Univerzita Tomáše Bati ve Zlíně
Fakulta technologická

Teze habilitační práce

Factors affecting the functional properties of processed cheeses

Faktory ovlivňující funkční vlastnosti tavených sýrů

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„Ἓν οἶδα ὅτι οὐδὲν οἶδα“
„I know one thing, that I know nothing“
Socrates

„The current work is dedicated to my wife for her never-ending patience and unlimited love“

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Abstract

The scope of the current work was to explain the importance of emulsifying salts, natural cheese (type and maturity degree) and selected technological properties during processed cheese production. Firstly, principles of emulsifying salts action in the system or processed cheese were described. The work was focused on phosphate- and citrate-based emulsifying salts. The role of solely applied phosphates and citrate (sodium salts) also was discussed. Additionally, more complicated systems consisted of binary and ternary mixtures of phosphate and/or citrate emulsifying salts were also described. Moreover, in the habilitation thesis are described basic/general natural cheese-making processing steps. In addition, selected natural cheese varieties (Edam, Mozzarella, Swiss-type, Cheddar and white brined cheeses, respectively), specific producing steps and their typical characteristics are presented, since these varieties are among the most applicable cheese types during the industrial production of processed cheese. Furthermore, the impact of natural cheese maturity degree (or in other words its intact casein content) on the resultant processed cheese properties (mainly textural and rheological characteristics) is also mentioned. On the whole, the effect of divergent cheese varieties on the above-mentioned properties of processed cheese is also described. Furthermore, the impact of selected target processing parameters (dry matter content, fat in dry matter content) and specific technological characteristics (melting temperature, holding time, speed of agitation) on processed cheese properties was discussed. On the whole, the habilitation thesis aimed to summarize the existing knowledge in the field of characteristics of raw materials for the production of processed cheeses, production technology of these products and factors influencing the consistency (a parameter described mainly by textural and rheological properties) of processed cheeses. Based on the results of the current thesis, it is possible to provide a more comprehensive point of view of the importance of the composition of a mixture of raw ingredients (including natural cheese, emulsifying salts – phosphates and/or citrates) and processing parameters in influencing the textural and rheological properties of processed cheeses.

Key words

Natural cheese; processed cheese; processed cheese sauce; emulsifying salts; maturity degree; storage.

Abstrakt

Záměrem této práce bylo vysvětlit význam tavicích solí, přírodního sýra (druh a stupeň zralosti) a vybraných technologických parametrů při výrobě taveného sýra. Byly popsány principy působení tavicích solí ať již v modelovém systému mléka, tak v reálné matrici taveného sýra. Práce byla cílena na tavicí soli na bázi fosforečnanů a citronanů. Diskutována byla také role samostatně aplikovaných sodných solí fosforečnanů a citronanů. Dále byly popsány složitější systémy sestávající z binárních a ternárních směsí fosforečnanových a/nebo citronanových tavicích solí. V habilitační práci jsou dále popsány obecné kroky výroby přírodního sýra. Kromě toho byly v textu představeny vybrané druhy přírodních sýrů (konkrétně Edam, Mozzarella, sýr švýcarského typu, čedar a bílé sýry zrající v solném nálevu), specifické kroky výroby a jejich typické vlastnosti. Dále byl zmíněn dopad stupně zralosti přírodního sýra, nebo jinými slovy obsahu intaktního kaseinu, na výsledné vlastnosti taveného sýra (zejména texturní a reologické vlastnosti). Byl také popsán vliv odlišných druhů sýrů na výše uvedené vlastnosti taveného sýra. Dále byl diskutován vliv vybraných cílových procesních parametrů (obsah sušiny, obsah tuku v sušině) a specifické technologické charakteristiky (teplota tavení, doba výdrže tavicí teploty a rychlost míchání taveniny v průběhu tavicího procesu) na vlastnosti taveného sýra. Habilitační práce jako celek měla za cíl shrnout dosavadní znalosti v oblasti charakteristiky surovin, technologie výroby a faktorů ovlivňujících konzistenci tavených sýrů (parametr popsaný zejména texturními a reologickými vlastnostmi). Výsledky této práce poskytují komplexnější pohled na důležitost složení směsí surovin (včetně přírodního sýra, výběru tavicích solí – fosforečnanů a/nebo citronanů) a procesních parametrů, tedy faktorů, které mohou následně významně ovlivnit texturní a reologické vlastností konečných výrobků, tavených sýrů.

Klíčová slova

Přírodní sýr; tavený sýr; tavená sýrová omáčka; tavicí soli; procesní parametry; stupeň zralosti; skladování.

List of selected publications which are part and parcel of the habilitation

A. The impact of emulsifying salts composition and natural cheese on the functional properties of processed cheese and similar products.

A1. Nagyová, G., Buňka, F., **Salek, R. N.**, Černíková, M., Mančík, P., Grůber, T., & Kuchař, D. (2014). Use of sodium polyphosphates with different linear lengths in the production of spreadable processed cheese. *Journal of Dairy Science*, *97*(1), 111-122. https://doi.org/10.3168/jds.2013-7210

A2. Salek, R. N., Černíková, M., Nagyová, G., Kuchař, D., Bačová, H., Minarčíková, L., & Buňka, F. (2015). The effect of composition of ternary mixtures containing phosphate and citrate emulsifying salts on selected textural properties of spreadable processed cheese. *International Dairy Journal*, *44*, 37-43. https://doi.org/10.1016/j.idairyj.2014.12.009

A3. Salek, R. N., Černíková, M., Maděrová, S., Lapčík, L., & Buňka, F. (2016). The effect of different composition of ternary mixtures of emulsifying salts on the consistency of processed cheese spreads manufactured from Swiss-type cheese with different degrees of maturity. *Journal of Dairy Science*, *99*(5), 3274-3287. https://doi.org/10.3168/jds.2015-10028

A4. Salek, R. N., Černíková, M., Pachlová, V., Bubelová, Z., Konečná, V., & Buňka, F. (2017). Properties of spreadable processed Mozzarella cheese with divergent compositions of emulsifying salts in relation to the applied cheese storage period. *LWT – Food Science & Technology*, *77*, 30-38. https://doi.org/10.1016/j.lwt.2016.11.0198

A5. Salek, R. N., Černíková, M., Lorencová, E., Pachlová, V., Kůrová, V., Šenkýřová, J., & Buňka, F. (2020). The impact of Cheddar or white brined cheese with various maturity degrees on the processed cheese consistency: A comparative study. *International Dairy Journal*, *111*. https://doi.org/10.1016/j.idairyj.2020.104816

A6. Salek, R. N., Vašina, M., Lapčík, L., Černíková, M., Lorencová, E., Li, P., & Buňka, F. (2019). Evaluation of various emulsifying salts addition on selected properties of processed cheese sauce with the use of mechanical vibration damping and rheological methods. *LWT – Food Science & Technology*, *107*, 178-184. https://doi.org/10.1016/j.lwt.2019.03.022

B. The impact of selected processing parameters on the functional properties of processed cheese

B1. Černíková, M., Nebesářová, J., **Salek, R. N.**, Řiháčková, L., & Buňka, F. (2017). Microstructure and textural and viscoelastic properties of model processed cheese with different dry matter and fat in dry matter content. *Journal of Dairy Science, 100*(6), 4300-4307. https://doi.org/10.3168/jds.2016-12120

B2. Černíková, M., **Salek, R. N.**, Kozáčková, D., & Buňka, F. (2018). The effect of different agitations and temperature maintainings on viscoelastic properties of full-fat processed cheese spreads. *LWT – Food Science & Technology*, *89,* 244-247. https://doi.org/10.1016/j.lwt.2017.10.054

B3. Přikryl, J., Hájek, T., Švecová, B., **Salek, R. N.**, Černíková, M., Červenka, L., & Buňka, F. (2018). Antioxidant properties and textural characteristics of processed cheese spreads enriched with rutin or quercetin: The effect of processing conditions. *LWT – Food Science & Technology*, *87,* 266-271. https://doi.org/10.1016/j.lwt.2017.08.093

B4. Černíková, M., **Salek, R. N.**, Kozacková, D., Běhalová, H., Luňaková, L., & Buňka, F. (2017). The effect of selected processing parameters on viscoelastic properties of model processed cheese spreads. International Dairy Journal, 66, 84-90. https://doi.org/10.1016/j.idairyj.2016.11.007

B5. Pluta-Kubica, A., Černíková, M., Dimitreli, G., Nebesářová, J., Exarhopoulos, S., Thomareis, A. S., **Salek, R. N.**, & Buňka, F. (2021). Influence of the melt holding time on fat droplet size and the viscoelastic properties of model spreadable processed cheeses with different compositions. *International Dairy Journal, 113*. https://doi.org/10.1016/j.idairyj.2020.104880

B6. Černíková, M., Nebesářová, J., **Salek, R. N.**, Popková, R., & Buňka, F. (2018). The effect of rework content addition on the microstructure and viscoelastic properties of processed cheese. *Journal of Dairy Science, 101*(4), 2956-2962. https://doi.org/10.3168/jds.2017-13742

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Introduction

The term “processed cheese” characterizes a dairy product produced by heating a mixture of various cheese types/varieties of different degrees of maturity in the presence of appropriate emulsifying salts (mostly sodium phosphate, polyphosphates, citrates and/or their combinations), usually under reduced pressure (vacuum) with constant stirring, commonly in the temperature range of 90 – 100 °C, until a smooth and homogenous compact mass is formed with desired textural and organoleptic properties. Optional dairy (butter, anhydrous milk fat, skim milk powder, whey powder, coprecipitates, caseinates, etc.) and non-dairy (water, vegetables, spices, flavorings, colorings, salt, hydrocolloids, preservatives, etc.) ingredients can be added into the blend (Guinee et al., 2004; Kapoor & Metzger, 2008). The discontinuous production of processed cheeses includes the following steps: (i) determining the composition of ingredients (with respect to the desired parameters of the final product); (ii) placing the determined amounts of ingredients and additives into the melting equipment and the actual melting process is run (at a usual temperature of 85 to 105 °C with a holding time of several minutes); and (iii) packaging in different materials (Guinee et al., 2004; Mizuno & Lucey, 2007). Furthermore, emulsifying salts play a key-role during the manufacturing of processed cheese. In particular, they possess the ability to sequester calcium from the casein matrix by exchanging sodium ions, which results in the conversion of insoluble calcium paracaseinate into the more soluble sodium paracaseinate (Guinee et al., 2004; Kapoor & Metzger, 2008). Thus, within the matrix sodium paracaseinate acts as a “true” emulsifier at the oil-in-water interface. The control and stabilization of the pH level and an influence on the formation of a final structure after cooling are some of the additional effects of emulsifying salts (Dimitreli & Thomareis, 2009; El-Barky et al., 2011; Guinee et al., 2004). Nevertheless, not all emulsifying salts have the same calcium ion-exchange ability. The phosphate ion-exchange ability increases with increasing P_2O_5 content in the following order: monophosphate < diphosphate < triphosphate < polyphosphate (Buňka et al., 2013, Guinee et al., 2004; Shirashoji et al., 2006). In addition, El-Bakry et al. (2011) and Mizuno & Lucey (2005) stated that trisodium citrate presents better calcium chelating ability and casein peptization properties than do sodium mono- and diphosphates. Furthermore, the consistency of processed cheese can be influenced by many factors, including the following: (i) raw material composition – the type and chemical profile of the natural cheese applied (dry-matter, fat, protein, and calcium ion contents, and maturity degree), composition and concentration of emulsifying salts, addition of other optional dairy and non-dairy ingredients and also the pH of the mass to be melted; and (ii) processing and storage conditions – agitation speed, target melting temperature and holding time, cooling rate and also storage temperature. Nowadays, hydrocolloids, regularly are applied during the production of processed cheese, are important components affecting the consistency of processed cheese (Dimitreli & Thomareis, 2007; Shirashoji, et al., 2006).

1. Current state of the examined topic

1.1 Processed cheese classification

Processed cheese is a multicomponent dairy complex matrix also described as stable oil-in-water emulsion/system (Chen & Liu, 2012; Hanaei et al., 2015; Lee et al., 2004). In a physicochemical point of view, processed cheese could be characterized as a dispersion of fat droplets in a concentrated, gelled protein network (Hasenhuetl & Hartel, 2008). Therefore, the multilateralism of the above-mentioned system (processed cheese) derives from the fact that it can contain plethora of interacting components and relatively high amount of water content (Marchesseau et al., 1997). Thus, the processed cheese matrix can be formed by blending shredded natural cheese (of different types/varieties and maturity degrees) in the presence of appropriate amount and type of emulsifying salts (mainly sodium salts of phosphates, polyphosphates and/or citrates), under partial vacuum, constant stirring and upon heat treatment; resulting in the constitution of a homogeneous and smooth mass with desired functional and organoleptic properties (Buňka, et al., 2009; Guinee, 2011; Sádlíková et al., 2010; Zerfiridis, 2001). Additionally, the application of heat during the processing can inactivate starter culture microorganisms and other bacteria, including also enzymes, all present in the natural cheese applied; resulting in the shelf-life extension of the final product (Tamime, 2007; 2011).

However, there is no specific European Union legislation on processed cheese. For a more complete oversight on the legislation of processed cheese and related products within the European Union, it is necessary to look at a selection of Member States. In addition, processed cheese according to international standards is grouped by the following characteristics: composition, water content and consistency. According to these characteristics, exist three main categories (mainly in the USA): processed cheese blocks, processed cheese foods and processed cheese spreads. Moreover, additional subcategories can be processed cheese slices and smoked processed cheese (Tamime, 2007; 2011). Furthermore, according to the decree 397/2016 Collection of Laws, edited by the Czech Ministry of Agriculture, defining the requirements for milk and milk products, ice creams and edible fats and oils, processed cheese (*tavený sýr* – in Czech) is defined as a dairy product which was thermally treated. In addition, if the product contains more than 5 % (w/w) of lactose and in which the applied natural cheese constitutes at least 50 % (w/w) by weight of dry matter, therefore, should be designated as processed cheese product (*tavený sýrový výrobek* – in Czech).

1.2 Manufacture principles and steps in processed cheese manufacture

In general, processed cheese production protocol is characterized by complexity and thus, it is primarily influenced by the chemical interactions occurring between the applied ingredients of dairy origin and the emulsifying salts utilized. On the contrary, the principal technological operations of processed cheese manufacture are rather characterized as "simple"; however, they require skillful and professional handling in order to control all the used ingredients, their concentrations in the blend and processing parameters/conditions.

The basic theory of processed cheese manufacture is based on the change of the state of casein protein from the coarsely dispersed calcium-paracaseinate which is present in the natural cheese, due to application of heat, stirring and the presence of particular salts (as emulsifying/peptizing agents), into a homogeneous free-flowing condition (the sol state) with desired functional and organoleptic properties. The basic technological steps for processed cheese production could be summarized by the following operations: natural cheese/cheeses selection, formulation of the blend, blending, shredding, emulsifying salts addition, processing (thermal treatment), and packaging, cooling, storage of the final product. Moreover, in a simplified point of view, the principles of processed cheese manufacture could be described by two stages/phases. During the first stage/phase, present caseins are liberated and dispersed, by the action of emulsifying salts and then could serve as "*true*" emulsifiers. This phase is also known by the term "ion-exchange phenomenon". During the second stage/phase, the new developed protein network is established, in which the proteins are hydrated and the fat is emulsified. This phase is also known as the "creaming phase".

In addition, the release of caseins is caused by the action of emulsifying salts, during which the ion-exchange phenomenon occurs. Polyvalent anions (especially phosphates, polyphosphates and citrates) and monovalent cations (especially sodium, but in some cases also potassium and/or aluminum) are applied as emulsifying salts. Multivalent anions have more intensive affinity to bivalent ions (in the case of processed cheese are calcium ions) in comparison to monovalent ions. Therefore, when e.g. sodium salt of phosphate is added into the mixture of the raw materials, in the presence of sufficient amount of water, under heat treatment and agitation, the ion-exchange of calcium to sodium ions will occur. Moreover, the insoluble calcium paracasein (from the natural cheese protein network) is transformed into the more soluble sodium paracasein and the latter might be dispersed in the processed cheese melt. Therefore, the dispersed proteins (caseins) within the system could effectively act as emulsifiers. In particular, the first role of emulsifying salts is to adjust the environment of the raw materials mixture in a manner that the caseins will enhance their emulsifying properties. Hence, this attribute is defined as the "main role" of emulsifying salts (Carić et al., 1985; Guinee et al., 2004; Kapoor & Metzger, 2008; Kawasaki, 2008; McIntyre, et al., 2017; Mizuno & Lucey, 2005a; 2005b; Shirashoji et al., 2006; Templeton & Sommer, 1936).

Furthermore, emulsifying salts (salts of phosphates, polyphosphates and citrates) are sometimes labelled as emulsifiers (substances presenting surface activity). However, this is not the exact (or correct) definition because these substances do not have an amphiphilic character. In general, the term emulsifying salts/agents, is the most appropriate for use (Carić et al., 1985; Guinee et al., 2004). Moreover, during processing (so called also "melting" process) under heat treatment (90 – 100 °C) and mechanical stirring in the presence of water, polyvalent anions (especially phosphates) start binding onto the caseins via calcium ions resulting in the incensement of the hydrophilic character of the proteins. Caseins possess the ability to bind water and caseins modified in the above-mentioned way are more effective in terms of binding water. In addition, parallel, the fat is emulsified by dispersed caseins and partially by the residues of original lipoprotein membranes (covered fat droplets before processing). Caseins with bonded phosphates have more intensive negative charge leading to their dispersion. Generally, the addition of emulsifying salts can also increase the pH value from approximately 5.0 – 5.5 (in the mixture of the raw materials) to the target values which are in the range of 5.6 – 6.0 (typical for spread-type processed cheese), something which is also supported by the raising of caseins negative charge. These phenomena (proteins hydration and fat emulsification) cause the increase of viscosity in the resultant melt. Additionally, during holding time (generally a process lasting several minutes) under a target melting temperature and during the cooling of the hot melt, the new structure is established – the new hydrated protein network is developed, in which the fat is emulsified. The above-mentioned processes are named as the "creaming" process. There are many interactions, which participate in the process of the final network structure formation and thus, supporting creaming (new calcium bridges, phosphates-calcium complexes, hydrophobic interactions, hydrogen bonds and/or disulfide bonds are taking place). The establishment of the new protein network can be observed as the intensive increase of melt viscosity (Awad et al., 2004; Berger et al., 2002; Bowland & Foegeding, 2001; Buňka et al., 2013; Hoffmann, 2012; Fu et al., 2018; Kalliapan & Lucey, 2011; Kapoor et al., 2007; Kapoor & Metzger, 2008; Kawasaki, 2008; Lucey et al., 2003; Lucey et al., 2011; Mizuno and Lucey 2005b, 2007).

The successful melt creaming for the establishment of the network (final product) with desirable properties for the consumers requires some time, in which a target melting temperature is maintained under continuous stirring. In particular, during the first phase, the ion exchange phenomenon and casein dispersion occur by the activity of emulsifying salts together with heating and stirring. Furthermore, caseins are hydrated, fat is emulsified and the new protein network starts to develop. During the prolonging of the processing time, the viscosity of the melt is increasing due to interactions connected with caseins chains in the newly formed matrix, resulting in the decrease of the fat droplets (up to the maximum available viscosity under actual conditions). The heat treatment and holding time under the target melting temperature is stopped when the melt possesses the appropriate properties for the manufacture of a final product with desirable properties for the customers. However, when the processing time is extended, the aggregation between the proteins (increasing number of interactions between caseins) will continue and from a certain point the fat and later also the water phases will be released from the matrix. Thus, the developed system will become unstable with lower values of viscosity. Nevertheless, this undesirable phase is commonly labeled as "over creaming" and is a significant quality defect. (Kawasaki, 2008; Lee et al., 2003; Shirashoji et al., 2006).

Cheese ripening (mainly the proteolysis) can affect the new protein network establishment. When high amount of short-chained caseins and low concentration of intact casein are available (proteolysis is very intensive), the danger of an unstable protein matrix development would exist, resulting in an undesirable final product in which fat or even also water might be released (Buňka et al., 2013; Brickley et al., 2007; Carić 7 Kaláb, 1997; Salek et al., 2016).

According to Lee et al. (2003), the creaming process (the formation of a new network) is mainly described by the interactions of caseins. The authors supported their statement in an experiment with full-fat processed cheese and also with a sample with no fat content. In both products, the creaming phenomenon occurred. On the other hand, the presence of fat significantly influenced the protein matrix and therefore the consistency of the final product. Generally, fat can disturb the continuity and the compactness in such way that decreases the number of interactions between caseins. The latter can lead to the development of a processed cheese with lower viscosity (more spreadable). The proteins interact with fat – emulsify it – and in those places cannot interact with each other (caseins) and a compact network is obtained. A similar effect on the continuity and the compactness of the protein matrix possesses water. Also the cooling rate of the processed cheese has a significant effect on the network formation. When the cooling rate is too fast, the viscosity of the products decreases especially because the interactions between proteins take place especially when the melt is hot (Awad et al., 2002; Bowland & Foegeding, 2001; Guinee, 2003; Kapoor & Metzger, 2008; Lee et al., 2003; Lucey et al., 2011; Piska & Štětina, 2004).

It could be concluded that the final structure of processed cheese is affected by the following factors: the effectiveness of ion-exchange and proteins dispersion (including the actual pH-value and its impact on the negative charge of caseins); the intensity of protein hydration; the intensity of fat emulsification; the number of interactions between caseins. Moreover, the addition of other ingredients (optional) can contribute to the increase of the above-mentioned interactions among proteins. On the other hand, the presence of other ingredients can also disrupt the continuity and the compactness of the developed casein network.

2. Natural cheese in processed cheese production

The basic ingredient for the production of processed cheese is natural cheese. Therefore, by the selection of this main raw material, it is possible to influence the consistency of the final processed cheese product. During the selection of the natural cheese, it is necessary to take into account the type of natural cheese (variety), the degree of maturity, its composition (dry matter, fat, protein, and calcium contents, respectively), the pH value and the required properties (organoleptic or functional) of the final product.

Natural cheese varieties which are predominantly applied in various countries of the world vary (including mainly Cheddar, Dutch-type, Swiss-type, Mozzarella-type (or other pasta-filata cheeses) and white brined cheeses). In general, in the English-speaking countries (e.g., Britain, USA, Canada, Australia, and New Zealand) the basic natural cheese as the main raw material for processed cheese manufacture is commonly Cheddar and Mozzarella cheese. On the other hand, in regions around the Mediterranean basin, Balkan, the Near and Middle East, white brined cheeses belong among the most preferable and consumed natural cheese types and are utilized as the basic ingredient for the production of processed cheese and related products (Černíková et al., 2017; Moatsou & Govaris, 2011; Salek et al., 2020). For the past years several studies have been performed in which divergent natural cheese varieties were implemented for the production of processed cheese and similar products. In particular, Abdel-Hamid, El-Sharaby & Awad (1999); Awad et al. (2002) used *Ras* cheese, Adhikari et al., (2009); Biswas et al. (2014); Brickley et al. (2007); Guinee & O'Kennedy (2009; 2012); Rafiq & Ghosh (2017), Salek et al. (2020); Hassan et al. (2007); Fu & Nakamura (2020); Fu et al. (2018); Janevski et al. (2012); Shirashoji et al. (2010; 2016; 2006); Guinee & O'Callaghan (2013); Kommineni et al. (2012); Fagan et al. (2007); Hoffmann et al. (2012) applied *Cheddar* cheese, Chavhan, Kanawja, Khetra & Puri (2015); Seth & Bejwa (2015); Salek et al. (2017); Chen & Liu (2012) used *Mozzarella* type cheese, Cunha & Viotto (2010); Cunha, Alcandara & Viotto (2012); Nogueira et al. (2018); Ferrão et al. (2018); Torres et al. (2017); Belsito et al. (2017); Alves et al. (2007) used *Requeijão cremoso* cheese, Černíková et al. (2017); Salek et al. (2016; 2017; 2020a; 2020b); Hauerlandová et al. (2014); Nagyová et al. (2014); Macků et al. (2009); Buňka et al. (2004; 2013); Weiserová et al. (2011); Schädle et al. (2020); Přikryl et al. (2018); Sádlíková et al. (2010); Piska & Štětina (2004) utilized *Edam* cheese, Stangieski, Weiss & Kaczmarek (2019); Weiss, Stangieski, Baranowska & Rezler (2018); Dimitreli & Thomareis, (2004; 2007; 2008); Fu & Nakamura (2020); Fu et al. (2018); Mozuraityte et al. (2019) applied in their studies *Gouda* cheese, Chatziantoniou, Thomareis & Kontominas (2015, 2019); Kontou, et al. (2019) used a *Myzithra*-type whey cheese, Hanna (1999) applied *Halloum* cheese, Kaminarides & Stachtiaris (2000); Topcu et al. (2020) utilized in their work *Kasseri/Kashar* (pasta-filata) cheese, Ghods Rohani & Rashidi, (2019), Kontou, et al. (2019) used *Feta* cheese, Salek et al. (2016) used *Swiss*-type cheese, Burgos, et al. (2020) used goat cheese, Yılmaz et al. (2011) applied white cheese.

Proteolysis is one of the principal biochemical changes during cheese ripening leading to physical, chemical, flavor, and functional changes in natural cheese. Proteolysis occurs when various enzymes hydrolyze proteins in cheese during ripening. The sources of enzyme for proteolysis originate from chymosin and bovine pepsin in rennet residual, indigenous and endogenous heat-stable proteinases in milk, and various proteinases from starter and non-starter bacteria (McSweeney &Fox, 1997). In other words, during the ripening process of natural cheeses, the phenomenon of proteolysis occurs, where the casein (*para*-casein) component is hydrolyzed into peptides and free amino acids. In particular, proteolysis of casein occurs by the action enzymes involving the residual activity of native milk enzymes, rennet and the exogenous enzymatic system of acidic and non-acidic lactic acid bacteria. The ratio of cleaved and practically non-cleaved (intact) casein is an important criterion influencing the consistency of processed cheeses (Kapoor & Metzger, 2008). The term intact casein in cheese refers to the casein that has not been hydrolyzed during the ripening process, which is used as a non-specific method to measure cheese proteolysis. Intact casein content in the final product has been also correlated to cheese rheological and functional properties. Experimentally, intact casein is determined by the difference between total protein and the pH 4.6-soluble protein using nitrogen-based quantifications. Furthermore, intact casein and total protein contents can provide indirect essential information for processed cheese manufacturing as they both can determine its functional and textual properties (Kapoor & Metzger, 2008). Furthermore, it is generally accepted that the intact casein content of natural cheese is inversely related to the age of the natural cheese. Hence, as a natural cheese is matured, its intact casein content (Kapoor et al., 2007).

Moreover, Guinee et al. (2004) stated that "*young*", unripe natural cheese containing 70-95 % of intact casein is suitable for the production of sliceable processed cheese products, whilst medium-ripened cheese with 60-75 % of intact casein is suitable for the manufacture of spreadable processed cheese products. Thus, if an immature raw material with a low degree of proteolysis is used, the processed cheese product obtained is characterized by a stiffer, gummier consistency and an empty taste. On the other hand, the advantage of using this kind of raw material (unripen natural cheese) is the reduction of raw material costs. In addition, by using a ripened natural cheese with a high degree of proteolysis, an easily melting mixture is obtained and the final processed cheese product has a fine and spreadable consistency. During a longer maturing process, plethora of sensory active substances are formed, giving to the developed processed cheese melt a full and distinctive aroma (Guinee et al., 2004; Guinee, 2003; Lee & Klostermeyer, 2001; Swenson, et al., 2000). In practice, a mixture of more or less matured natural cheeses is usually applied during the production of processed cheese. If the raw material composition contains older, very mature natural cheeses with excessively hydrolyzed proteins, the addition of intact casein (in the form of young cheeses or curd) is usually included, which will help to form a stable protein matrix. Otherwise, there is a risk of an unstable to liquid-like consistency of the product. This phenomenon would occur due to the low binding of water and its release and the inability to form a stable protein matrix. The effect of increasing the degree of proteolysis of natural cheese (Cheddar natural cheese was the basic raw material) on the textural and viscoelastic properties of processed cheese was demonstrated in a study by Brickley et al. (2007). The degree of proteolysis of Cheddar increased most rapidly in the first 28 days after its production, which was in line with the highest decrease in the firmness of the processed cheese produced. Piska & Štětina (2004) produced processed cheeses from mixtures of natural cheeses with different degrees of proteolysis. It was found that the sample showing the highest stiffness, adhesiveness and gumminess was made from natural cheese with a low proteolysis index (ratio of water-soluble nitrogen at pH 4.6 to total nitrogen). On the contrary, the values of these textural parameters were the lowest for the product manufactured from the raw material with a higher proteolysis index.

Additionally, Bunka et al. (2013) studied the dependence of natural cheese (Edam block; 2, 4 and 8 weeks of maturity) maturity level on selected textural properties (hardness, cohesiveness and relative adhesiveness) of spreadable processed cheese (40 %, w/w, dry matter, 50 % w/w fat in dry matter contents) during a 30 day storage period. The above-mentioned authors stated that hardness of processed cheese decreased when a raw material with higher level of maturity was applied for the production of the examined samples. Furthermore, with the raising maturity degree of the cheese, the values of processed cheese cohesiveness slightly decreased. On the other hand, the obtained values of relative adhesiveness of the processed cheese samples increased. Furthermore, significant changes in the relative adhesiveness values were reported when cheese with 8-week maturity was used. A possible explanation of this could be due to a shortening length of casein fractions caused by the rising intensity of proteolytic changes during the ripening of the utilized raw material (natural cheese) (Brickley et al., 2007; Piska & Stětina,2004). The results are in accordance to the findings reported by Brickley et al. (2007) and Guinee et al. (2004). In general, the values processed cheese relative adhesiveness should increase with the rising maturity level of the used natural cheese. Moreover, according to the work of Hladká et al. (2014) model samples of processed cheeses (40 % w/w dry matter content and 50 % w/w fat in dry matter content; Edam cheese block was applied as the main raw material; 50 % w/w dry matter, 30 % w/w fat in dry matter) were homogeneous regardless of the maturity degree of cheese (the maturity of the applied raw material was in the range of 1–16 weeks). Moreover, the latter authors developed processed cheese samples without emulsifying salts addition. The highest values of hardness were observed in the products made of cheese with 1–2 weeks of maturity. Moreover, with the increasing degree of cheese maturity (4–12 weeks), the hardness of the tested samples was gradually decreasing. Additionally, the hardness of processed cheese made of natural cheeses with maturity of 12–16 weeks did not differ. Nevertheless, the lowest values of hardness were observed in the samples examined on the production day and this parameter was significantly increasing with the prolonging of the storage period. In addition, other series of studies performed by Salek et al. (2016; 2017; 2020) examined the effect of various natural cheese varieties and their degree of maturity on selected textural and rheological properties of model processed cheese samples. The above-mentioned authors applied Mozzarella-type (42 % w/w dry matter content; 35 % w/w fat in dry matter content; 0, 2, 4 weeks of maturity), Swiss-type (60% w/w dry matter content, 30% w/w fat in dry matter content; 4, 8, 12, and 16 weeks of maturity), Cheddar (dry matter content 62 % w/w, fat in dry matter content, 50 % w/w; 4, 8, 12 and 16 weeks of maturity) and White brined cheeses (dry matter content 48 % w/w, fat in dry matter content 38 % w/w; 2, 4, 8, 16 and 24 weeks of maturity) respectively, as the basic raw material in the formulation of the resultant processed cheese samples. The hardness of the samples obtained decreased with the rising maturity degree of the natural cheese used (regardless of the applied cheese variety). Moreover, the monitored values of the gel strength and interaction factor decreased with the increasing maturity degree of the cheese used. According to Kapoor & Metzger (2008), the reported decrease in the gel strength values could be probably due to fewer number of interactions within the processed cheese matrix. In general, it could be stated that a more rigid end-product can be expected with higher gel strength values. The intensity of rigidity of the processed cheese samples has an analogous relationship to the intensity of the gel strength; the higher the gel strength of the sample, the more firm the product that can be expected (Salek et al., 2016; 2017). Moreover, higher values of gel strength were reported for the processed cheese samples produced with Cheddar cheese in comparison to those made from White brined cheese. According to Salek et al., (2020) from the comparison of processed cheese samples manufactured from two different natural cheeses (Cheddar and White brined cheese) of the same maturity level (4-, 8- and 16-week maturity), it can be seen that the White brined processed cheese samples were significantly less hard that those made of Cheddar cheese. Moreover, this important difference in the hardness values development could be due to different chemical composition (including pH, calcium content, NaCl content, residual lactose content and mainly the amount of intact casein) and cheesemaking process of the applied natural cheese (Piska & Štětina, 2004). As it was mentioned previously, the level of intact casein present in the natural cheese can influence the properties of the resultant processed cheese. Furthermore, the structural network of processed cheese is mainly formed by intact casein and thus, a high level of intact casein could be a potential indicator for abundant interactions between the present proteins and fat. According to the literature, processed cheese produced from natural cheese with lower intact casein level is soft, whereas processed cheese made from natural cheese with a higher level of intact casein is firm. On the same token, probably the level of intact casein in the utilized White brined cheese was lower in comparison to that in Cheddar cheese, resulting in significant differences in their values of hardness. On the whole, from the reported results of rheological and hardness analyses it could be concluded that the processed cheese gel strength and hardness development follow a contiguous trend which is not affected by the type of applied natural cheese.

In addition, Burgos et al. (2020) evaluated the effect of the maturity degree of natural goat cheese on the textural, rheological and organoleptic properties of processed cheese products. Furthermore, the developed processed cheeses were formulated using goat cheeses with 10, 20 and 40 days of ripening. In particular, the hardness, adhesiveness and complex modulus (G*) of the tested samples decreased as the maturity degree of the utilized natural cheese (raw material) increased, which is probably associated with the intact casein content. According to the latter authors, increasing the amount of natural goat cheese having hogher amount of intact casein in the formulation can influence the complex modulus of the resultant end product. Finally, a general statement could be reported that as the maturity degree of natural cheese applied in process cheese manufacture increased, the hardness of the resultant processed cheese decreased (Kapoor, et al., 2007; Piska & Štětina, 2003).

Active acidity (pH value) has a significant effect on the textural and rheological properties of processed cheeses. In particular, these properties are mainly influenced by the type and concentration of emulsifying salts used and the pH value of the applied natural cheese. Moreover, processed cheese with low pH values (in the range of 4.8 - 5.2) can be expected to have a stiffer consistency than less acidic processed cheeses with higher values of pH (pH> 6.0), which are usually more spreadable under otherwise identical circumstances (Awad, et al., 2004). The closer the pH value of the processed cheese is to the value of the isoelectric point of casein (pI ≈ 4.6), the more attractive protein interactions occur, resulting in a stiffer product (Lee, et al., 2004). Marchesseau et al. (1997) investigated the effect of increasing pH on the rheological properties of processed cheeses with a constant content of emulsifying salts. The result was decreasing values of loss and elastic moduli of elasticity, indicating a decrease in viscosity and elasticity of the tested processed cheese with an increasing pH.

Moreover, Lee & Klostermeyer (2004) also achieved the same results in processed cheese spreads with reduced fat content (12.0 % w/w of fat). In contrast, Swenson et al. (2000) found a decrease in the firmness of processed cheese containing only 0.6 % w/w of fat with a decreasing pH. However, these different results are probably due to the analysis of different types of matrices (e.g. different processing equipment and conditions, chemical composition of the matrix). Moreover, factors such as natural cheese calcium and phosphorus contents and salt-moisture-content can influence the functional properties of processed cheese. According to Kapoor at al. (2006) high calcium and low phosphorus content in natural cheese can influence the processed cheese melting properties, viscous modulus and apparent viscosity. Moreover, decreased levels of calcium content can affect the rheological properties of processed cheese in such a way that the resultant end product will present lower values in fracture test, strain and hardness. In general, such products are characterized as soft and brittle in texture (Guinee & O'Kennedy, 2009). In addition, the salt-in-moisture content of natural cheese can influence the proteolysis degree. In particular, an increase in the salt-in-moisture content can result in a decreased degree of proteolysis. Thus, the hardness of the resultant processed cheese should be higher, whereas the values of cohesiveness will be lower. Furthermore, according to Biswas et al. (2015) processed cheese produced from Cheddar cheese with high calcium and phosphorus contents and high salt-in-moisture content is harder with lower tendency to flow.

3. Emulsifying salts in processed cheese production

3.1 The role of emulsifying salts within the processed cheese matrix

Emulsifying salts (or emulsifying agents) are more commonly known in the dairy industry as "*melting*" salts and are of great importance during the production of processed cheese and similar products (processed analogues or processed cheese sauces). Emulsifying salts are ionic compounds made up of monovalent cations (sodium, potassium) and polyvalent anions (phosphates, polyphosphates and/or citrates) (Chen & Liu, 2012; Buňka et al., 2014). The most commonly applied emulsifying salts are sodium citrates, sodium hydrogen monophosphates, diphosphates and polyphosphates. Nowadays, during the industrial production of processed cheese, emulsifying salts are rarely used as individual compounds, whereas they are applied rather in the form of phosphate and phosphate-citrate blends (binary, ternary or even quaternary mixtures) (Chen & Liu, 2012; Hasenhuetl & Hartel, 2008). Nevertheless, emulsifying salts cannot be characterized as "*true*" emulsifiers (low molecular-weight surfactants) since they do not indicate any surface-activity properties. In the narrow sense, emulsifying salts cannot be used for the preparation of oil-in-water (O/W) or water-in-oil (W/O) emulsions, whereas they play important role in modifying the emulsifying activity of the present surface-active proteins (mainly caseins from the applied natural cheese which is the main raw material) within the processed cheese matrix. (Carić et al., 1985; Hasenhuetl & Hartel, 2008; Lucey, 2002; Tamime, 2007; 2011; Zehren & Nusbaum, 2000). In addition, caseins possess calcium binding ability, which has the effect of reducing their solubility and thus enhancing their emulsifying properties. Furthermore, emulsifying salts present higher affinity for calcium than do the caseins, and thus, are able to improve the solubility and emulsifying ability of the caseins. In general, two types of emulsifying salts exist; those that bind calcium relatively „*weakly*", and those that bind calcium more „*strongly*". "*Weak*" emulsifying salts have a modest effect on the emulsifying properties of the caseins, leading to the formation of a softer processed cheese with relatively large fat droplets. On the other hand, "*strong*" emulsifying salts give a greater improvement in the emulsifying capacity and result in a firmer processed cheese with smaller droplets of fat (Hasenhuetl & Hartel, 2008). According to the European Union legislation (Commission Regulation No. 1333/2008) the maximum permitted level of emulsifying salts (expressed as P_2O_5) in processed cheese is up to 20,000 mg/kg (or mg/L). In general, depending on the nature of the applied emulsifying salt, the latter are added in an amount ranging from 1 to 3 % (w/w) (Tamime, 2007; 2011). The essential role of emulsifying salts is to solubilize calcium paracaseinate, sequester calcium and thus, dispersing the present proteins. Calcium in the calcium-paracaseinate complex of natural cheese is removed by the ion-exchange properties of the emulsifying salts; solubilizing the paracaseinate, usually as sodium paracaseinate (Fox, 2000; Hui, 2005). Moreover, pH adjustment and calcium sequestering are among the main functions of emulsifying salts during processed cheese production. Emulsifying salts possess the ability to sequester calcium from the casein matrix; by exchanging sodium ions, resulting in the conversion of the insoluble calcium paracaseinate into the more soluble sodium paracaseinate (Guinee et al., 2004; Chen & Liu, 2012; Kapoor, et al., 2007; Salek et al., 2015). Within the matrix, sodium paracaseinate acts as an emulsifier leading to the stabilization of the oil-in-water interface. Some of the additional properties of emulsifying salts are the control and stabilization/adjustment (an upward trend) of the pH level and an effect on the formation of the final product structure after cooling (Dimitreli & Thomareis, 2009; El-Bakry et al., 2011). Moreover, application of the appropriate blend of emulsifying salts increases (due to their buffering capacity) the pH from the typical values in the range of ~4.6 – 5.5 for natural cheese to values ranging from 5.6 to 6.2 in the spreadable processed cheese. Furthermore, the increase in pH extends the calcium-sequestering ability of the emulsifying salts and the negative charge on the paracaseinate. The dispersed hydrated paracaseinate contributes to the emulsification of the free fat by coating the surfaces of the dispersed free fat globules, resulting in the formation of an artificial (recombined) membrane. The high water-binding capacity of the paracaseinate enhances the developed emulsion stability as it leads to higher values of viscosity of the aqueous phase and thus a reduction in the collision frequency of the emulsified particles is observed. However, not all emulsifying salts have the same calcium ion-exchange ability. The phosphate ion-exchange ability increases with increasing P_2O_5 content in the following order: monophosphate < citrates ≈ diphosphate < triphosphate < polyphosphate (Shirashoji et al., 2006; Buňka et al., 2013). According to El-Bakry et al. (2011) and Mizuno & Lucey (2005), trisodium citrate (TSC) presents better calcium chelating ability and casein peptization properties than do sodium monophosphates and diphosphates. Moreover, on a mole for mole basis, phosphates possess higher calcium chelating ability than citrates (Dimitreli & Thomareis, 2009) The ion-exchange ability increases with the increasing length of the polyphosphate chain, resulting in better casein dispersion and better fat emulsification and water stabilization, which leads to better crosslinking of the matrix network in the final product (Chen & Liu, 2012; El-Bakry et al., 2011; Shirashoji et al., 2010; Molins, 1991). Nevertheless, absence of emulsifying salts (during heating and shearing of the applied mixture of various ingredients), could lead to the formation of an undesired inhomogeneous mass (Guinee et al., 2004; Buňka et al., 2014). In particular, heating (to temperatures utilized during processed cheese production) and stirring of the used natural cheese together with the other ingredients of the formula (in the absence of emulsifying salts); might result in the membrane destruction of the emulsified fat globules, leading to their clustering into larger units. Moreover, the combined effect of low pH and high processing temperatures application would cause aggregation and contraction of the casein molecules, resulting in water release followed by the separation of hydrophilic and hydrophobic phases. The latter phenomenon is a defect known also under the term oiling-off (Guinee et al., 2004). Furthermore, another important property of emulsifying salts is their bacteriostatic effects. Traditional manufacture of processed cheese normally involves processing temperatures in the range of 70 – 95 °C, which are lower than those which are used over sterilization. Hence, processed cheese might contain viable microbial spores (e.g. *Clostridium* genus) mainly from the utilized raw materials. Nevertheless, spore germination during storage could lead to serious technological problems (or defects – such as blowing of the packaging material, protein putrefaction and off-flavors). Generally, bacterial spoilage can be eliminated by the addition of preservatives. In addition, some emulsifying salts possess bacteriostatic properties. Particularly, polyphosphates can inhibit many microorganisms (*Staphylococcus aureus*, *Bacillus subtilis*, *Clostridium sporogenes* and some *Salmonella* spp.). Moreover, monophosphates and diphosphates have been found to inhibit the growth of *Clostridium botulinum* (depending on the levels of moisture, NaCl and pH of the processed cheese). However, on the contrary, citrates do not possess bacteriostatic properties, and may be even degraded by bacteria and thus reducing the shelf-life of the final processed cheese product (Carić & Kaláb, 1997; Buňková, et al., 2008; Fox et al., 2000; Tanaka et al., 1986; Zerfiridis, 2001).

3.1.1 Phosphate emulsifying salts

Phosphates are the salts derived from phosphoric acid (H_3PO_4). Phosphates constitute a large group of compounds, in which the anion consists of PO_4 tetrahedra which may be linked together by the sharing of corners. The phosphate series begins with single PO_4 group, which may exist as the triply charged monophosphate (named also as orthophosphate) anion PO_4^{-3}, or may participate in molecules where one, two or three of the four oxygens are covalently bonded to other atoms. Additionally, the PO_4 group is a tetrahedron formed by four oxygen atoms surrounding a phosphorus atom. The present oxygen molecules may constitute a bridge between the phosphorus atom and other atoms (including also other phosphorus atoms). Therefore, a long series of two- or three-dimensional phosphates can originate through P-O-P linkage. A term often used in relation to polyphosphates (including also diphosphates) is that of "condensed phosphates". The origin of the term lies in some of the industrial processes used in the manufacture of polyphosphates, which involve elimination of water at high temperatures. Under very specific conditions, monophosphates or longer-chain phosphates having terminal OH groups lose water between hydroxyl groups. The dehydrated tetrahedra are thereby brought together through a sharing of corners (are condensed) in order to form polymers. Similar processes may result in two monophosphate molecules coming together to form a diphosphate (previously named as pyrophosphates), or a monophosphate and a diphosphate may link into a triphosphate, and so on. Moreover, the term ultraphosphate includes any phosphate having a tridimensional structure. On the other hand, in processed cheese industry, linear (two-dimensional) polyphosphates are used (Molins, 1991).

In addition, sodium, potassium, ammonium, calcium, magnesium and sodium aluminum salts of phosphates can be used in the food industry. In particular, in the processed cheese industry, sodium salts of phosphates are predominantly utilized. Potassium salts of phosphates are generally not applied due to possible bitter flavor of the end-products (Guinee et al., 2004; Kapoor & Metzger, 2008; Mayer, 2001). In some countries, the sodium aluminum salt of phosphate is permitted and some recent papers showed that bitter flavor could not be a problem and pointed out lower sodium content (Chavhan et al. 2015; Nogueira et al., 2018; Schatz et al., 2014).

3.1.2 The effect of phosphate emulsifying salts on processed cheese properties

Phosphate and polyphosphate salts with varying number of linearly bonded phosphate units in the molecule possess different properties. The first property that is significantly influenced by the above-mentioned factor is the ability to exchange ions. The capability of sodium phosphate-based emulsifying salts to exchange sodium and calcium between emulsifying salts and a natural cheese casein structure is affected by two factors: (i) the length of phosphate-based salt; and (ii) the target temperature during processed cheese production. When longer phosphate-based salt is used and higher target melting temperature applied, the ion-exchange ability and subsequently the casein dispersion are enhanced (Buňka et al., 2013; Carić & Kaláb, 1997; Gupta et al., 1984; Molins, 1991; Nagyová et al., 2014; Sádlíková et al., 2010; Salek et al., 2015; Templeton & Sommer, 1936; Weiserová, et al., 2011).

The ion-exchange activity of sodium phosphate emulsifying salts is also affected by the actual pH of the mixture of the raw materials, the latter in turn might be influenced by the selection of (i) natural cheese type; (ii) other ingredients; (iii) and also by the applied emulsifying salts (type and/or concentration). In general, when the pH value of the mixture of the raw materials is low, the ion-exchange ability of sodium phosphate-based emulsifying salts is enhanced. The pH-values of aqueous solution (1 % w/v) prepared using different sodium phosphate-based ES (commonly applied by industrial practice) are presented in the work of Nagyová et al. (2014) and Salek et al. (2015) could be ordered as follows:

$Na_2H_2P_2O_7 < NaH_2PO_4 < (NaPO_3)_n < Na_2HPO_4 < Na_5P_3O_{10} < Na_4P_2O_7 < Na_3PO_4$

According to the above-mentioned authors, the pH-values of processed cheese manufactured with the individual sodium phosphate emulsifying salts (in concentration of 3 % w/v) are also presented. The order of pH-values of processed cheeses produced is the same (as in the case of the aqueous solutions of 1 % w/v). It should be added that the actual pH-value of the raw material mixture influences also the aggregation processes of caseins. The caseins aggregation is more intensive, when the pH-value of the mixture is near to its isoelectric point ($pH \approx 4.6$). Furthermore, excessive aggregation of caseins and "*poor*" ion-exchange ability of the used emulsifying salts and therefore, insufficient dispersion of proteins could lead to unsatisfactory fat emulsification and protein hydration. On the whole, it could be reported that the selection of proper emulsifying salts is very important for reaching the target pH-value of the final product (Carić & Kaláb, 2004; Guinee et al., 2004; Marchesseau et al., 1997; Lee & Klostermeyer, 2001; Molins, 1991; Muslow et al., 2007; Nagyová et al., 2014; Shirashoji et al., 2016; Sádlíková et al., 2010; Salek et al., 2015).

With respect to the above-mentioned relations and all references presented above, the ion-exchange ability of sodium phosphate-based emulsifying salts could be presented by the following order:

$NaH_2PO_4 < Na_2HPO_4 < Na_3PO_4 < Na_2H_2P_2O_7 < Na_4P_2O_7 < Na_5P_3O_{10} <$ sodium polyphosphates ($n \leq 9$) < sodium polyphosphate ($n > 9$).

The use of sodium phosphate-based emulsifying salts is not limited only by the pH-value adjustment of the final products but some of these salts possess also buffering capacity and are able to stabilize the pH of the processed cheese. The buffering capacity of sodium phosphate-base emulsifying salts is lower when the length of phosphates and polyphosphates is decreasing. Therefore, monophosphates and partially diphosphates are evaluated as substances with higher buffering ability (Carić & Kaláb, 2004; Guinee et al., 2004; Lucey et al., 2011; Muslow et al., 2007).

The length of phosphate-based emulsifying salts influences also the ability to support the casein network (gel) formation. Diphosphates are evaluated as very effective substances for dairy gels forming, followed by triphosphates. According to some literal sources, it was reported that also units consisting of calcium with diphosphates or triphosphates also help to establish stable casein networks, due to the support to hydrophobic interactions between the proteins. On the other hand, polyphosphates are considered that do not support gel forming, due to their bonding with caseins and thus increasing their negative charge (Buňka et al., 2013; Carić & Kaláb, 2004; El-Bakry et al., 2011; Guinee et al., 2004; Lu et al., 2008; Lucey et al., 2011; Mizuno & Lucey, 2007; Muslow et al., 2007; Shirashoji et al., 2010, 2016).

The effect of individual (sodium) phosphate-based emulsifying salts on the properties of processed cheese has been studied in detail for last years. On the other hand, the most of the referenced studies are not directly comparable because different manufacturing conditions were applied (different raw materials, divergent target dry matter and fat in dry matter values, processing parameters – target temperature and holding time, agitation speed). On the other hand, indirectly, the observed trends could be compared.

In the work of Nagyová et al. (2014) are depicted the results of hardness values of processed cheese samples with solely applied nine emulsifying salts (target parameters: 40 % w/w dry matter content; 50 % w/w fat in dry matter content), which were produced under the same conditions. Each emulsifying salt was added in an amount of 2 % w/w. In general, the hardness of the resultant model processed cheese samples increased with the rising number of phosphate units in the sodium phosphate and polyphosphate molecule. According to the latter authors, the hardness increased between samples with sodium polyphosphates in comparison with products in which was used pentasodium triphosphate. Moreover, hardness of processed cheeses also increased when the number of phosphates units in sodium polyphosphates raised up to $n \approx 13$ (the mean value of the polyphosphate length). Therefore, the hardness of the samples, in which sodium polyphosphate with $n \approx 13$, $n \approx 20$ and $n \approx 28$, remained practically similar. When the emulsifying salts were used individually, the effect on the hardness of the samples was controlled and explained by the ability of the certain phosphate and polyphosphates texchanging ions. Furthermore, monophosphates are substances (low-molecular) with the ability to bind onto caseins and increase their hydration. Diphosphates cause the gel formation of caseins and act as cross-linking agents, while bonding with calcium ions. The latter mentioned phenomenon leads also to the reduction of charge repulsion, which can help to facilitate hydrophobic interactions between the hydrophobic segments of the present proteins. On the other hand, excessive amounts of diphosphates could bond too much amount of calcium, making it unavailable for cross-linking. Moreover, long-chain polyphosphates can strongly bind calcium and intensively support the dispersion of caseins. However, it is accepted that they do not support gel formation (due to their multiple negative charges). In addition, it could be reported that emulsifying salts are not used in practise as sole ingredients but as mixtures of several phosphate salts, sometimes also in the combination with citrate salts.

As it was mentioned above, under industrial conditions, mixtures of individual phosphates and also citrates are commonly applied during the production of processed cheese. Mixtures for commercial production of processed cheese contain two or even more individual types of emulsifying salts.

El-Bakry et al. (2011) reported the influence of the binary mixture consisting of disodium phosphate (DSP) and trisodium citrate (TSC). Moreover, the current authors reported that the firmness of processed cheese increased when the relatively amount of TSC raised (within the mixture of emulsifying salts). Moreover, Weiserová et al. (2011) studied the effect of six binary mixtures contained DSP, tetrasodium diphosphate (TSPP), pentasodium triphosphate (PSTP) and sodium salt of polyphosphate in which the mean amount of phosphate units was $n \approx 20$ (POLY20). The hardness of the processed cheese samples (target parameters: 40 % w/w dry matter content; 50 % w/w fat in dry matter content) in which the above-mentioned six binary mixtures (DSP:POLY20, TSPP:POLY20, PSTP:POLY20 and TSPP:PSTP) were applied increased, when the relative amount of phosphate with higher ion-exchange capability raised. Furthermore, a different trend of hardness development was observed when the binary mixtures of DSP:TSPP and DSP:PSTP were studied. In samples produced using DSP:TSPP or DSP:PSTP, the maximum values of hardness were reported, when the ratio of DSP and TSPP or DSP and PSTP ranged in the range of 1:1 – 2:3. These findings leaded to the conclusion that the ion-exchange ability (and subsequently the intensity of caseins dispersion) is not the only role of emulsifying salts. Weiserová et al. (2011) suggested the hypothesis that emulsifying salts especially in some ratios can influence the creaming process and generally, the new protein network forming.

Buňka et al. (2013) continued with the ternary mixture of emulsifying salts consisted of DSP, TSPP and POLY20. In particular, in samples when the relative amount of POLY20 was lower than 60 %, processed cheese hardness rapidly increased (in comparison with products with the same relative amount of POLY20), when the mutual ratio of DSP and TSPP was in the interval of 1:1 – 2:3. When the ratio of DSP and TSPP was different or (<2:3 or >1:1) the hardness of processed cheese decreased. The effectiveness of the specific combination DSP:TSPP was increasing, when the relative amount of POLY20 was decreasing. The hardest processed cheese were obtained when the ratio of DSP:TSPP was 2:3 and POLY20 was not included. However, in samples with the relative amount of POLY20 > 60 %, the significant influence of the specific ratio of DSP:TSPP was not observed (Buňka et al., 2013).

Nagyová et al. (2014) followed up with a research study, in which they used sodium salts of polyphosphates with different mean number of phosphate units (n ≈ 5 – POLY05; n ≈ 9 – POLY09; n ≈ 13 – POLY13; n ≈ 20 – POLY20; n ≈ 28 – POLY28) in ternary mixtures composed also with TSPP and DSP. Generally, the trend of the influence of the ternary mixtures composition, described by Buňka et al. (2012) only for POLY20 (wit DSP and TSPP), on the firmness of processed cheese remained unchanged also for the other polyphoshate with 5 – 28 units of phosphates linearly bonded in the molecules. When the relative amount of the above mentioned phosphates were ≤ 60 %, the effect of the DSP:TSPP combinations 1:1 – 2:3 still existed regardless of the polyphoshate used. However, the absolute values of hardness were affected. When POLY05 or P0LY09 were added, the absolute values of the model processed cheeses hardness were slightly higher with the application of ternary mixtures with the relative amount of polyphosphate < 50 % compared to those samples with POLY13, POLY20 and POLY28 (excluding for the amount of polyphosphates was zero). The latter mentioned phenomenon was observed not only in products, where the combination of DSP and TSPP was at 1:1 – 2:3, but also in samples with the same ratio consisting of DSP and PTPP. With the application of the relative amount of polyphosphate ≥ 60 %, the hardness of the processed cheeses decreased in relation to the decreasing number of phosphate units.

Furthermore, the explanation of the above-mentioned results in processed cheese samples manufactured using the binary and ternary mixtures of monophosphates, diphosphates and polyphosphates could be based on the specific properties of the individual phosphates or their mutual synergic relationship (Buňka et al., 2013; El-Bakry et al., 2011; Lu et al., 2008; Mizuno & Lucey, 2005b, 2007; Molins, 1991; Nagyová et al., 2014; Shirashoji et al., 2010, 2016; Weiserová et al., 2011; Salek et al., 2015). In particular, when zero or the low relative amount of polyphosphates (< 10 %) were in the mixtures of emulsifying salts composed of monophosphates and diphosphates (1:1 – 2:3), the strong gel formation of caseins was probably affected by the presence of diphosphates. Thereafter, small molecules of monophosphates have the ability to permeate among cross-linked proteins and thus strongly bind water. A sufficiently short distance between caseins supports their hydrophobic associations resulting in the increase of the final processed cheeses hardness. However, zero or low levels of polyphosphates do not provide caseins a strong negative charge resulting in the repulsion of proteins. Moreover, a low relative amount of diphosphates does not provide sufficient concentration of cross-linking agents and a strong gel cannot be developed. On the contrary, a high relative amount of diphosphates could lead to very effective binding of calcium, which could negatively affect the formation of diphosphate-calcium cross-links (bridges) between caseins more difficult. In addition, a relative low level of monophosphate permeating among cross-linked caseins is probably not sufficient to bind water.

When the relative amount of polyphosphates is higher (≤ 60 %), the effect of polyphosphates on the processed cheese network could be more effective. Hence, their ability to provide caseins multiple negative charges could diminish the effect of the specific ratio of monophosphates with diphosphates. Furthermore, the rising relative amount of polyphosphates, could result in the decrease of the hardness of processed cheese when the specific ratio of monophosphates with diphosphates (1:1 – 2:3) is decreasing. When the relative amount of polyphosphates is > 60 %, the effect of polyphosphate on the caseins network formation is predominant. In addition, polyphosphates strongly disperse caseins, leading to their hydration. Hence, the dispersed caseins emulsify the fat present within the matrix. The above-mentioned phenomena lead to the production of processed cheese with similar values of hardness regardless of the ratio of monophosphates with diphosphates.

When the mean number of phosphate units linearly bonded in the polyphosphates decreases (in the case when the relative amount of polyphosphates is below 50 %), the hardness of the processed cheese could increase. The explanation could be based on the different ability of polyphosphates (with a varying mean number of phosphate units) to give caseins multiple negative charges. In general, short-chain polyphosphates (the mean number of phosphate units $n < 10$) cause a lower intensity on the negative charge on caseins, leading to a higher intensity of hydrophobic interactions among the dispersed caseins and thus increase of hardness of the processed cheese might be expected. When the relative amount of polyphosphates is above 60 % (regardless of the length), the influence on the processed cheese hardness is related to the mean number of phosphate units (which are linearly bonded in the polyphosphate). In particular, processed cheese hardness slightly increases with the increasing phosphate units in the molecule (up to $n \approx 13$). In addition when the length is higher than 13 ($n \geq 13$) the values of processed cheese hardness are similar (regardless of the polyphosphate length).

Among the factors which could affect the consistency of processed cheese, are the type and the maturity degree of the applied natural cheese, the total concentration of emulsifying salts applied and also the length of the storage time. Buňka et al. (2013) and Salek et al. (2016; 2017; 2020) utilized different types of natural cheese with divergent maturity degree (Dutch-type cheese, matured 2 – 8 weeks; Swiss-type cheese, matured 4 – 16 weeks; Mozzarella-type cheese, matured up to 4 weeks; Cheddar-type cheese, matured 4 – 16 weeks; and white brined type cheese, matured 2 – 24 weeks). In particular, the absolute values of the processed cheese hardness varied in dependence to the applied natural cheese. On the other hand, the general trend (as it was previously described) of the influence of the phosphate-based ternary mixtures of emulsifying salts composition on the hardness of processed cheese remained unaffected. In general, the increasing degree of maturity (in other words the intact casein concentration decreased), the hardness of the resultant processed cheese decreased, whereas the general trend of functionality of emulsifying salts (mixtures) was not influenced. Moreover, processed cheese hardness increased over the storage time (up to 60 days), without significant effect on the general trend of functionality of emulsifying salts.

3.2 Citrate emulsifying salts

Citrates are the salts obtained from citric acid. To obtain citrate salts the acidic hydrogen atoms must be replaced by cations from the tribasic citric acid.

Particularly, the H^+ ions of citric acid are neutralized with Na^+ resulting into three types of salts: mono-, di-, and trisodium citrates, respectively. The most important emulsifying salt applied in the manuafcture of processed cheese is the trisodium citrate (TSC). In addition, one percent aqueous solution of TSC had a pH-value 8.4 ± 0.1 and the pH value of processed cheese (target parameters: 40 % w/w dry matter content; 50 % w/w fat in dry matter content; solely applied TSC in amount of 2 % w/w.) was 6.4 ± 0.1. Monosodium and disodium citrates are not applied as sole ingredients because the final product might be acidic with poor textural properties and oiling-off could be expected. Additionally, citrates are applied in blends for processed cheese pH modification. TSC is used for the manufacture of processed cheese in slices or sliceable processed cheese (block-type). Moreover, potassium or ammonium citrates are applied in the production of processed cheese with reduced sodium content. Nevertheless, at high levels extensive bitterness of the final processed cheese can be reported. TSC presents better calcium ion-exchange ability and casein dispersion properties compared to sodium monophosphates and diphosphates. However, TSC is not able to bind on caseins and therefore, is not directly part of the novel protein network. In general, the main role lies on the ability of ion-exchange ability (Dimitreli & Thomareis, 2009; El-Bakry et al., 2011; Lu, Shirashoji & Lucey, 2008; Mizuno & Lucey, 2005, Shirashoji et al., 2006; Molins, 1991; Salek et al., 2015; Tamime, A. Y., 2011).

The development of hardness in processed cheese samples, depending on the composition of emulsifying salt mixtures consting of DSP, TSPP and TSC was studied by Salek et al. (2015). The specific ratio of DSP:TSPP (approximately 1:1–2:3) was once again identified, leading to a significant increase in the hardness of the tested processed cheese samples. However, the influence of this specific ratio rapidly decreased, when the proportion of TSC in the mixture of emulsifying salts increased. Nevertheless, the influence of this specific ratio (DSP:TSPP) on the hardness of the processed cheese samples even at 40 % levels of TSC was insignificant. On the other hand, if the proportion of TSC was higher than 40 % (in the ternary mixture of emulsifying salts), the hardness of the samples increased with the increasing proportion of TSPP and TSC and with the decreasing levels of DSP (Salek et al., 2015).

In addition the latter authors reported the development of hardness values in processed cheese samples, depending on the composition of the emulsifying salts in ternary mixtures composed of DSP, TSC and POLY20. It could be stated that the increasing level of TSC and POLY20 (and with the reducing level of DSP in the mixture), the hardness of the processed cheese samples increased (Salek et al., 2015). Furthermore, in the same study were also presented the values of hardness of processed cheese samples composed by the ternary mixtures of emulsifying salts of TSC:TSPP:POLY20. When the proportion of POLY20 was at zero levels in the ternary mixtures of emulsifying salts, firmer processed cheese samples were reported than samples composed of TSC and TSPP (at a ratio 1:1). In addition, any deviation from this ratio resulted in significant hardness decrease. Hence, this phenomenon was only observed in the absence of POLY20 (in the mixture of emulsifying salts). On the contrary, with the increasing proportion of POLY20, the hardness of the processed cheese samples increased. Moreover, at constant levels of POLY20 the hardness of the samples slightly decreased as the level of TSC decreased (Salek et al., 2015).

The explanation of the above-mentioned results in processed cheese samples developed by the application of ternary mixtures of monophosphates, diphosphates, polyphosphates and trisodium citrate could be based on the specific properties of the individual phosphates and/or citrates or their mutual synergic relationship (El-Bakry et al., 2011; Lu et al., 2008; Mizuno & Lucey, 2005b, 2007; Shirashoji et al., 2010; Salek et al., 2015). The development of processed cheese hardness with the use of ternary mixtures containing TSC could be explained by the ability of the mixture of emulsifying salts to disperse casein. Generally, when a mixture of emulsifying salts with more intensive ability to disperse casein was applied, the harder processed cheese was produced. (Buňka et al., 2013; Lu et al., 2008; Mizuno & Lucey, 2007; Shirashoji et al., 2010). In case of TSC and TSPP binary mixtures, a specific ratio was observed (1:1) which led to the increase of the hardness of the processed cheese samples. However, there is no clear explanation about the interactions among TSC and TSPP and their involvement in the development of the casein network. TSC does not participate in the creation of new networks, and therefore the effect of diphosphates on casein crosslinking would not be influenced by the presence of TSC (Kalliapan & Lucey, 2011; Lu et al., 2008; Mizuno & Lucey, 2005). Additionally, a possible explanation may be that diphosphates are effective on enhancing casein proteins gel formation ability only when their concentration is at some optimum level relatively to the protein content (Mizuno & Lucey, 2007). In general, TSC is applied in mixtures of trade emulsifying salts and is used in the production of block-type and processed cheese in slices (Gupta, et al., 1984; Carić & Kaláb, 1997; Guinee et al., 2003; Mizuno & Lucey, 2005a, 2007; Shirashoji et al., 2006).

Salek et al. (2015, 2016, 2017; 2020) used different types of natural cheese with varying maturity degrees. The absolute values of processed cheese hardness were dependent on the natural cheese type applied. On the other hand, the general trend of the effect of the ternary mixture composition (DSP, TSPP, POLY20 and

TSC) on the processed cheese hardness was not affected. Therefore, when the maturity degree increased, the hardness of processed cheese decreased, but the general trend of the functionality of ternary mixtures (including also TSC) of emulsifying salts was not affected. In addition, processed cheese hardness increased with the prolonging of the storage time (up to 60 days), with no significant impact on the general trend of functionality of the ternary mixtures of emulsifying salts (Dimitreli & Thomareis, 2009; El-Bakry et al., 2011; Lu et al., 2008; Salek et al., 2015, 2016, 2017; Shirashoji et al., 2006).

Citrates can present some inhibitory effect on microorganisms which can cause spoilage of processed cheese (mainly due to production of gas). On the whole, the antimicrobial effect of polyphosphates, orthophosphates or monophosphates is characterized as better in comparison to citrates (Buňková & Buňka, 2017).

4. Selected factors affecting the functional properties of processed cheese

4.1 The impact of natural cheese and dairy fat source on the textural and rheological properties of processed cheese

Many types of natural cheeses could be used during the production of processed cheeses. Different geographical regions (countries) usually use various types of natural cheese. Hence, Dutch-type, Swiss-type, Cheddar-type, Mozzarella-type chesses are commonly applied in the European countries. However, white-brined cheeses are frequently used in the regions of Near East and Middle East. For processed cheese structure and consistency is not important only the type of natural cheese, but also "age" of natural cheese so-called degree of maturity (Guinee et al., 2004; Carić et al., 1985; Kapoor et al., 2007; Kapoor & Metzger, 2008; Salek et al., 2015, 2016, 2017). Many biochemical and microbiological changes are taking place during the natural cheeses ripening. These biochemical and microbiological changes are responsible for organoleptic and physicochemical properties of natural cheeses, which are crucial for the processed cheese properties. In the work of Brickley et al. (2007, 2008), Buňka et al. (2013, 2014), Salek et al. (2016, 2017) was reported that, when the maturity degree of natural cheese increases, the hardness of the resultant processed cheese is decreasing. Furthermore, more mature natural cheese with more hydrolyzed protein chains entail better meltability and fullness of aroma in the final processed cheese. However, there is a risk of a sharp to pungent taste and above all, instability of the final product, because short protein chains are not able to bind water sufficiently. In addition, there is a risk of fat release (oiling-off), because short protein chains do not have the required emulsifying capacity (Berger et al., 2002; Guinee et al., 2004; Piska & Štětina, 2004). In practice, this problem is usually solved by processing raw material with a higher level of intact (non-hydrolyzed, or very slightly hydrolyzed) casein. Young natural cheeses (less mature) are cheaper compared to middle or high maturity cheeses. These young natural cheese result in lower values of meltability in processed cheese, but the resulting network of processed cheeses is stable without a tendency to release water. Consistency of processed cheese manufactured from young raw material is from stiff to very firm (solid). Therefore, a larger proportion of younger natural cheese is applied for the production block-type processed cheese or processed cheeses intended for slicing (Guinee et al., 2004; Brickley et al., 2007; Lu et al., 2007; Salek et al., 2016). Moreover, for the manufacture of spread-type processed cheese it should be used a more mature natural cheese (as the main raw material).

Additionally, processed cheese manufacturers apply different sources of milk fat (containing various amounts of surfactants, such as phospholipids, lipoproteins, glycoproteins). In case of butter and anhydrous milk fat, is possible (due to the technology of their production) to except extremely small amounts of surfactants. On the other hand, in case of cream and natural cheeses, fat is in the form of fat globules (with lipoproteins membranes containing surfactants). These surface-active substances can cooperate over fat re-emulsification during processed cheese production and their concentration can affect final product rheological and textural characteristics. In addition, the influence of dairy fat source with various surface-active compounds content on the viscoelastic properties of processed cheeses was studied by Černíková et al. (2018b). Natural cheese with various level of fat (ingredient containing fat globules coated in surface-active compounds) and butter (fat without surface-active compounds) were used in different ratios to reach 50% w/w fat content in the final product. According to the above-mentioned authors the effect of various fat content originating from natural cheese was not significant. The latter observation is important for processed cheese producers, because they can utilize the basic raw material (natural cheese) with different fat in dry matter content without consistency defects.

4.2 The effect of melt pH on the textural and rheological properties of processed cheese

Spreadable processed cheeses have optimum pH in the range of 5.6 – 6.0, processed cheese blocks are characterized by pH from 5.0 to 5.5 (Berger et al., 2002) and in processed cheese sauces the pH lies within the range of 6.5 – 7.0. This means that a lower pH of processed cheese than the optimum might lead to a solid or even crumbly consistency of processed cheeses (Marchesseau et al., 1997; Lee & Klostermeyer, 2001; Muslow et al., 2007; Barth et al., 2017). Higher pH than optimum value can cause not only a watery texture but there exists also the risk of undesirable microflora development. Therefore, it is necessary to carefully consider the choice of emulsifying salts for each type of product and choose specific combination for the achievement of the optimal pH. If the raw materials have low pH (e.g. curd), it is absolutely essential to choose emulsifying salts which will increase the pH of the system and vice versa, if more mature natural cheeses are used for production, it is advisable to apply emulsifying salts adjusting the pH to a more acidic range. Barth et al. (2017) studied how pH influences the hydrolysis of sodium polyphosphate in dairy matrices and the structure of processed cheeses. The latter authors stated that hydrolysis of sodium polyphosphates increased with the decreasing pH value. In processed cheeses with initial pH of 5.2 and 5.6, the final pH remained practically constant, because the higher rate of hydrolysis (at these pH values) increased the percentage of monophosphates (buffering agents), which are able to maintain unaffected the pH values. Decreasing the pH value caused the rising of hardness, adhesiveness, and gumminess of the processed cheese. Higher pH (above 6.0) values resulted in a concentrated protein emulsion with "long" texture and less adhesiveness. The negative charges of the caseins allow the formation hydrogen bonds and electrostatic interactions, which enhance the absorption and binding of water by the protein matrix. The long chain polyphosphates added multiple negative charges to the caseins and thus, the hardness of the matrix decreased.

However, emulsifying salts do not affect the consistency only due to pH value. Many studies established specific ratios of emulsifying salts. Furthermore, each emulsifying salt has specific properties and in selected/specific ratios can influence the consistency of the developed processed cheese differently (Mizuno & Lucey, 2005, 2007; Shirashoji et al., 2006, 2010; Sádlíková et al., 2010; El-Bakry et al., 2011; Weiserová et al., 2011; Buňka et al., 2013; Nagyová et al., 2014; Salek et al. 2015, 2016, 2017). In the above-mentioned studies, the authors described the use of individual emulsifying salts, binary and ternary mixtures of emulsifying salts and their effect on processed cheese textural and rheological properties. Additionally, the total concentration of the applied emulsifying salts can influence the consistency of processed cheese. Buňka et al. (2014) stated that the hardness of processed cheese increases with the rising concentration of emulsifying salts in the mixture. The same statement was also reported by Mozuraityte et al. (2019).

4.3 The impact of target parameters, holding time, agitation speed, melting temperature, cooling rate and storage time on processed cheese textural and rheological properties

Target parameters

A very large group of factors affecting the consistency of processed cheeses are target parameters, especially dry matter content, fat in dry matter content, non-fat dry matter, percentage of protein content in dry matter content, and pH. As a source of protein could be applied natural cheeses (which are also source of fat), curd, caseins, and other ingredients described above. Similarly, it can be talk about the fat sources. In particular, it can be used milk fat in different forms, such as butter, cream, natural cheeses, and anhydrous milk fat and then could be used non-dairy fat sources as different type of oils. Moreover, about the target parameters are not many comparable works performed in the last 20 years. Articles which exist about that issue (Dimitreli & Thomareis 2004; 2007; 2008), Bayarri et al. (2012), Guinee & O'Callaghan (2013), Chatziantoniou et al. (2015), Lee et al. (2015) and Černíková et al. (2017a), are relatively difficult to compare to each other and to look for clear trends in influencing the consistency of processed cheese, because individual experiments were usually performed under different conditions (in terms of final processed cheese parameters, especially dry matter and fat in dry matter content) and the composition of the raw material mixture, as well as in terms of the process parameters applied in the production of processed cheese model samples.

In general, the dry matter content and the fat in dry matter content are one of the most important parameters. The limits or ranges of these parameters are usually incorporated to the food legislation. These are also the basic parameters according to which processed cheese is often divided in terms of consistency into processed cheese blocks, processed cheeses intended for slicing, spreadable processed cheeses, or processed cheese sauce.

Lee et al. (2015) studied the influence of protein content (10 – 20 % w/w) and fat content (0 – 40 % w/w) on the viscoelastic properties of model samples of processed cheeses produced from rennet casein, at a melting temperature of 85 °C and stored for 24 hours. With the increasing protein content and decreasing fat content (at constant ratio protein: water, various dry matter content) the firmness of the processed cheese increased. Moreover, the influence of protein content was found out to be more significant in comparison to that of fat content. Guinee & O´Callaghan (2013) prepared for their work processed cheeses from Cheddar and fat-free cheese at a melting temperature of 80 °C. The final processed cheeses had different contents of fat (14 – 33 % w/w) and different contents of protein (25 – 12 % w/w), whereas the dry matter content (46 – 47 % w/w) was constant. In addition, the cheeses were stored for a maximum of 4 days. Furthermore, the firmness of the above-mentioned samples decreased with the increasing fat content (reducing ratio protein: fat). Dimitreli & Thomareis, (2004; 2007; 2008) examined viscosity, textural and viscoelastic properties of processed cheeses (manufactured from Gouda at a melting temperature of 80 °C) with different dry matter content (38 – 62 % w/w), protein content (11 – 30 % w/w) and fat content (12 – 23 % w/w). All these processed cheeses were storied only for 24 hours. The firmness of the processed cheeses decreased with the decreasing dry matter content and protein content and with the increasing fat content. Bayarri et al. (2012) and Chatziantoniu et al. (2015) produced and examined specific processed cheeses made from whey cheese and at the same time evaluated the influence of the content components on the consistency of commercially available processed cheeses. Both authors presented increasing firmness with decreasing fat content. Additionally, Černíková et al. (2017a) studied the consistency and microstructure model processed cheese with two dry matter levels (35 % and 45 % (w/w), respectively), two fat in dry matter content (40 % and 50 % (w/w), respectively), at a melting temperature of 86 °C, and stored for 14 days. When assessing the effect of one parameter (e.g. dry matter content), the other parameter (in this case the fat content) was constant and vice versa. It was found that with increasing dry matter content (with constant fat in dry matter content) and decreasing fat in dry matter content (while maintaining dry matter content) the gel strength increased. At the same time, the interaction factor (z) increased, which means that the number of interacting structural units in the protein network was higher. Furthermore, it has been shown that with increasing fat in dry matter content (at a constant dry matter content), the diameter of the fat globules increased and at the same time the firmness of the processed cheeses decreased. The protein to fat ratio was ~ 1:0.85 for the model processed cheeses with 40 % (w/w) fat in dry matter content and ~ 1:1.30 for products with 50 % (w/w) fat in dry matter content. The relatively lower amount of proteins, especially caseins, which act as emulsifiers in processed cheeses, caused a lower degree of fat emulsification, which was reflected in increasing fat globule's diameter. Larger fat globules can disrupt the continuity of the protein matrix much more than smaller fat globules and produce a softer, more spreadable processed cheese. On the other hand, processed cheeses containing a larger number of smaller fat globules give rise to firmer and less spreadable processed cheese (Kapoor & Metzger, 2008; Lee et al., 2003). Increasing the dry matter content (with a constant fat dry matter content) and thus increasing the relative concentration of proteins, which increase the emulsifying ability of the system, was reflected in a decrease in the diameter of the fat globules and at the same time an increase in the hardness of processed cheese. Lee et al. (2015) also established that with increasing protein content the fat globules size is lower. Increasing of apparent viscosity with rising protein concentration in model samples was described also by Lenze et al. (2019), who proved the necessity minimally 15 % (w/w) of protein to reach an apparent viscosity over then 1.0 $Pa \cdot s^{-1}$ at time approximately 200 minutes.

In general, increasing of nitrogenous substances (under the same condition) content results in higher firmness and decreased spreadability. This fact is explained by the rising intensity of the protein network, because nitrogenous substances are primarily "network building or supporting agents" and they need to meet other protein molecules to increase the number of interactions followed by protein aggregation and gel (network) formation. Lenze et al. (2019) also assumed that increasing protein concentration (including para-caseinate) can lead to better and quicker adsorbtion at surface of the oil-water interface, thereby decreased interfacial tension and emulsion stabilization might be expected. On the other hand, under the same condition, by increasing water and in water soluble saccharides, or fat content, or other water content ingredients, the protein matrix is much more damaged leading to a more spreadable processed cheese.

Holding time

Sutheerwattananonda et al. (1997) studied the effect of the holding time 0 – 15 minutes at the melting temperature of 65.5 °C on the distribution of fat globules in processed cheese blocks and observed the decreasing fat globules size (the emulsification improved) in the first five minutes of holding time (at the melting temperature). The reduction of fat globules size had as a consequence a harder processed cheese. Further extension of holding time did not have any significant effect on the size of fat globules. Bowland & Foegeding (1999) studied the effect of processing time on the consistency of processed cheese blocks prepared from rennet casein. The latter authors chose 10, 20, and 30 minutes of holding time, 80 °C melting temperature and constant agitation speed. The strength of the processed cheese gel increased with the prolonged melting time. On the other hand, Swenson et al. (2000) studied the effect of holding time (0 – 20 minutes) on the final consistency of a fat-free processed cheese spread. They used lower melting temperature (specifically 75 °C) than Bowland & Foegeding (1999), the agitation speed was constant but not exactly mentioned. In this case, the opposite result occurred, extending the holding time caused decreasing of firmness of the processed cheese product. Lee et al. (2003) described viscosity changes of the hot melt. In this work, the viscosity riced for the first 25 minutes and then, after reaching the local maximum, the viscosity of the hot melt slowly decreased. Moreover, the final consistency of the cooled products was not evaluated. Norohna et al. (2008a) investigated the effect of different agitation speeds in the range of 100 – 1500 rpm at 80 °C. The final consistency of the processed cheese blocks was stiffer with the increasing agitation speed. Similar results were published by Přikryl et al. (2018), who reported rising values of complex modulus with prolonged holding time (0, 5, 10 minutes) at 80 °C and 90 °C (constant agitation speed 1500 rpm).

A comprehensive work, which would consider both the effect of the mixing speed and the holding time of the melting temperature was published by Černíková et al. (2017b, 2018c). The difference in these articles was in the usage of the processed cheese samples with various fat in dry matter content (40 % and 50 % (w/w)). In addition, Černíková et al. (2017b) dealt with the combined effect of the agitation speed of the knives of the production equipment (1000, 1500 and 3000 rpm) and the holding time (0 – 20 minutes) at a melting temperature of 90 °C on the consistency of spreadable processed cheeses with 35 % (w/w) dry matter content and 40 % (w/w) fat in dry matter during 60 days of storage. The holding time of the melting temperature was divided into shorter intervals than in previous publications. First, a holding time of 0 minutes was selected, when the melting process was completed after reaching the target melting temperature. Furthermore, the holding time at the melting temperature was extended to 1, 3, 5, 7, 9, 11, 13, 15, 17 and 20 minutes, respectively. The monitored residence time intervals were chosen to map the effect of agitation time more precisely on the consistency of processed cheeses. It was found that both tested parameters significantly affected the consistency of processed cheeses. As the melting temperature invreased, the firmness of the processed cheese increased, similarly to Bowland & Foegeding (1999). The exception was the lower holding times, when model samples produced with a holding time of 1 min showed lower stiffness compared to samples produced without holding time. A downward trend in stiffness (weakening of the protein matrix) for one minute was observed for all three mixing speeds used. The decreasing complex modulus which determinates hardness of processed cheese, was supported by the detected decrease in the gel strength (A_F) and interaction factor (*z*) values. With a further increase in the holding time (above 3 minutes) the stiffness of the model samples increased with the increasing holding time for all observed agiataton speed intensities (1000, 1500 and 3000 rpm). There was an exception at 3000 rpm 1 day after storage. In this case was noticed further decrease of the complex modulus value. However, there was no significant difference between processed cheese production at the agitation speeds of 1000 and 1500 rpm (melting temperature of 90 °C; 1 and 3 minutes of holding time). A similar trend was observed by Sutheerawattananonda et al. (1997) and Bowland & Foegeding (1999). However, Černíková et al. (2017b, 2018c) used higher melting temperatures than the above-mentioned authors. The increase in firmness of processed cheeses is explained by Sutheerawattananonda et al. (1997) by reducing the size of fat globules, where a larger number of small fat globules disrupt the continuity of the protein matrix less intensively compared to the presence of a smaller number of order of magnitude larger fat globules. Simultaneously with the decrease in the size of fat globules, the authors found that the stiffness of the monitored samples also increased with increasing holding time. However, the above-mentioned authors also stated that the reduction in the size of the fat globules stops after about 5 minutes. Therefore, in this matrix, where the increase in stiffness occurred even with longer melting temperature durations (in addition to the effect of decreasing the size of fat globules), other interactions can be expected (especially more intensive hydration of caseins and increase in the number of their bonds), which contribute to increasing stiffness and are supported by more extensive melt mechanical stress (Bowland & Foegeding, 1999; Lee et al., 2003). The biggest and rapid increase of *z*-factor was observed at the highest used agitation speed (3000 rpm) from 10 min and higher.

Agitation speed

As it was mentioned above, not only the holding time, but also the agitation speed fundamentally can affect the consistency of the processed cheeses. In the literature exist two different conclusions. Noronha et al. (2008a) reported more rigid final products of processed cheese block with increasing agitation speed. They used a melting temperature of 80 °C, agitation speed in the rabge of 100 – 1500 rpm and constant holding time 2 min. On the other hand, Černíková et al. (2017b, 2018c) reported softest samples (made at 1500 rpm, then at 1000 rpm agitation speed). With using the highest agitation speed (3000 rpm) were prepared the most solid spreadable processed cheese samples. The difference in the results may be due to the type of processed cheese blocks versus spreadable processed cheeses, various melting temperatures, lower agiatation speeds and shorter holding times.

Melting temperature

Processed cheeses can be produced at many melting temperatures in ranges of 85 – 110 °C (Kapoor & Metzger, 2008) or 72 – 145 °C (Tamime, 2011). Some authors described also lower temperatures of 65 °C, including Sutheerawattananonda et al. (1997). Higher temperatures (135 – 145 °C) are used especially during continuous production in comparison to lower melting temperatures applied in discontinuous equipments. Generally, for processed cheese blocks can be used lower temperatures than for spreadable processed cheeses. But it is also possible to produce block-type processed cheeses by higher melting temperature with adaptation of other production conditions, such as type of melting equipment and agitation speed (Guinee, 2003; Guinee et al., 2004; Tamime, 2011).

The influence melting temperature on the consistency of processed cheese was studied by Kaláb et al. (1987) and Dimitreli & Thomareis (2004), who reported that when the melting temperature increases, the viscosity of the melt decreases and the size of the fat globules decreases, which is accompanied by an increase in the stiffness of the final product. On the other hand, Swenson et al. (2000) studied the effect of melting temperature in the range of 60 – 90 °C on the consistency of fat-free processed cheese spreads. Their work showed decreasing firmness of fat-free processed cheeses, with increasing melting temperature in the range of 60 – 80 °C. The increasing firmness of these fat-free processed cheese spreads was observed at melting temperature of 90 °C. An increase in the processed cheese spread firmness at 90 °C was observed by Přikryl et al. (2018) in all batches produced (control samples without biologically active substances as well as samples containing rutin and quercetin) compared to samples produced at 80 °C. This conclusion was valid for the fat-free processed cheeses and also for full-fat processed cheeses with 50 % (w/w) fat in dry matter content. Similarly, Mozuraityte et al. (2019) studied different creaming times (1.5, 3.0, 4.5 minutes) at various temperature (45, 55, 65 °C), at a final melting temperature of 95 °C for 6 min. However, the latter authors stated that temperature was not crucial for the final consistency of processed cheese product.

Cooling rate and storage time

The final consistency (described by textural and rheological characteristics) of processed cheese can be influenced by the cooling rate. Piska & Štětina (2004) reported that higher cooling rate can lead to a less firm, more spreadable and with elevated stickiness processed cheese. In addition, high cooling rate of processed cheeses provides more extensively presence of the β´ polymorph compared to the β polymorph, which is usually observed in bulk milk fat (Ramel & Marangoni, 2017). The above-mentioned authors also reported that the presence of protein and/or other ingredients can led to higher changes of the β´ polymorph to the β polymorph (Ramel & Marangoni, 2017). On the other hand, when the conditions and other parameters (including the dry matter content, fat in dry matter content, melting temperature, holding time and agitation speed) are the same, lower cooling rate provides rise to stiffer final processed cheese products. In the same token, higher cooling rate is required for the production of spreadable processed cheese, while relatively low cooling rate is suggested for the manufacture of block-type processed cheese (Muslow et al., 2007). In the case of processed cheese spreads and/or processed cheese sauces, lower rate of cooling can cause the product to remain at temperatures suitable for the growth of microorganisms for a longer period of time, and this may lead to undesired multiplication of contaminating microflora (Görner & Valík, 2004).

Processed cheese storage, together with some other processing parameters (agitation speed, holding time, melting temperature), is one of the most important factors affecting textural and rheological properties, and thus also the structure of processed cheeses. The possible sources of processed cheese changes (appearance, structure, color and flavor) during storage are: loss of water, hydrolysis of polyphosphates, changes in ionic equilibria, fat polymorphism, formation of crystals, oxidation, non-enzymatic browning, enzyme activity, interactions with the applied packaging materials. The intensity of changes occurring with the prolonging of processed cheese storage period can be induced by the ingredients applied, selected processing parameters, packaging type and storage conditions.

In the work of Černíková et al. (2017b, 2018c) the basic trends, the effect of agitation speed and holding time at a melting temperature, did not change during the two months of storage time. However, there was an increase in the absolute values of the elastic and loss moduli of the model processed cheese samples, a result corresponding to the findings of other studies (Awad et al., 2002; Weiserová et al., 2011; Nagyová et al., 2014 and Salek et al., 2015). The explanation of this phenomenon could be the possible hydrolysis of emulsifying salts – diphosphates and polyphosphates, possible change in the dissociation of the salts or other compounds present, the decrease in the pH values of processed cheeses and also the polymorphism of milk fat and ongoing changes in its crystalline form where β´ polymorph might change to β polymorph (Awad et al., 2002; Muslow et al., 2007; Dimitreli & Thomareis, 2009; Shirashoji et al., 2010; Weiserová et al., 2011; Nagyová et al., 2014). Increasing dry matter content during processed cheese storage period (due to water evaporation in dependence on permeability of packaging material) could be another reason resulting in increasing the firmness of processed cheese (Fox at al., 2004; Mohammadi & Fadaei, 2018). Weiss et al. (2018) wanted to prepare mathematical models as predictive tool for the assessment of changes in quality parameters during the storage of spreadable processed cheese at 8, 20 and 30 °C. During 4 months of storage (at 8 °C) were reported only minor and no significant changes. At the storage temperature of 20 °C were identified dynamic changes which significantly decreased the quality of the tested processed cheese product, but the highest rate of changes was registered at the storage temperature of 30 °C. Reactions in the processed cheeses led to "*negative*" taste, smell and changes in consistency and the authors recommended the approximate shelf-life limit for spreadable cheeses stored at 8, 20 and 30 °C without significant changes in its quality on the 49, 28 and 4 days (Weiss et al., 2018). Which does not fully correspond to the commonly storage condition for processed cheeses, because processed cheeses can be stored also at room temperature for 4 months and/or 6 months in a refrigerator. Moreover, Schär & Bosset (2002) described processed cheese as "semi-preserved food" with a limited shelf-life. They also reported that products without bacterial spoilage retain their quality for 4 – 12 months at room temperature and at a lower storage temperature for longer time, because lower temperature cause more slowly changes in quality processed cheeses.

4.4 The effect of rework addition on processed cheese textural and rheological properties

Rework (sometimes also reported as a pre-cooked cheese) is a processed cheese that has already been produced and the creaming process has previously occurred. Manufacturers apply rework as a raw material during the production of processed cheese. In addition, exist different types of rework. In particular, rework is produced in the industry either intentionally (expected) or unintentionally. Hence, intentionally means that the production of processed cheese was targeting the development of rework or the rework is the residual amount of processed cheese in production apparatus. The term unintentionally rework describes a processed cheese originally intended for the retail market network, however it was not "*released*" in the market, due to some deficiencies (incorrect dry matter content and/or fat in dry matter content or because of unsuitable packaging, or excessive stickiness on the packaging material). Rework is usually applied as fresh (a processed cheese residue in the production apparatus), 3 or up to 14 days after manufacture (it could be intentionally or unintentionally produced processed cheese) in a level up to 20 % (w/w), depending on the type of rework and also the desired target parameters of the final processed cheese (Kaláb et al., 1987; Guinee et al., 2004). The reasons for rework application are on the one hand economic and on the other hand to increase processed cheese viscosity, increase hardness, improve meltability and possibility of emulsifying salts reduction (rework contains emulsifying salts) (Lauck, 1972; Kaláb et al., 1987; Pluta et al., 2000; Kapoor & Metzger, 2008; Černíková et al., 2018a). According to Kaláb et al. (1987) are available three types of rework: (i) the fresh rework (produced and rapidly frozen immediately after production), (ii) the regular rework from previous manufacture batches and (iii) the so-called hot melt, simulating processed cheese subjected to extreme stress conditions (cooled from 82 °C to 4 °C in 5 hours). Furthermore, another rework type according to Mayer (1973) is referred as over-creamed. In general, each type of rework can be utilized for various reasons and in various levels. Hence, the first type of rework (fresh) can be applied in an amount ranging from 1 to 2 % (w/w) in order to increase the creaming of processed cheese spreads, containing higher level of matured natural cheese. In addition, the second type of rework is used for processed cheese blocks or block-type processed cheese in concentration ranging from 2 up to 30 % (w/w), to increase hardness and elasticity. Finally, the third type of rework (over-creamed) is applied in a quantity less than 1 % (w/w), because it has an extraordinarily strong creaming effect and could easily lead to deficiencies the final processed cheese (over-creaming). However, Lauck (1972) recommended that the rework addition should be between 2 to 15 % (w/w). Guinee et al. (2004) evaluated the application of individual rework types in processed cheese production. Furthermore, additional heating of rework can cause a higher degree of temperature-induced dehydration and aggregation of the para-casein (mainly the third type of rework) and increase the elasticity of the final product. Moreover, the emulsifying salts in rework can create more effective dispersion resulting in faster hydration, higher degree of emulsification (effective dispersion of fat) leading to product viscosity increase. The impact of different rework amount on the viscoelastic properties and microstructure of spreadable processed cheeses was reported in the work of Černíková et al. (2018a). The later authors applied rework at a range of 0 – 20 % (w/w) using real industrial conditions. The rework was prepared from the same raw material (natural cheese) as processed cheeses and was added to the blend of ingredients 3 days after the production day and was stored at 6 ± 2 °C. The consistency of processed cheeses without rework was characterized as soft (samples reported the lowest values of elastic and loss moduli and the largest size of fat droplets) compared to samples prepared without addition of rework. The processed cheese firmness increased with the quantity of rework and simultaneously, the size of fat droplets gradually decreased, which means increasing emulsification properties with the further rework addition. Higher concentration of rework from 10.0 to 20.0 % (w/w) did not provide significant changes in the processed cheese consistency, express by viscoelastic properties (complex modulus; G*) and also the median of fat droplet size was smaller than in the case of lower rework addition. Moreover, the addition of rework affected the intensity of the emulsification of the present fat, which was demonstrated by the reduction of the fat droplets size. As the degree of fat emulsification grew, the processed cheese firmness increased, corresponding with the findings presented by Kapoor & Metzger (2008), Lee et al. (2015) and Černíková et al. (2017a). However, these results are contradict to those reported by Kaláb et al. (1987), who used block-type processed cheese with higher dry matter content.

In addition, Fu et al. (2018) used four types of rework (pre-cooked cheeses) in amounts 1.5 % (w/w) with different agitation speeds [400 or 1500 rpm (rounds per minute)] and total stirring times (10 or 30 minutes), PCLS (400 rpm, 10 min), PCLL (400 rpm, 30 min), PCHS (1500 rpm, 10 min), PCHL (1500 rpm, 30 min), the final melting temperature was 90 °C and rework was used 24 hours after production and was stored at 5 °C. The viscosity of products with short time mixing were smooth without any increasing of viscosity (the creaming effect did not occur). The addition of long time (30 minutes) rework (PCLL, PCHL) influenced the viscosity of processed cheese samples which increased and the creaming effect was enhanced during processing. The application of PCHL showed higher increase probably due to advanced agitation speed. This study showed that the rework in amount less than 1.5 % (w/w) produced with longer mixing time had a capacity to increase the viscosity (to induce the creaming effect) of processed cheese manufacturing only for 15 minutes, due to fine-stranded network. Lenze et al. (2019) stated that rework addition [5 and 10 % (w/w)] dramatically accelerated the creation of the new structure. During creaming was observed very short initial phase (to 10 minutes), when exponential phase started earlier, and the apparent viscosity of the matrix increased very sharply. Plateau phase was shorter and in higher level of apparent viscosity in comparison to samples developed without rework addition. Rework addition in amount 10 % (w/w) caused faster structure formation than 5 % (w/w) supplement (Lenze et al., 2019).

5. AIM OF THE HABILITATION THESIS

The main aim of the habilitation thesis was to evaluate the influence of selected factors affecting the functional properties (mainly in terms of consistency) of processed cheese during storage. The work was focused on factors that are important for industrial scale production of processed cheese and whose effect on the consistency of processed cheeses was not sufficiently up to now described in the literature.

Hence, in order to achieve the main objective of the thesis, it was necessary to establish the following sub-objectives:

- Evaluate the effect of various phosphate-based and/or citrate-based emulsifying salts application (as sole ingredients) on the functional properties of processed cheese and processed cheese sauces,
- To deal with changes in the consistency of processed cheeses depending on the different composition of ternary mixtures of phosphate and/or citrate emulsifying salts,
- To describe the effect of various natural cheese (different types and degrees of maturity) application in processed cheese production and describe their influence on processed cheese functional properties,
- To evaluate changes in the consistency of processed cheeses during their storage,
- To describe the effect of various levels of dry matter content and fat content in dry matter content on the consistency of processed cheese,
- To evaluate the impact of selected processing parameters (holding time and speed of agitation) on the consistency of spreadable processed cheese. In particular, to describe the effect of increasing holding time of the melt at the so-called melting temperature on the consistency of processed cheese manufactured at different regimes of agitation speed of knives,
- To investigate the effect of rework addition (at divergent levels) on the rheological and textural properties of processed cheese.

6. MAIN RESULTS OF THE WORK

A. The impact of emulsifying salts composition and natural cheese on the functional properties of processed cheese and similar products

Processed cheese can be manufactured by mixing natural cheese (of various types and degrees of maturity), water, emulsifying salts, and other optional dairy/non-dairy ingredients, commonly under vacuum, in the presence of heat and shear. The desired compact structure of processed cheese is obtained by the addition of appropriate emulsifying salts. Hence, the ability of emulsifying salts to sequester calcium from the casein matrix (exchanging Na+ for Ca2+) and the pH adjustment cause protein hydration and dispersion, and the casein present acts as the "true" emulsifier within the matrix (El-Bakry et al., 2011, Kapoor & Metzger, 2008, Lee et al., 2003, Lee & Klostermeyer, 2001; **A1**; **A2**; **A3**; **A4**; **B2**; **B5**; **B6**). However, the ion-exchange ability is not identical for all emulsifying salts. Therefore, the phosphate ion-exchange ability increases with the increasing content of P_2O_5 (Buňka et al., 2014, Shirashoji et al., 2006; **A1**; **A2**; **A3**; **A4**; **A5**). The consistency (a critical processed cheese property described by textural and rheological parameters) of processed cheese can be affected by many factors, including the type, composition, and chemical profile of the natural cheese utilized (dry matter, fat, protein, and calcium ion content, and maturity degree), the type and concentration of emulsifying salts, the presence and concentration of ions (especially calcium, sodium, and potassium), other optional dairy and nondairy ingredients, the pH of the mass to be melted, the processing and storage conditions (processing and storage temperature, stirring speed, time and temperature of the fusion, and cooling rate) and a possible use of some hydrocolloids (Shirashoji et al., 2006; Dimitreli & Thomareis, 2007; Gustaw & Mleko, 2007). Given the huge range of processed cheeses in terms of their consistency, flavor and possibilities of use in gastronomy, it is clear that the choice of the final parameters of the product and the derived raw material composition should be the first step in determining the final consistency of the product.

Furthermore, as it was mentioned above, the essential role of emulsifying salts is the exchange of sodium ions for calcium ions in the casein matrix (gel) of the cheese; insoluble calcium paracaseinate transformes into more soluble sodium paracaseinate, whose molecules (chains) can move within the melt system and thus enhance fat emulsification and water binding (Guinee et al., 2004; Shirashoji et al., 2006; Muslow et al., 2007). The ability of individual emulsifying salts to support the exchange of sodium for calcium ions can vary. Generally, the ability to support ion exchange occurs in the following order (considering of sodium salts): long polyphosphates (> 10 phosphorus atoms in a molecule) > short polyphosphates (< 10 phosphorus atoms in a molecule) > triphosphates >

diphosphates > monophosphates (A1; A2; A3; El-Bakry et al., 2011). In addition, citrates might support ion-exchange to a similar extent that to of monophosphates. However, El-Bakry et al. (2011) and Mizuno & Lucey (2005) stated that citrates support ion exchange to a greater extent than monophosphates. The role of polyphosphates in processed cheese manufacture with different chain lengths was described in the study **A1**. Sodium salts of polyphosphate with different mean lengths of chain are often used under real industrial practice. Furthermore, chains of different length could affect the intensity of the exchange of sodium ions for calcium ions and thus, casein dispersion (Mizuno & Lucey, 2007; Lu et al., 2008; Sádlíková et al., 2010). A different number of phosphorus atoms in a linear chain could also affect the creaming process by means of interactions with casein fractions of varying intensity. Moreover, the use of polyphosphates with different chain lengths can also affect the pH of the product (Lu et al., 2008). Based on the above-mentioned statements, the aim of the study **A1** was to observe selected textural parameters (large deformation properties) of model samples of processed cheese with the addition of ternary mixtures containing DSP, TSPP, and polyphosphates with different mean lengths ($n \approx 5$, 9, 13, 20, and 28). The current study would provide valuable information about the role of phosphate-based emulsifying salts in processed cheese properties, something that was missing from the scientific literature. Furthermore, in the second part of the current work (**A1**), TSPP was replaced with PSTP. The samples were produced without and with pH adjustment (to achieve pH values typical of processed cheese spreads, in the range of 5.60 – 5.80). Thus, with a low content of polyphosphate, hardness of the processed cheese increased and cohesiveness and relative adhesiveness decreased at a ratio of DSP to TSPP approximately 1:1 to 3:4. An increasing amount of polyphosphate (in the ternary mixture) led to a decrease in hardness of the processed cheese at this specific ratio. With the relative amount of polyphosphates reaching ≥ 60 %, the influence of this specific ratio became insignificant. This trend was observed in all ternary mixtures; the only differences were found in the absolute values of the textural parameters of the processed cheeses examined. Replacing TSPP with PSTP did not affect the general trend either. However, the absolute values of hardness of model samples with the addition of PSTP were lower compared with that in which TSPP was used. Duration of the storage period increased hardness of the processed cheeses. In the samples in which the pH was intentionally increased, hardness and cohesiveness decreased and relative adhesiveness increased slightly. However, the reverse trend was observed in samples in which pH was decreased. The more significant the pH adjustment was, the more noteworthy changes were observed. However, pH adjustment did not affect the value of the specific ratio of DSP:TSPP and DSP:PSTP and its general influence on textural parameters of the processed cheeses. The influence of the specific ratio of DSP:TSPP and DSP:PSTP and the general trend concerning the dependence of composition of the ternary mixtures of phosphate emulsifying salts on the textural parameters of processed cheeses cannot be attributed only to the effect of phosphates on the dispersion of casein. In addition, the dispersion of casein is closely related to the processed cheese matrix formation. Hence, an intensive dispersion of casein allows caseins to develop their emulsifying and hydrating abilities and thus, stabilize the fat and water present in the mixture. Increasing the range of protein hydration and fat emulsification results in higher intensity of casein crosslinking. A more rigid processed cheese will occur with a greater number of cross linkages in its matrix (**A1**; **A2**). Over the past few years, several studies (Awad et al., 2002; Weiserová et al., 2011; Buňka et al., 2012, 2013) have shown the dependence of textural parameters of processed cheeses on the composition of binary and ternary mixtures of phosphate emulsifying salts (consisting mainly of disodium phosphate, tetrasodium diphosphate, and sodium salt of polyphosphate). In these studies, a specific ratio of disodium phosphate to tetrasodium diphosphate was determined (approximately 1:1 to 3:4), at which hardness of the processed cheeses increased rapidly but cohesiveness and adhesiveness decreased. The influence of this specific ratio decreased with an increasing relative amount of sodium salt of polyphosphate. When the amount of sodium salt of polyphosphate exceeded 60 %, the influence of this specific ratio became insignificant. However, existing studies are limited to linear-chain polyphosphates with mean length (n; the number of phosphorus atoms bound in a linear molecule of polyphosphate) of about 20 (Sádlíková et al., 2010; Weiserová et al., 2011; Buňka et al., 2012, 2013).

In addition, as it was mentioned above trisodium citrate presents better calcium chelating ability and casein peptization properties than do sodium mono- and diphosphates (El-Bakry et al., 2011; Mizuno & Lucey, 2005). The effect of the individual phosphates and composition of phosphate binary and ternary mixtures on textural properties of processed cheese has been previously studied (Buňka et al., 2013, Dimitreli & Thomareis, 2009, El-Barky et al., 2011, Lu et al., 2008, Sádlíková et al., 2010, Shirashoji et al., 2006, Weiserová et al., 2011). Processed cheeses in which the content of DSP is predominant have softer consistency. On the other hand, when polyphosphates are dominant in the binary and ternary mixtures, the hardness of the product increases (Buňka et al., 2013, Weiserová et al., 2011).

Nevertheless, in the above-mentioned studies the effect of TSC on processed cheese consistency was not evaluated, thus providing space for further scientific work. To the best of our knowledge, we have evaluated the effect of TSC on the processed cheese properties. Moreover, a specific ratio of DSP to TSPP exists that significantly affects textural parameters of processed cheese (**A1**). In the latter study it was found that when the polyphosphate content was at low levels (less than 60 %) and the ratio of DSP to TSPP ranged from 1:1 to 3:4, the hardness of the resultant processed cheeses increased, while their cohesiveness and relative adhesiveness decreased. Based on the results in work **A1**, a study providing information about how ternary mixtures containing parallel phosphate and citrate emulsifying salts influence textural properties of processed cheese was performed

(**A2**). The impact of the ternary mixtures composition on textural parameters of processed cheese and on the casein micelle dispersion (in a simplified model milk system) was also studied. Moreover, the impact of a specific ratio of DSP:TSPP and TSC:TSPP on processed cheese hardness was also described. Hence, at constant content of POLY20 ≤ 60 % or TSC ≤ 40 % in the emulsifying salt ternary mixtures a rapid increase in hardness of the product was observed, especially at a specific ratio of DSP to TSPP of approximately 1:1 to 2:3. When the content of POLY20 or DSC was absent in the ternary mixture, the products consisting of TSC and TSPP in a range of approximately 1:1 were the hardest (among samples with the binary mixture of TSC and TSPP). Also the hardness of all processed cheese samples increased with the increasing storage period.

Furthermore, processed cheese with diverse textural and rheological characteristics and alternative functional properties may be manufactured as a result of the use of different types (phosphate, citrate, or both) and combinations of emulsifying salts. In practice nowadays, the individual application of emulsifying salts is very rare. In fact, emulsifying salts are applied in ternary or even more componential mixtures (Guinee et al., 2004; Kapoor & Metzger, 2008; **A2**. Generally, the effect of different composition of ternary mixtures of the individual sodium salts of phosphates (especially disodium hydrogenphosphate, tetrasodium diphosphate, and sodium salt of polyphosphate) has been described in the papers by Weiserová et al. (2011) and Buňka et al. (2012, 2013), but only for Dutch-type cheese as the main raw material for the processed cheese samples tested.

On the whole, it could be concluded that the type and composition of applied emulsifying salts can affect the rheological and textural properties of processed cheese and similar products. In general, when the tested emulsifying salts were applied as sole ingredients the storage and loss moduli decreased (parameters describing the consistency of processed cheese) in the following order: POLY20 > TSPP ≈ TSC > DSP. This specific trend was also confirmed by observing of the processed cheese hardness. With respect to our studies (**A2**; **A3**; **A4**; **A6**), we are able to infer that the general trend of the effect of the ternary mixtures composition is the same when different natural cheeses are used, as the main raw material.

Moreover, significant parameters influencing the textural and rheological properties of processed cheeses are the type and the maturity degree of the utilized natural cheese. To the best of our knowledge, a series of studies providing information about how the type and maturity degree of natural cheese (other than Edam type cheese) can affect the properties of processed cheese was performed. Swiss-type cheese is a group of hard or semi-hard cheeses in texture, with desired propionic acid fermentation caused by propionic acid bacteria (especially Propionibacterium freudenreichii ssp. freudenreichii and Propionibacterium freudenreichii ssp. shermanii). Therefore, their flavor is characterized as sweet and nut-like. This is due to free fatty acids, peptides, amino acids, carbonyls, or their mutual interactions (Beuvier et al., 1997; Bouton et al., 2009). However, processed cheese manufacture using Swiss-type cheese as the main raw ingredient in the available literature is scare. Under real industrial practice, Swiss-type cheese is often used as part of the raw material for processed cheese manufacture. On the other hand, the individual usage of Swiss-type cheese in processed cheese production was described in the study **A3**. Moreover, the influence of different maturity degrees of Swiss-type cheese associated with different combinations of emulsifying salts ternary mixtures affecting processed cheese consistency was also reported. According to the findings in the work **A3** the application of the binary mixture of DSP:TSPP (in a ratio of 1:1) resulted in products with the highest values of hardness (regardless of the maturity degree of the Swiss-type cheese applied). Furthermore, the hardness of the samples obtained decreased with the rising maturity degree of the Swiss-type cheese used (regardless of the emulsifying mixture applied). However, on the contrary, the hardness of all processed cheese samples increased with prolonging the storage period. Admittedly, the results of texture profile analysis corresponded to those of the rheological analysis. The highest overall rigidity (G*), gel strength, and interaction factor values were found in the samples prepared with DSP:TSPP (1:1), followed by the samples prepared with POLY20, TSPP, TSC, and DSP, respectively. The monitored values of the gel strength and interaction factor decreased with the increasing maturity degree of the Swiss-type cheese used. The intensity of rigidity of the processed cheese samples has an analogous relationship to the intensity of the gel strength; the higher the gel strength of the sample, the more inflexible the product that can be expected.

In the same token, the combined effect of Mozzarella cheese storage period and different emulsifying salts (type and/or composition) on the textural and rheological characteristics of spread-type processed cheese during its storage was studied (**A4**). Furthermore, during the storage of Mozzarella cheese (a pasta-filata cheese), complex biochemical events determine its final quality and acceptance. Proteolysis is the major phenomenon that occurs during cheese aging (besides glycolysis and lipolysis) that greatly affects the physical characteristics of nearly all cheeses. Generally, cheeses show similar proteolytic trends. On the other hand, differences in cheese nature and manufacturing processes influence the proteolytic pattern. In the case of processed cheese, rheological and textural properties are influenced by the age of the applied cheese and/or also by specific technological operations during cheese manufacturing. Hence, more intensive proteolytic reactions result from an increasing cheese maturity level. The combined impact of the Mozzarella cheese age and different ternary mixtures of emulsifying salts on the textural and viscoelastic properties of processed cheese were evaluated in the study A4. According to the latter research paper (**A4**), the increasing storage period of the processed cheese samples resulted in an increase in hardness. On the contrary, the hardness of the samples decreased with expanding Mozzarella cheese storage time. Model samples with diverging properties were obtained by the application of different types of ternary mixtures of emulsifying salts. The hardest samples were those comprised of DSP:TSPP (1:1). However, when DSP or TSPP were replaced by TSC, this ratio was not observed. The rising amount of POLY20 in the mixtures led to a decrease in the samples' hardness (up to ≥ 50%). The results obtained from the rheological analysis were in accordance to those of the hardness analysis. Hence, the ratio of DSP:TPSS resulted in processed cheese with the highest values of gel strength and interaction factor. Moreover, with increasing Mozzarella cheese storage periods the values of gel strength and interaction factor decreased.

Last but not least, a research providing direct comparison of the viscoelastic properties of processed cheese produced under identical processing parameters and similar experimental design from two technologically very different varieties of natural cheese (Cheddar cheese and white brined cheese, respectively), and additionally with various degrees of maturity, was performed (**A5**). The impact of the Cheddar cheese and white brined cheese maturity and different compositions of ternary mixtures of emulsifying salts on the hardness and gel strength of processed cheese during 60 days of storage was investigated in the latter work. Hence, with raising the storage period of the processed cheese samples, an increase in hardness was observed. On the other hand, the hardness and gel strength of the samples decreased with prolonging of cheese ripening period for both tested natural cheeses (Cheddar and white brined cheeses, respectively) used as the basic raw material. The hardest samples were those composed of DSP:TSPP (1:1). However, when the relative amount of DSP and TSPP (in the ratio of 1:1) were replaced by TSC or POLY20, the influence of the latter mentioned ratio diminished. Furthermore, higher values of hardness and gel strength were reported for the processed cheese samples produced with Cheddar cheese in comparison with those made from white brined cheese.

In conclusion, four types of natural cheese (Dutch type – **A1**; **A2**; Swiss-type – **A3**; Mozzarella-type – **A4**; Cheddar-type – **A5**; and white brined cheese-type – **A5**, respectively), which are used through the whole world, were tested. In all examined cheese varieties/types the mechanisms of function of the above mentioned ternary mixtures were practically very similar regardless of the ripening period of the cheese utilized. Moreover, with raising storage period of the processed cheese samples, an increase in hardness was observed. On the other hand, the hardness and gel strength of the samples decreased with prolonging of cheese ripening period for all applied natural cheeses which used as the main raw material. Hence, the hardest samples were those composed of DSP:TSPP (1:1). However, when the relative amount of DSP and TSPP (in the ratio of 1:1) were replaced by TSC or POLY20, the influence of the latter mentioned ratio diminished cheese were performed (**A1**; **A2**; **A3**; **A4**; **A5**).

Processed cheese sauces are novel cheese products and commercially can be found in many forms (frozen, semi-liquid, shelf-stable dry mixtures). Processed cheese sauces can serve as flavor enhancers, dipping sauces, act as the main attractiveness in many dishes or help to intensify or round out an appetizer flavor profile. However, at the moment there are no standards of identity or definitional legal for processed cheese sauce. Thus, the latter dairy products can be manufactured by applying many ingredients such as natural cheese, cheese powder, processed cheese and other ingredients of dairy or non-dairy origin. Processed cheese sauces and processed cheeses could be described as stable oil in water emulsions. Hence, their manufacture could be realized by mixing natural cheese, milk fat, water, emulsifying salts, other optional ingredients, commonly under vacuum in the presence of heat (temperature range of 85 up to 110 °C) and constant shear (**A3**; **A6**; **B4**; **B5**; Shalaby et al., 2017). It could be stated that the hardness of the processed cheese sauce samples was affected by the applied type of emulsifying salt, pH of the melt and storage period. Furthermore, it was found that the samples prepared with DSP:TSPP (1:1) and POLY20 (without pH adjustment) presented the higher values of hardness. The latter phenomenon was also confirmed by the increasing samples complex viscosity and storage modulus. On the contrary, the remaining tested samples showed lower hardness, storage modulus and complex viscosity values. The hardness of all examines samples increased with the increasing storage period (**A6**).

B. The impact of selected processing parameters on the functional properties of processed cheese.

As it was reported above (**A1; A3; A4; A5; A6**) the basic raw materials for the production of processed cheese (and/or processed cheese sauces) are natural cheeses of Dutch- and Swiss-type, whereas in the Anglo-American countries it is usually Cheddar and Mozzarella cheeses, respectively. In areas of the Near and Middle East white brined cheeses are also widely used. Other dairy raw materials (butter, cream, anhydrous milk fat, curd, milk powder, whey powder) and non-dairy raw materials (water, flavoring agents, and hydrocolloids) as well as emulsifying salts (especially sodium salts of phosphates, polyphosphates and citrates) are added to this cheese-base of different maturity degrees.The ingredients are usually heated under constant agitation at a temperature of 90–110 °C until a homogenous mass with desired properties is developed (Kapoor & Metzger, 2008, Khetra et al., 2015, Muslow et al., 2007).

One of the most important and very critically evaluated parameters of processed cheeses is their consistency, which can be, in the form of blocks, slices, spreads, or sauces (Kapoor & Metzger, 2008). Consistency of processed cheese can be affected by three main groups of factors: (i) the composition of the raw material mixture, (ii) processing parameters during the production of processed cheese (especially agitation speed, holding time, target temperature during the melting process and cooling rate), and (iii) the storage temperature and length (Bayarri et al., 2012, Dimitreli & Thomareis, 2004, Kapoor & Metzger, 2008, Khetra et al., 2015, Muslow et al., 2007, Schatz et al., 2014, Subramanian et al., 2006). In general, it could be stated that the processing parameters during the production of processed cheese (and similar products) are of great significance.

In the industry, the consistency of processed cheese regularly is evaluated by sensory analysis. Additinally, the instrumental evaluation using small (dynamic oscillation rheometry) or large (texture profile analysis) deformations or their combinations is increasing for the past few years (Lee et al., 2003; Kapoor & Metzger, 2008; Buňka et al., 2013; 2014). Rheological parameters measured in the area of small or large deformations are given mainly by the microstructure of processed cheeses and mutual bonds between the individual components (especially the properties of the protein network and its interactions with other components; Hosseini-Parvar et al., 2015; da Silva et al., 2016). To explain the nature of the current state of consistency, it is therefore useful to have the data about the mechanical properties of the processed cheese and also its microstructure. The microstructure of processed cheeses may be studied by several methods, the most common of which are optical microscopy (Hladká et al., 2014; da Silva et al., 2016), scanning electron microscopy (Noronha et al., 2008; Cunha et al., 2010), transmission electron microscopy (Lee et al., 2003), and confocal laser scanning microscopy (Lee et al., 2015).

Although the final parameters of processed cheeses (especially dry matter, fat in dry matter, and fat-free dry matter content) affect their consistency to a large extent, they have not been given sufficient attention in the literature over the past 10 years. One of the few studies, by Lee et al. (2015), dealt with the effect of protein content (10 – 20 % w/w) and fat content (0 – 40% w/w) on the viscoelastic properties of model samples of processed cheeses made from rennet casein (melting temperature of 85 °C) and stored for 24 h. With the increasing protein content and decreasing fat content (constant protein-to-water content, variable dry matter content), the rigidity of the processed cheeses increased. A more significant effect of the protein content was observed compared with the fat content. In the work **B1** was evaluated the effect of different dry matter contents (35 and 45 % w/w) and fat in dry matter contents (40 and 50 % w/w) on the textural and viscoelastic properties and microstructure of model processed cheeses made from „*real*“ ingredients regularly used in the dairy industry. Apart from the basic chemical parameters, textural and viscoelastic properties of the model samples were measured and scanning electron microscopy was realized. With increasing dry matter content, the rigidity of the products increased and thus the size of the fat globules in the model samples of the processed cheeses decreased. With increasing fat in dry matter content, the rigidity of the processed cheeses decreased and the size of the fat globules increased.

However, full-fat products with a significantly higher fat content are also commonly produced. The question therefore remains whether the trends observed in **B1** also apply to full-fat processed cheeses. In particular, in work **B2** was evaluated the effect of the holding time at a given melting temperature, and agitation speed, on the consistency of full-fat processed cheeses. It was found that, after an initial decrease in the firmness of the processed cheeses (during holding times of up to 3 min), the firmness of the products increases significantly as the holding time is extended at the melting temperature. As the storage period extended to 60 days, the firmness of the processed cheeses also increased, regardless of either the applied holding time at the melting temperature, or the agitation speed. Although the effect of extended holding times at the given melting temperature on the consistency of semi-fat and full-fat processed cheeses seems clear under the given conditions, this cannot be said for the effect of agitation speed, which remained unclear and required further studies.

The work **B3** described the effect of melting temperature and holding time on the rheological properties of spreadable processed cheese. The values of G* significantly increased with the increase of holding time. Elevated temperature of melting also caused the increase of G*. The higher levels of observed processing parameters led to development of a denser structure and therefore the model processed cheese became more rigid (**B1**).

Furthermore, in the work **B4** were studied the viscoelastic properties of model processed cheese spreads at various agitation speeds and holding times at the melting temperature over 60 days of cold storage. Under the conditions of the experiment mentioned above it was found that at all agitation speeds tested (1000, 1500 and 3000 rpm) the firmness of the samples increased steadily from the 3rd to the 20th minute of holding time. The most striking increase was observed in the model processed cheeses manufactured at 3000 rpm, especially from the 10th minute of the holding time onwards. However, a clear trend in the development of viscoelastic properties of the observed samples depending on the agitation speed could not be determined. This trend changed according to the particular holding time of the melt at the melting temperature. During the 60 day storage period, the firmness of all the observed processed cheeses increased. In recent years, other works have been published which attempt to contribute to the clarification of, in particular, the role of agitation speed and holding time at a given melting temperature on the consistency of the resulting processed cheese (Shirashoji et al., 2016; **B4**). The conclusion drawn as a result of the studies conducted for both these works was the same, namely that when the holding time at the given melting temperature is extended, the firmness of the processed cheeses increases. From the article **B4**, it can be assumed that this trend is not linear. Furthermore, it was demonstrated that the effect of agitation speed on the consistency of the product is not clear, and depends significantly on the holding time. During short holding times (up to 3 min), processed cheeses produced at lower agitation speeds (1000 rpm) were more solid than those products produced at higher speeds (1500 and 3000 rpm). However, as the holding time was extended, this trend changed significantly.

The effect of processing parameters, such as holding time, on the consistency of processed cheese spreads has been studied extensively. Swenson et al. (2000) investigated fat-free processed cheese (with 40 %, w/w, dry matter content) and stated that, the longer the holding time, the lower the firmness of the product. However, Bowland & Foegeding (2001) examined the effect of processing time (10, 20 and 30 min) on the viscoelastic properties of model processed cheese (49.5–52.5 %, w/w, dry matter; 51.4–54.5 %, w/w, fat in dry matter) over a decreasing temperature regime from 25 °C to 80 °C (to determine sample solidification). The authors concluded that there was no relationship when the small strain analyses (G′, G′′, G* and δ) were performed at temperatures lower than 80 °C. Moreover, Lee et al. (2003) found that the apparent viscosity of spreadable processed cheese melt containing 50 % (w/w) dry matter and 50 % (w/w) fat in dry matter rose until 25 mins of processing at 80 °C and then decreased. Furthermore, the studies **B1**, **B2** and **B4** investigated the effect of holding time of the melt in a selected temperature on the viscoelastic properties of processed cheese with 35 % (w/w) dry matter and 40 % (w/w) and 50 % (w/w) fat in dry matter content. The conclusion in these studies was that the firmness of processed cheese decreased up to the 3rd minute of holding time but then increased significantly (the maximum holding time applied was 20 min). Nevertheless, the above-mentioned results are contradictory and the effect of holding time on the consistency of processed cheese spreads with different dry matter and fat in dry matter contents remains unclear. Especially, the effect of holding times below 10 min (in close gaps within the holding time range) on spreadable processed cheese samples (with different dry matter and fat in dry matter contents; produced under identical processing protocol) viscoelastic properties described by the complex modulus and phase shift was missing from the existing scientific literature. In general, it is accepted that short duration of holding time is economically advantageous. According to the results presented in the work **B5** (were studied six different types of model spread-type processed cheese prepared and stored for 30 days); the viscoelastic properties depend on the holding time, time of storage and dry matter and fat in dry matter contents. For most of the produced spread-type processed cheese, it was demonstrated that, on the 1st, 14th and 30th day of storage, G* (a measure of consistency) decreased in the first 2 or 3 min of the holding time and gradually increased afterwards. In the most cases of dry matter and fat in dry matter contents, prolonging the holding time from the 3rd min up to the 10th min and storage for 30 days increased the G* in all samples examined. Also, G* increased with increasing dry matter content at constant fat in dry matter and also with decreasing fat in dry matter. The same dry matter content and increasing fat in dry matter content caused decreasing value of G*. Nevertheless, inverse relationships were observed in the case of the phase shift evaluation. In addition, most of the spread-type processed cheeses produced exhibited more elastic than viscous consistency (solid-like behavior). It could be concluded that dry matter and fat in dry matter contents, holding time and length of the storage time affected the rheological properties of the processed cheese samples. In particular, increasing fat content reduced the values of complex modulus, resulting in more soft processed cheese final products. Moreover, the processed cheeses with low dry matter content were more viscous than the samples with higher level of dry matter content. This information may be relevant to industry practice. Moreover, longer holding times of the melt can result in smaller diameter of milk fat droplets in the final product. However, a significant decrease in size was observed after 2 or 3 min. Furthermore, the size of milk fat droplets decreased as the dry matter content increased and the fat in dry matter content decreased. In general, from an economic point of view, shorter holding times could be evaluated as more advantageous for producers of processed. In addition, processed cheese formulation can have an impact on final product price, as higher dry matter content can result in higher processed cheese price. Comparing processed cheeses with the same dry matter content, the higher the fat in dry matter content, the lower the price of the final product.

In addition, the work **B6** described the impact of rework content (utilized rework "age" of 72 h) on the consistency of processed cheese. It was discovered that a lower quantity of added rework, up to 10.0% (w/w), caused a gradual increase in the firmness of the processed cheese; simultaneously, the size of the fat droplets gradually decreased, which results primarily from the improvement of the emulsification properties as the amount of rework added increases. As the rework concentration in the raw material composition increased further (from 10 – 20 % w/w), the firmness of the processed cheese no longer increased, and the median size value of the fat droplets, which was smaller than that in samples with less added rework, also no longer differed significantly. Additionally, the term rework is describing a processed cheese that has already been processed once and in which creaming has already occurred; it is, used as a raw material for the production of processed cheese. Therefore, its consistency is affected by all of the aforementioned factors. Rework is created in the industry either (1) intentionally (production of processed cheese for rework or residue of processed cheese in production equipment) or (2) unintentionally (production of processed cheese originally intended for the market network but ultimately not released for market; for example, due to unsuitable packaging or incorrect dry matter or fat in dry matter content). Rework is usually used fresh (processed cheese residue in production equipment) or 3 to 14 days old (processed cheese unsuitable for the market network due to unsuitable fat or dry matter content, or an incorrect packaging weight. The reasons for using rework can be economic, but also to increase viscosity (with the increasing age of the rework and with its increasing concentration) after production, increase firmness, improve meltability, or reduce emulsifying salt content, since rework already contains emulsifying salt (Lauck, 1972; Meyer, 1973; Kaláb et al., 1987; Pluta et al., 2000; Kapoor & Metzger, 2008).

In general, it could be concluded that the factors including the dry matter and fat in dry matter contents, holding time and length of the storage time influence the rheological and textural properties of the processed cheese. In particular, increasing fat content reduced the values of complex modulus, resulting in “softer” (or more spreadable) processed cheese final products. Moreover, the processed cheeses with low dry matter content were more viscous than the samples with higher level of dry matter content. Finally, based on our results it was found that the applied agitation speed during the production of processed cheese affects the firmness of the samples.

7. CONTRIBUTION TO SCIENCE AND PRACTICE

The principal contributions of the current habilitation thesis to science and practice could be summarized in the following points:

- It was found that when the phosphate and citrate emulsifying salts were applied individually the hardness of the model processed cheeses increased in the following order: POLY20 > TSPP ≈ TSC > DSP (regardless of the type of natural cheese applied and its maturity degree or length of the storage time).
- It has been documented that the basic trend of the dependence of the consistency of processed cheeses on the composition of mixtures (including binary or ternary mixtures) of phosphate and citrate emulsifying salts (consisting of sodium hydrogen phosphate, sodium diphosphate, sodium polyphosphate and sodium citrate) is not significantly affected by the natural cheese applied, maturity degree or length of storage time. In all studied combinations of all the above-mentioned factors, the existence of specific ratios of sodium hydrogen phosphate and sodium diphosphate was identified, as well as sodium diphosphate and sodium citrate, which significantly increase the hardness of processed cheeses.
- It was found that with a low content of polyphosphate, hardness of the processed cheese increased and cohesiveness and relative adhesiveness decreased at a ratio of DSP to TSPP around 1:1 to 3:4. Moreover, the increasing amount of polyphosphate (in the ternary mixture) led to a decrease in hardness of the processed cheese (at this specific ratio). With the relative amount of polyphosphates reaching ≥ 60 %, the influence of this specific ratio became insignificant. This trend was observed in all ternary mixtures; the only differences were found in the absolute values of texture parameters of the processed cheeses.
- The influence of different compositions of ternary mixtures of phosphate and citrate emulsifying salts on the consistency of processed cheeses made from Dutch-type, Swiss-type, Mozzarella-type, Cheddar-type and white brined-type cheeses was described. It has been shown that the choice of a particular natural cheese will significantly affect the consistency of processed cheeses and must be taken into account when "modeling" the raw material composition formula. Furthermore, it has been found that as the maturity degree of natural cheeses (for all types of the tested natural cheeses) increases, the hardness of processed cheeses decreased, regardless of the applied phosphate and citrate emulsifying salts applied (sole, binary or ternary mixtures). In addition, the maturity degree of the applied natural cheese affects hardness "absolute" values development, whereas the main trends remain unaffected.
- The effect of different dry matter and fat content in dry matter on the consistency of processed cheeses was described (values of dry matter and fat

in dry matter content which are typical for the Central Europe region). In particular, increasing fat content reduced the values of complex modulus, resulting in more soft processed cheese final products. Moreover, processed cheese with lower values of dry matter content were more viscous than the samples with higher level of dry matter content. Furthermore, the size of milk fat droplets decreased as the dry matter content increased and the fat in dry matter content decreased.

- The effect of the longer holding time at the so-called melting temperature on the consistency of processed cheeses has been documented for such a range of stirring speeds in the production protocol as is typical for the production of spreadable processed cheeses. The existence of a limit time for the developed melt to remain at a specific melting temperature has been proved, until which the hardness of the final processed cheeses decreases. It has been found that if the hardness of processed cheeses is to be increased only by extending the residence time of the melt at the melting temperature (assuming a constant level of other factors affecting the consistency of these products), then the total residence time must be more than 3 minutes. Furthermore, the latter phenomenon was also identified in processed cheese samples with various fat in dry matter level.
- It has been found that the hardness of processed cheeses increases during cold storage, with these changes being most intense during the first 14 days of storage.
- The effect of rework addition on the viscoelastic properties of model processed cheeses samples was evaluated. The addition of rework (in spread-type processed cheese) up to 10.0 % (w/w) increased the hardness and elastic character of the processed cheese. However, with the further addition of rework, the consistency of the processed cheeses did not differ significantly.
- The obtained results could be used as models describing the dependence of processed cheese functional properties (mainly textural and rheological) on the composition of mixtures of emulsifying salts for the production of final products with different consistency. Under real industrial conditions the obtained models could be applied as a description of the samples textural and viscoelastic properties arising from the type and maturity degree of the utilized natural cheese in combination with the applied type and concentration of emulsifying salts. From the obtained data could be proposed an appropriate mixture of emulsifying salts, a suitable type and maturity level of natural cheese in order to produce final processed cheese products with desirable consistency and organoleptic properties.

8. CONCLUSIONS

Taking into account that processed cheese is one of the youngest dairy products and also due to the complexity of the processed cheese matrix (system), there is still insufficient relevant information in the scientific literature about the effect of individual factors on the consistency of processed cheeses. Hence, this provides enough “space” not only for the producers but also for the researchers in order to perform high-quality research studies to understand the exact course of reactions/phenomena evolved in the production of processed cheeses (or similar products). The presented habilitation thesis aimed to summarize the existing knowledge in the field of characteristics of raw materials for the production of processed cheeses, production technology of these products and factors influencing the consistency (a parameter described mainly by textural and rheological properties) of processed cheeses. Based on the results of the current thesis, it is possible to provide a more comprehensive point of view of the importance of the composition of a mixture of emulsifying salts (phosphates and/or citrates) in influencing the textural and rheological properties of processed cheeses. The habilitation thesis also dealt with the use of different natural cheeses with divergent maturity degrees and how this factor for industrial practice affects the consistency of processed cheeses. Last but not least, the development of the consistency of processed cheeses manufactured from different raw materials, with different compositions of emulsifying salts and cheeses produced under different processing conditions was described. Changes in the consistency of processed cheeses occurring during their storage (under refrigeration conditions) have also been reported.

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- *Oborový seminář I (Vede seminář)*
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QK1920190 - *Hmotnostní ztráty masa po tepelné úpravě: vliv vlastností čerstvého masa, použitého zařízení a parametrů kulinární úpravy (2019 – 2021).*

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1. Nagyová, G.; Buňka, F.; **Salek, R. N. (20)**; Černíková, M.; Mančík, P.; Grůber, T.; Kuchař, D.(5). Use of sodium polyphosphates with different linear lengths in the production of spreadable processed cheese. *Journal of Dairy Science*, 2014, *97*. ISSN 0022-0302.
2. **Salek, R. N. (45)**; Černíková, M.; Nagyová, G.; Bačová, H.; Mynarčíková, L.; Buňka, F. The impact of ternary mixtures composed of sodium phosphate and citrate salts on processed cheese spreads hardness. In *Sborník příspěvků z XVI. Konference mladých vědeckých pracovníků s mezinárodní účastí*. Brno: Veterinární a farmaceutická univerzita, 2014. ISBN 978-80-7305-670-4.
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4. Lapčík, L.; Lapčíková, B.; Otyepková, E.; Otyepka, M.; Vlček, J.; Buňka, F.; **Salek, R. N. (5)**. Surface energy analysis (SEA) and rheology of powder milk dairy products. *Food Chemistry*, 2015, *174*. ISSN 0308-8146.
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6. **Salek, R. N. (35)**; Černíková, M.; Nagyová, G.; Kuchař, D.; Bačová, H.; Mynarčíková, L.; Buňka, F. The effect of composition of ternary mixtures containing phosphate and citrate emulsifying salts on selected textural properties of spreadable processed cheese. *International Dairy Journal*, 2015, *44*. ISSN 0958-6946.
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9. Černíková, M.; Nebesářová, J.; **Salek, R. N. (10)**; Řiháčková, L.; Buňka, F. Srovnání různých metod přípravy vzorků tavených sýrů pro elektronovou mikroskopii. In *Hygiena a technologie potravin - XLV. Lenfeldovy a Höklovy dny*. Brno: Veterinární a farmaceutická univerzita Brno, 2016. ISBN 978-80-7305-782-4.
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processing conditions. *LWT- Food Science and Technology*, 2018, *87*. ISSN 0023-6438.

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18. Černíková, M.; Nebesářová, J.; **Salek, R. N. (10)**; Popková, R.; Buňka, F. Vliv přídavku nátavku na viskoelastické vlastnosti a mikrostrukturu tavených sýrů. In *Výsledky přehlídek a sborník příspěvků konference Mléko a sýry*. Praha: Vysoká škola chemicko-technologická v Praze, 2018. ISBN 978-80-7592-030-0.
19. Lazárková, Z.; Sumczynski, D.; **Salek, R. N. (10)**. Effect of cooking and germination on antioxidant activity, total polyphenols and flavonoids, fiber content, and digestibility of lentils (Lens culinaris L.). *Journal of Food Processing and Preservation*, 2018, *42*. ISSN 0145-8892.
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26. Buňka, F.; Černíková, M.; Míšková, Z.; Pachlová, V.; **Salek, R. N. (5)**; Buňková, L.; Pleva, P. Spolupráce při inovaci technologických procesů výroby mléčných výrobků. LACRUM Velké Meziříčí, s.r.o., 2019. 1.
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Teze habilitační práce

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