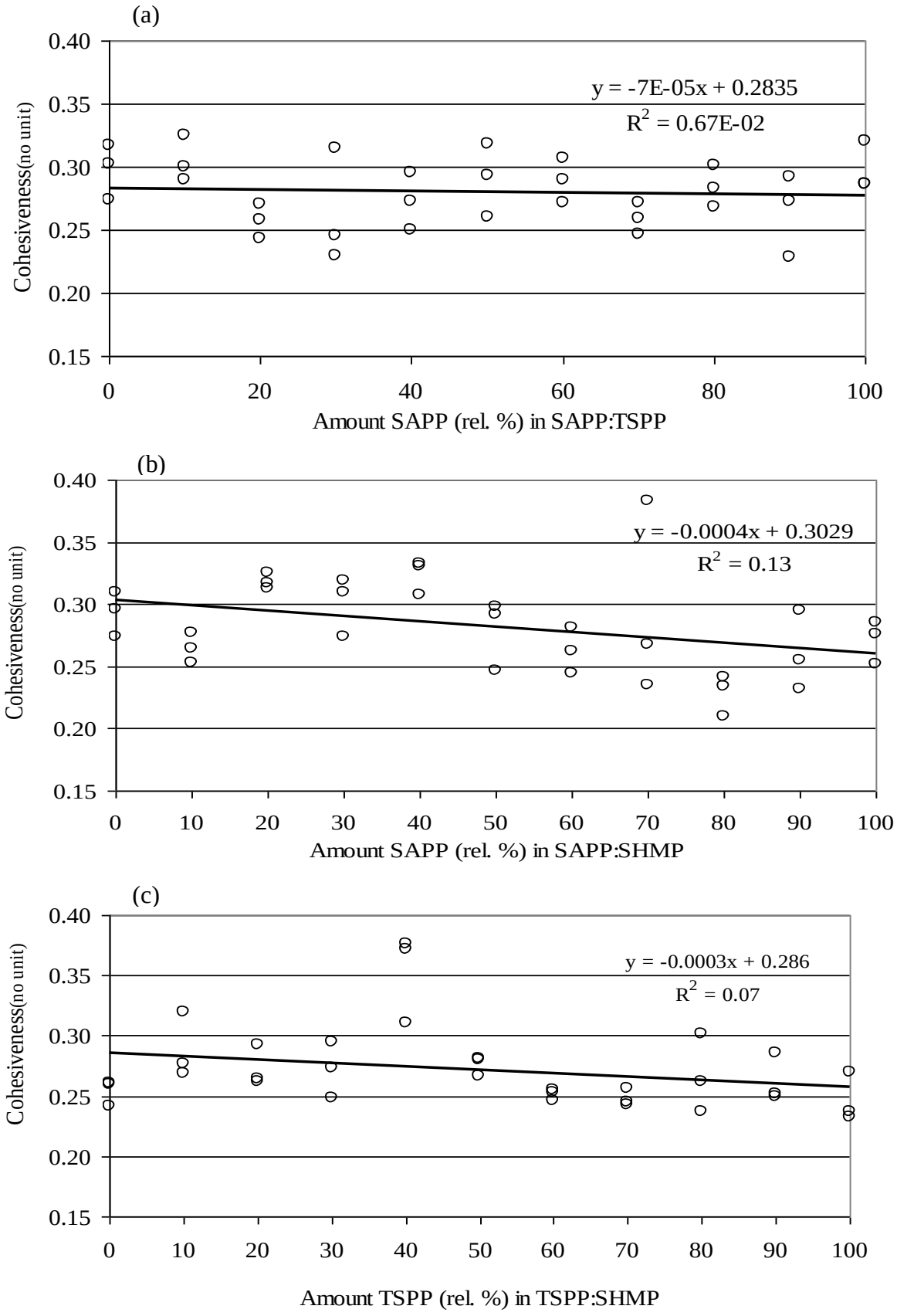

Figure 23. The dependence of cohesiveness values on binary phosphate with different ratios: (a) SAPP:TSPP, (b) SAPP:SHMP and (c) TSPP:SHMP.

As can be seen in Fig. 21-a), when the ratio of SAPP in TSPP increased, the hardness values decreased significantly from values around 9 N up to 6 N. The dependency of the hardness on the ratio of SAPP:TSPP showed to be significant as given by the coefficient of determination of 0.57. Considering the average of the determinations, there was not change with the minimum amount of SAPP tested but after adding 20%, the hardness value decreased significantly approximately up to 6 N, then rose with the next 30% and again decreased in the next two levels of concentration followed by an slightly increase to 6.3 N approximately. The use of a percentage from 70 to 100% SAPP increased also the hardness value but not significantly. The maximum hardness value (average) obtained was 8.9 N using 10% of SAPP and 90% of TSPP.

Fig. 21-b) presented the case of SAPP and SHMP binary phosphates, were the dependency of hardness was low (R^2=0.17), the range of hardness values were between 10.0 and 6.0 N. Small amounts of SAPP caused a decrease in hardness value (average of 6.6 N). However, when the concentration was increased to 20%, the maximum value for hardness was obtained (10.3 N) which was followed by just a slightly decrease to 9.7 N with 40% of SAPP. Using a concentration of 50% SAPP caused as well a suddenly drop in hardness value down to 6.5 N. After, an increase was observed using 60% (7.8 N) but was followed by a downward trend from 70% to 100%.

After, it is presented in Fig. 21-c) the results for binary phosphate TSPP:SHMP, where significant dependency was obtained (R^2=0.31). The hardness value decrease when using lower amount of TSPP and increases at higher TSPP:SHMP ratio. In particular, a decrease in hardness down to 6.5 N was observed using concentrations from 10 to 30% TSPP. After, the hardness increased to 9.4 N followed by a fluctuating trend to obtain the maximum hardness value at 80% (9.7 N). A concentration of 90 and 100% TSPP were found to be lower.

Fig. 22 presented the dependence of adhesiveness on binary phosphates SAPP:TSPP, SAPP:SHMP and TSPP:SHMP. The first plot presented in Fig 22-a) describes a lower influence of the SAPP:TSPP ratio (R^2=0.03) in adhesiveness. Values between $-0.5\cdot10^{-2}$ and $-0.5\cdot10^{-1}$ N·mm were obtained. In the case of SAPP:TSPP binary phosphate, and considering the average of adhesiveness for the different phases, an increase in adhesiveness was observed with 10% of SAPP and was followed by a decrease down to -0.26 N·mm. The use of 30% SAPP only increase slightly the hardness value and for concentrations in the range of 40 to 60% SAPP the value was also less but it change dramatically when plummeted at 70% concentration ($-0.4\cdot10^{-2}$ N·mm). Adding more SAPP caused that the hardness also increased until getting the maximum value of $\sim-0.1\cdot10^{-1}$ N·mm

In contrast, as presented in Figs 22 b) and c) for SAPP:SHMP and TSPP:SHMP ratios, the dependency was noticed (R^2 values of 0.29 and 0.33 respectively). In general, in both binary phosphates, the adhesiveness decreased

when a lower concentration of SHMP was used. The higher value for adhesiveness in Fig. 22-b) was obtained using a concentration of 40% SAPP. Using a concentration of 50% caused a sharply decrease to $-0.3 \cdot 10^{-1}$ N·mm and was followed by an increase to $-0.2 \cdot 10^{-1}$ N·mm. Following decrease of SHMP presented values of $-0.3 \cdot 10^{-1}$ to $-0.4 \cdot 10^{-1}$ N·mm. For TSPP:SHMP, Fig. 22-c), the higher adhesiveness value was achieved at 30% of TSPP and 70% SHMP. Either lower or higher concentrations of TSPP presented a downward trend and showed lower vales of adhesiveness were obtained. The use of 90% TSPP was characterized by the lower peak ($-0.4 \cdot 10^{-1}$ N·mm).

The dependency of cohesiveness values on binary phosphate SAPP:TSPP, SAPP:SHMP and TSPP:SHMP was presented in Fig. 23. As can be noticed, there is no dependency of this textural parameter and the ratio of two individual phosphates used because R^2 values found was very low (R^2 between $0.67 \cdot 10^{-2}$ and 0.13). In all cases the cohesiveness values reported were between 0.2 and 0.4. Therefore, no significant dependency was noticed. In particular, for SAPP:TSPP analysis, Fig. 23-a), a fluctuating behavior was presented, beginning with an increase in cohesiveness using 10% of SAPP (0.3) which was the highest value found for this binary mixture. After, a drastically drop was presented with the increase in concentration to 20% (≈ 0.3). Adding more SAPP (from 30 to 50%) caused an increase in the variable response up to ~ 0.3, then the cohesiveness decreased slightly with the next step in concentration and was followed by a zigzag behavior decreasing and increasing the cohesiveness with the next concentrations tested.

Fig. 23 b) shows the analysis of SAPP:SHMP binary phosphate, which revealed that two major peaks were obtained using different concentrations of SAPP and SHMP. One was obtained at 20% SAPP and the other one at 40%, with values of 0.3 approximately. In addition, a fluctuating behavior was observed with the rest of the concentrations analyzed. The minimum value of 0.2 was obtained with 80% of SAPP and 20% SHMP. When using a ratio of 70, a similar value to the absence of SAPP was observed (0.3).

Finally, as can be seen in Fig. 23 c), it was analyzed that for TSPP:SHMP, the highest cohesiveness value found was at ratio of 40 (~0.3). The use of other different concentrations of TSPP and SHMP did not alter significantly the variable response, showing average values of about 0.2 and 0.3. When using SHMP alone a cohesiveness of ~0.2 was found.

6.3.2. Discussion

The main purpose of the second study was to analyze the effects of binary phosphates on the textural properties through the hardness, cohesiveness, and adhesiveness values of the MDPM batters. Similar to the first study, it is clear that binary phosphates also adjusted the pH values in meat batters. Based on the pH values of phosphate salts (as shown in Table 7 and Table 11) when measured

in solution 1%, the increase or decrease of pH values of meat batters made from MDPM is related to the types and ratios as well as the concentrations of binary phosphates. In this study, the concentration of binary phosphates was constant, thus, the types and ratios of phosphates in binary mixtures significantly influenced the pH values of samples. Arcording to the pH values tested and obtained in Table 11, SAPP, SHMP and TSPP have a pH value 4.83, 10.07 and 10.56, respectively. Therefore, the mixtures of binary phosphate SAPP:TSPP and SAPP:SHMP gave a change of the pH values significantly compared to mixture of SHMP:TSPP. This explained for the changes of pH values of meat batter in Fig. 20 (a,b,c).

Additionally, this change of pH values also affects strongly textural properties of meat batters treated with binary phosphates. As mentioned in the first study, the textural properties of meat batters is dependent on many factors such as cooking temperature, added water content, processing temperature, raw meat quality, pH and especially the addition of phosphates. In addition, as previously discussed, meat proteins, especially actomyosin can be extracted to separative parts such as actin and myosin by sodium chloride and/or phosphates. After extracted, myosin exhibits a good functional property which forms a gel texture. Cross-linking is broken down by phosphates especially when combine along with sodium chloride. The pH values of raw meat and meat batter without phosphates was approximately 6.35. The isoelectronic point pH of meat protein is about 5.5 [33]. Hence, with the pH values of meat batter treated with binary phosphates were observed previously, textural properties must changed significantly. Alvarado and McKee [53] reported that the increment of pH values is lead to increase water binding capacity, whereas the decrease of pH is the result of decrease of WHC and yield relating directly to texture of meat batters. In particular, the decrease of pH adding binary phosphate causes the protein denaturation when the pH value reaches near isoelectronic point pH of meat protein.

Besides the influence of pH values, the formation of the gel network in meat batters is also influenced by the different types and concentrations of added phosphates. Variation of phosphates also results from the difference of the functional properties. In fact, Anjaneyulu et al. [55] stated that the effect of phosphate is not only for a pH effect, but also for the textural properties effect. As shown in the literature review, phosphates have a long chain and therefore the ability of sequestering divalent cations is more effective, that is, complexes formed by longer chain phosphates are stronger. The gel matrix formed by actin and myosin as well as the complexes of phosphates with Ca^{2+} and Mg^{2+} cations of meat proteins affect the textural properties of meat batters. Moreover, the functionality of phosphate is also dependent on the hydrolysis of phosphate in meat. The hydrolysis chemistry in meat is similar to that occurring in solution, that is, the phosphate activity decreases with time and depends on structure of phosphates. By this way, polyphosphates were hydrolyzed and/or converted step by step to other phosphate forms in meat batters. As in discussion of the first

study, TSPP is a phosphate acting right on the actomyosin complex of meat protein rather than other phosphates. Based on the solubility of phosphates showed in Table 7, every phosphate is different and has not a general rule for the solubility. The solubility of SAPP, SHMP and TSPP increases as follows: SHMP > SAPP > TSPP. In addition, the texture of meat products was also influenced by the cooking temperature. According to the study of Erdogdu et al. [25], the effect of temperature on denaturation of proteins can be attributed to theirs effects on textural properties. Ünal et al. [99] demonstrated that a barrier was formed with the presence of water, protein and phosphate. The formation of barrier was also dependent on cooking temperature. Overall, the combination of TSPP with SHMP and SAPP with TSPP and SAPP with SHMP not only gave a textural properties rather than the combination of SAPP with TSPP, but also prevented low texture resulting by TSPP addition alone.

6.4. Effects of different types and concentrations of caraageenans on textural properties of meat batter made from MDPM

6.4.1. Results

The analysis of the use of individual carrageenans as additive is presented in figures 24-26. Figure 24 showed the effect of both types of carrageenans on pH of meat batters made from MDPM. The pH of the different phases performed remained constant at a value of about 6.5 in the presence of carrageenans at several concentrations. This means that carrageenans did not have an influence on pH variation of meat batters.

Figure 25 presents the variation of hardness values according to the concentration of κ- and ι-carrageenans. The coefficient of determination of 0.86 and 0.65 was observed respectively and a dependency in hardness value was also observed in the samples treated with carrageenans (κ- and ι-). For the present analysis, the average of three determinations was considered. It was observed that the hardness value was increased by the addition of κ- carrageenan (see in Fig. 25-a)). Small addition of the carrageenan (0.1%) did not increase significantly the response. Following addition (0.2%) caused a small decrease in the value parameter. However, with higher amounts (0.3-0.5%), the hardness value rose. A considerable increase was presented with 0.4% (hardness value of 15.4 N ± 1.2) and it stabilized using 0.5%. The higher variation in hardness between one concentration and the previous one was between 0.3 and 0.4%, with a 2.1 N variation. In the case of ι-carrageenan (shown in Fig. 25-b)), the hardness values was also increased by the addition of concentration of ι-carrageenan, but decreased when the concentration overed 0.3%. The high hardness values were achieved when using ι-carrageenan with the concentration of 0.2-0.3% (approximately 14.0 N) compared to the control sample.

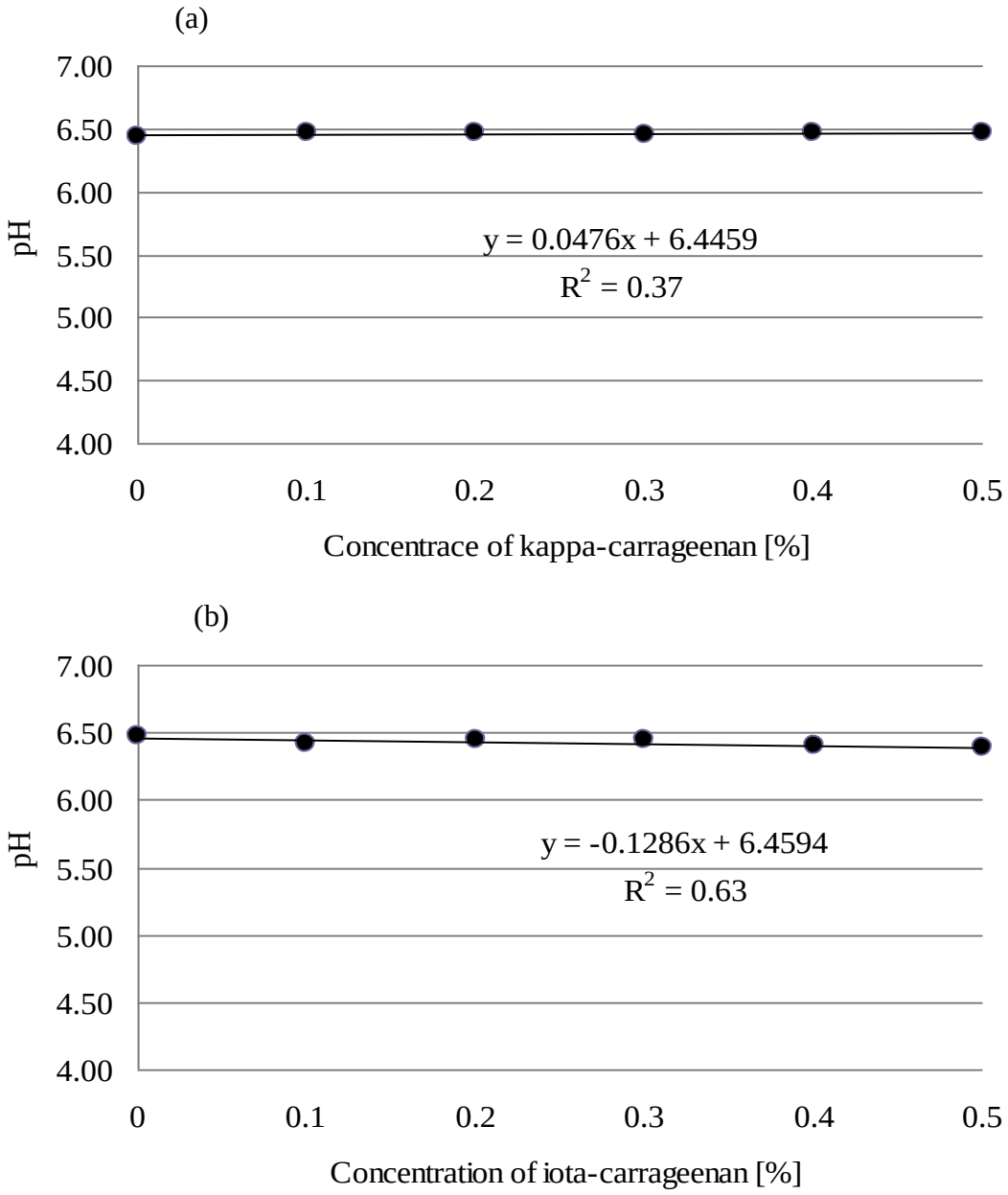

Figure 24. The dependence of pH values on carrageeenans with different concentrations: (a) κ-carrageenan and (b) ι-carrageenan.

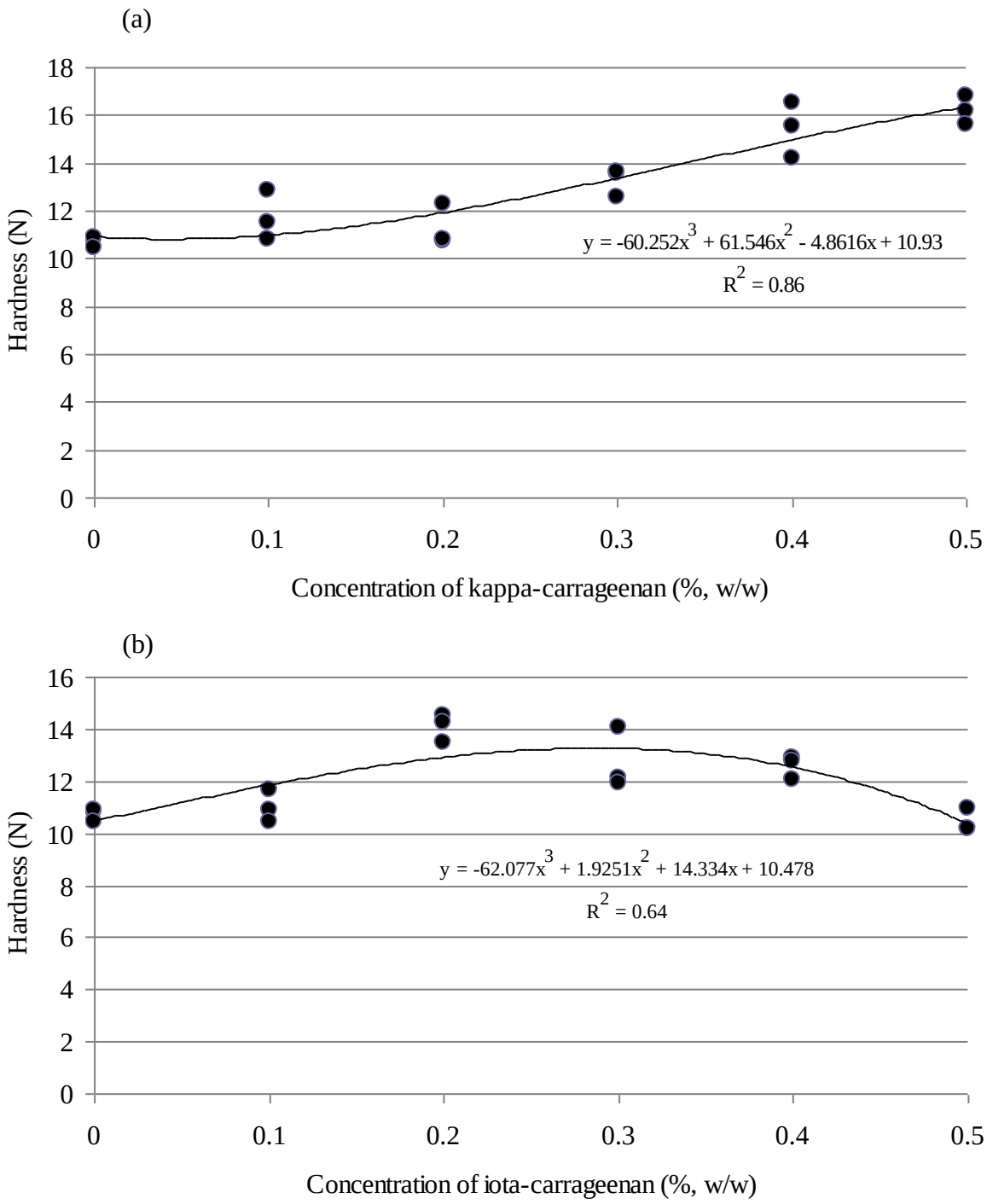

Figure 25. The dependence of hardness values on carrageeenans with different concentrations: (a) κ-carrageenan and (b) ι-carrageenan.

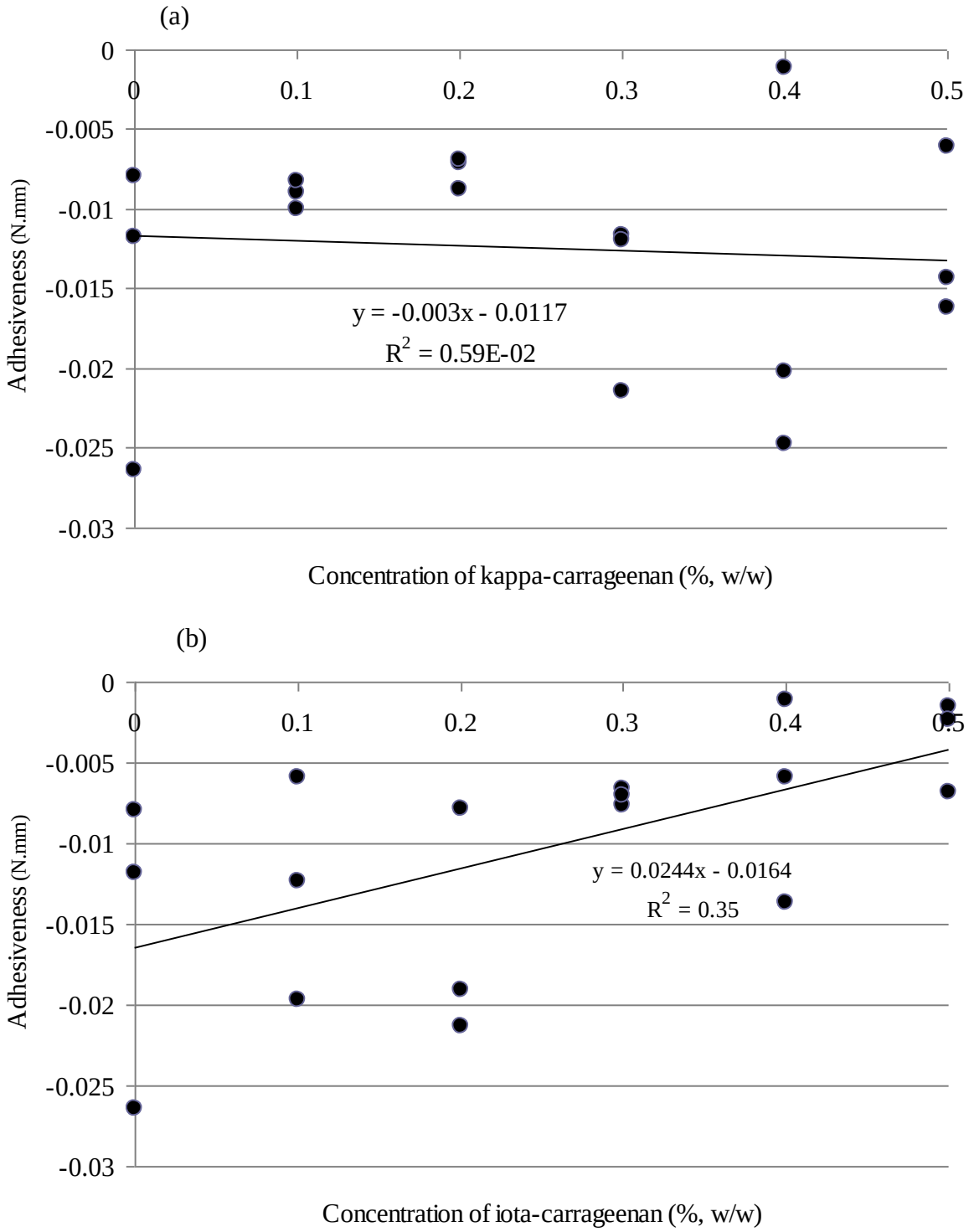

Figure 26. The dependence of adhesiveness values on carrageeenans with different concentrations: (a) κ-carrageenan and (b) ι-carrageenan.

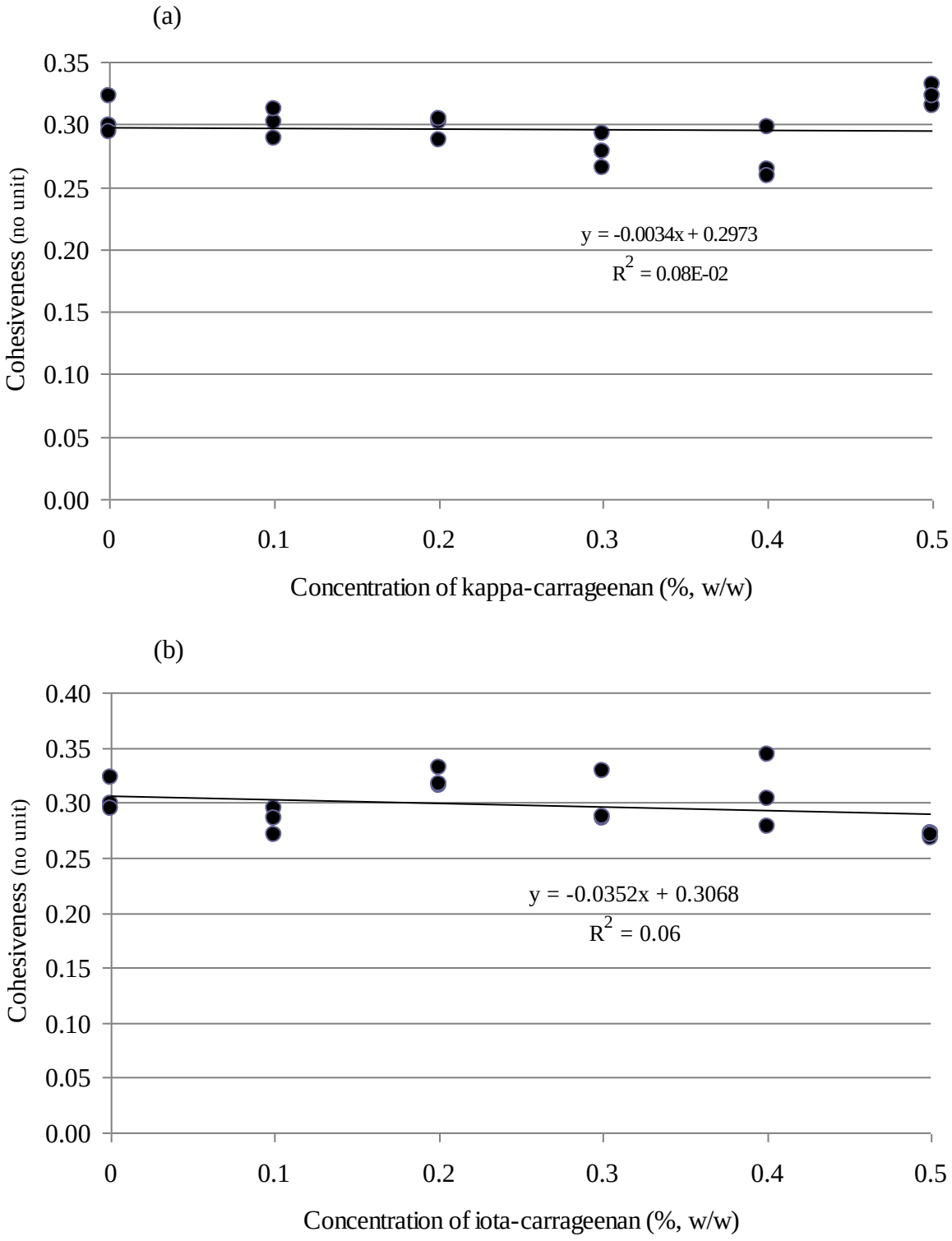

Figure 27. The dependence of cohesiveness values on carrageeenans with different concentrations: (a) κ-carrageenan and (b) ι-carrageenan.

It can be stated that the concentration that presented best results for hardness value was using 0.2%. The response was related to a third order equation with a coefficient of determination of 0.64, which means a rather strong dependence of hardness on ι-carrageenan concentration.

The analysis of adhesiveness is presented in Fig. 26. The results showed a weak influence of κ-carrageenan for these textural parameters. The independence in adhesiveness values was observed in the samples treated with κ-carrageenan ($R^2 = 0.59 \cdot 10^{-2}$, Fig. 26-a)). On the other hand, as shown in Fig. 26-b), the increment of concentration decreased the adhesiveness forces linearly. The minimum of adhesiveness was obtained using 0.5% of ι-carrageenan.

In the case of cohesiveness, the results are presented in Figure 27. The results showed that the cohesiveness values of κ- and ι-carrageenans nearly did not change. This was expressed the index of determination (R^2) of using κ- and ι-carrageenans ($0.08 \cdot 10^{-2}$ and 0.06, respectively) was low. Therefore, it can state that the dependence of cohesiveness values on the use of carrageenans with the different concentrations was not significant.

6.4.2. Discussion

The main purpose of the Phase III was to study the effect of κ- and ι-carrageenans on the textural properties as hardness, cohesiveness and adhesiveness of meat batters made form MDPM. From the results shown in 6.4.1, it is clear that carrageenans did not change pH values of meat batters through the range of concentrations tested in comparison to the samples treated without addition of carrageenans. Carrageenans are polysaccharides extracted from seaweeds. κ-carrageenan is a polymer consisting of D-galactose-4-sulfate and 3,6-anhydro-D-galctose. ι-carrageenan has a structure as same as κ-carrageenan, except for 3,6-anhydro-D-galactose sulfaterised in position of C_2. Therefore, in this study when using in meat batters, carargeenans had not any influences on pH values.

The results of the effects of carrageenans on the textural properties of meat batters were also presented in 6.4.1. As mentioned in the previous studies, meat proteins, especially actomyosin complex are extracted by the presence of sodium chloride. This extraction depends on the concentration of salt and affects the network formation which is mainly formed by actin and myosin, as described by Somboonpanyakult et al.[101] and Xiong [116]. Also shown in literature review, the addition of carrageenans improves cooking yields and the textural properties of meat products. In this study, all the textural changes can be explained in terms of the influence of the carrageenans on the gelling formation of meat batters. The mechanism of gel formation of carrageenans can be summarized as follows: firstly, a decrease of temperature causes a change in structure of the carrageenan molecule, a transfer from the random coil conformation to the formation of a helical conformation; secondly, after the

change of structure from coil to helical form, helical conformations combine and gather together orderly to form gel network three-dimension. The gel formation of carrageenans depends on the presence of ions. As shown in Table 9, κ-carrageenan forms a strong gel but brittle and firm with the presence of K^+ cation, whereas ι-carrageenan forms a strong gel but elastic with the presence of Ca^{2+} cation. Moreover, in meat batters, there may be an interaction of meat protein and carrageenans. This interaction happens due to the fact that the sulfate groups containing negative charge of carrageenans join to the positive charged cations of the protein molecules. Hongsprabhas and Barbut [117] reported that the presence of non-meat proteins affected the structure of the meat products which could interact with meat proteins directly. Therefore, in this study, the gel network was formed when carrageenans were applied. The use of carrageenans (κ- and ι-) increased hardness values. At concentration of 0.3%, the hardness had a down trend using ι-carrageenan. This can be the result of carrageenan gel network formation, that is, to have the presence of the second gel network [118]. By this way, the adhesiveness values of meat batter using ι-carrageenan changed. The cohesiveness values of meat batter using κ- and ι-carrageenans did not change significantly. This can be explained by gel formation of carrageenans. Verbeken et al. [79] showed that the influence of κ-carrageenan on the gelation of salt-soluble meat proteins was to cause an increase in hardness, gel strength and water holding capacity. They also stated that only salt-soluble meat proteins were responsible for the formation of a three-dimension gel network, but not κ-carrageenan. They claimed that κ-carrageenan did not interact with the meat protein to participate in the gel networking and was presented in the interstitial spaces of the protein network, where it bond water and may form gel fragments upon cooling. DeFreitas et al. [119] indicated that the addition of κ- and ι-carrageenans increased the hardness values. They also reported that ι-carrageenan caused a higher elasticity than κ-carrageenan did. They proposed that the functionality of carrageenans in meat products was due to carrageenan alone without obvious molecular interactions involving meat proteins.

7. CONTRIBUTION OF THE THESIS TO SCIENCE AND PRACTICE

Mechanically deboned poultry meat is currently used as an alternative in the meat products processing industry. Hence, this study is mainly concerned to produce meat products made from mechanically deboned poultry meat as commercial products by using the food additives.

Contribution to science:

- Obtaining the results of the effects of phosphate salts as monosodium phosphate, disodium phosphate, trisodium phosphate, tetrasodium diphosphate, disodium diphosphate, sodium tripolyphosphate, sodium hexametaphosphate, tripotassium phosphate and tetrapotassium diphosphate on the textural properties of meat batters made from MDPM.
- Obtaining the results of the effects of binary phosphates as tetrasodium diphosphate, sodium hexametaphosphate and disodium diphosphate on the textural properties of meat batters made from MDPM.
- Obtaining the results of the effects of κ- and ι-carrageenans on the textural properties of meat batters made from MDPM.
- The major influences using selected phosphate salts and hydrocolloids were presented for selected textural parameters of meat batters. However, complementary studies on sensorial evaluation should be realized to determine if these changes affect significantly the perception of consumers which will provide a full explanation of the effect of phosphates in MDPM batters. In addition, an optimization study using phosphate salts for improving textural properties of MDPM batters, using sensory values, namely odor, color, taste, appearance and acceptance values, as response variable will be useful. Therefore, recommendation to continue with further study on the effects of phosphates and hydrocolloids on sensorial properties of meat batters should be researched. Application of the results to development of meat products should be done as well.

Benefits for the practice:

- Base on the samples treated with individually and binary phosphates and evaluations obtained in Phase I and Phase II it is possible to use phosphate salts for manufacturing the meat products from mechanically debonded poultry meat.
- Base on the samples treated with κ- and ι-carrageenans and evaluations obtained in Phase III, there is a potential to use carrageenans for

manufacturing the meat products from mechanically debonded poultry meat.

- This work can be used as a base for further studies, like the use of mixtures of carrageenans or combination of carrageenans with phosphates or with binary phosphates to evaluate the effect of synergistic interactions on textural and sensorial properties.
- A relationship between the sensorial properties and the textural features can be performed from selected phase samples of the present work which can have an impact on further commercialization.
- The present work can have an influence on food production, especially from the point of view of chemical processes and interactions of phosphate additives and other components in mechanically deboned poultry meat.
- Similarly, further comparison of other meat products and the influence of selected phosphates can be performed with the results obtained from the present thesis.
- The results of the present thesis can have a potential impact on manufacturing processes, especially in the purpose of stabilization of meat and potentially dairy products for new food developments.

8. CONCLUSION

This research is focused on the study and determination of the effects of phosphate salts and hydrocolloids on textural properties of meat batter made from MDPM. The main objective of the performed phase as summarized in the thesis was to gain primary understanding of the impact of: (i) the addition of the different types and concentrations of phosphates, (ii) the addition of binary phosphate as SAPP:TSPP, SHMP:TSPP and SAPP:SHMP and (iii) the addition of κ- and ι-carrageenans to textural properties of meat batter made from MDPM.

To fulfill these objectives, three set of phases were realized. The pH values and textural parameters as hardness, cohesiveness, adhesiveness and gumminess were determined. The basic chemical analysis of mechanically deboned poultry meat was also performed. The individual parts of the thesis were obtained the following results:

Phase I:

- Individual types of phosphate salts influenced the textural parameters of samples in different ways.
- The concentration of added phosphate salts significantly affected the change in pH values.
- The textural properties of meat batter was also be affected by the concentration of added phosphate salts.
- The increase in hardness and gumminess of samples were observed at the concentration range of 0.20-0.35% of phosphate salts.
- The later mentioned increase was phosphate-type dependant.

Phase II:

- A comparative study between different binary phosphates was performed giving and insight about the effect of the mixtures on textural parameters (hardness, cohesiveness and adhesiveness).
- Higher hardness values were obtained using TSPP and SHMP and the lower with SAPP and TSPP.
- The binary phosphate of SAPP and TSPP did not show influences in cohesiveness force and adhesion, only in hardness.
- This binary phosphate SAPP and SHMP had a strong effect on hardness force, since the maximum values reported increased nearly 8% in comparison to the samples with other binaries and also showed the maximum adhesiveness value reported with an average value of 0.3 and

almost reached the maximum value for cohesiveness found using TSPP and SHMP (~0.3).

Phase III:

- The influences of κ- and ι-carrageenans with the different concentrations on textural properties as hardness, cohesiveness and adhesiveness were performed.
- The use of κ-carrageenan did not show influences in adhesiveness force but ι-carrageenan did. Both of κ- and ι-carrageenans did not show to affect significantly the cohesiveness force, only the hardness parameter.
- The highest hardness values were obtained using κ-carrageenan with concentration of about 0.4% and ι-carrageenan with concentration of about 0.2%.
- The pH values of meat batters were not influenced by the use of carrageenans.

Overall, the results point out a good potential of using phosphate and carrageenans in the poultry meat processing in industry**.**

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LONG, N. H. B. S., GÁL, R., & BUŇKA, F. Effects of different types and concentrations of phosphate salts on the textural properties of mechanically deboned poultry meat batters, 2012. *Submitted to Journal of Poultry Science.*

LONG, N. H. B. S., GÁL, R., & BUŇKA, F. The effect of selected phosphate salts on the textural properties of deboned poultry meat batters. *1st International Conference on Agricultural Science, Biotechnology, Food and Animal Science (ABIFA '12)*, 20-22.09.2012, Zlin, Czech, 219-223, ISBN: 978-1-61804-122-7.

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Professional experience

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